The effects of dopaminergic agents on the high potassium-evoked DOPA and dopamine release from PC12 cells

脱分極刺激誘発性ドーパ及びドパミン遊離に及ぼすドパミン作動薬の効果

○五嶋 良郎、青木 令奈、岡田 貴子、増川 太輝

横浜市立大・医

L-3,4-Dihydroxyphenylalanine (DOPA), a precursor of a neurotransmitter dopamine (DA), is synthetized by tyrosine hydroxylase in the cytoplasm of catecholaminergic neurons. We proposed that DOPA is a neurotransmitter. We previously reported that high K^+ -evoked release of DOPA and DA from cultured PC12 cells, both of which were similarly decreased by deprivation of extracellular Ca^{2+}. Using this system, we are attempting to elucidate the mechanism by which the DOPA release occurs. We found that bafilomycin inhibited the K^+-evoked-release of DA, but not DOPA, while brefeldin A suppressed the release of DOPA but not DA, thereby suggesting the release of DOPA may occur through a secretion pathway distinct from that for DA in PC12 cells (JPS95). To further characterize the release of DOPA, we here examined the effects of several agents on the evoked release of DOPA and DA from cultured PC12 cells. Proline, valine, phenylalanine, tyrosine and alpha-methyl-*p*-tyrosine (10^{-5} M) showed no effect on DOPA and DA release. Among reagents tested, 3-iodo-tyrosine inhibited the release of DOPA without affecting that of DA. This finding indicates a higher sensitivity of the release of DOPA to 3-iodo-tyrosine than that of DA.

α-tocotrienol binds to 67 kDa laminin receptor, resulting in activation of DGKα.

α-トコトリエノールは67kDaラミニン受容体に結合し、DGKαを活性化させる

○難波 朋花、上田 修司、福田 伊津子、白井 康仁

神戸大・院農・生命機能科学専攻 応用生命化学講座

Vitamin E is classified into two types: tocopherols (Toc) and tocotrienols. Although their structures are very similar, tocotrienols are known to have about 50 times the antioxidant capacity of Toc. Furthermore, it has been reported to have anticancer activity against various types of cancer. Recently, we have reported that α-Toc and epigallocatechin gallate (EGCg) activate diacylglycerol kinase α (DGKα), which is a lipid kinase to convert DG to phosphatidic acid, by direct binding to 67kDa laminin receptor (67LR) at respective different binding sites, contributing to improvement of nephropathy. The binding of αToc or EGCg to 67LR has been shown to induce palmitoylation of 67LR. These results suggest that α-tocotrienol also binds to 67LR and induces its palmitoylation, resulting in DGKα activation. Indeed, in this study, we showed that α-tocotrienol activated DGKα by binding to 67LR at the same site with α-Toc, and found that α-tocotrienol induced palmitoylation of 67LR with DGKα translocation (activation). In addition, the DGKα translocation rates of tocotrienol homologues were compared and found that d-tocotrienol had similar effect with a-tocotrienol. These results suggest that 67LR and subsequence activation of DGKa are involved in some functions of tocotrienols including anticancer effects.

Analysis of the molecular mechanism of neurite outgrowth under PACAP in PC12 cells.

PC12細胞におけるPACAPの突起伸長の分子制御機構解析

○山下 道生[1]、竹ノ谷 文子[1]、柴藤 淳子[2]、Rakwal Randeep[3]、平林 敬浩[2]、千葉 義彦[4]、塩田 清二[5]
[1]星薬科大・薬・運動科学、[2]湘南医療大・臨床医療研、[3]筑波大・体育系、[4]星薬科大・薬・分子生物、[5]湘南医療大・薬・解剖生理

We investigated the molecular mechanism of neurite outgrowth with PACAP treatments using a rat adrenal-derived pheochromocytoma cell line-PC12. This study was specifically investigated into the regulation of PACAP-induced CRMP2 previously identified in a mouse brain ischemia model and which could be recovered by PACAP treatment. We have previously revealed that PACAP-mediated neuroprotection involves not only CRMP2 but also pathways related to GSK-3β and other signaling components. To clarify whether CRMP2 acts directly on PACAP or through GSK-3β as part of the mechanism of PACAP-induced neurite outgrowth, we observed neurite outgrowth in the presence of GSK-3β inhibitors and activators. Post PACAP treatment in PC12 cells, immunostaining was used to confirm protrusion elongation, while RT-PCR, 2D Gel with Western blotting, and inhibition experiments were performed to confirm the expression of the PACAP gene, its receptors, and downstream signaling components. These results indicate that neurite protrusion elongation by PACAP follows a GSK-3β-regulated pathway through the PAC1-R, where the AKT and cAMP/ERK pathways are involved. These findings also provide a solid basis for future research to develop new therapies and therapeutic agents to treat neural disorders and may contribute to neurogenesis.

Significance of melatonin production in mast cells

マスト細胞のメラトニン産生の意義

○西 晴久[1]、ニヨンサバ フランソワ[2,3]

[1]東京慈恵会医科大・医、[2]順天堂大・院医・アトピーセ、[3]順天堂大・国際教養

[Background and objective]
Mast cells, the cells responsible for both immediate and delayed allergic reactions, are also one of the few melatonin-producing cells in the body. Melatonin, which is primarily released from the pineal gland and regulates circadian rhythm, has both hypnotic and antioxidant effects. However, the physiological function of melatonin produced by mast cells is unknown. We set out to investigate this phenomenon.
[Methods]
LAD2, a human-derived mast cell line, was used in all experiments. Melatonin production by mast cells was detected by ELISA using anti-melatonin antibodies. mRNA and protein expression of melatonin synthase proteins were analyzed by real-time PCR and Western blotting, respectively. Allergic stimulation of the cells was performed by sensitizing the cells with 1.0 μg/mL anti-NP IgE, followed by stimulation with 1.3 μg/mL NP-BSA.
[Results]
Expression of arylalkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT), key enzymes in melatonin production, was upregulated more than 2-fold at both mRNA and protein levels in mast cells treated by allergic-stimuli. The expression of the two enzymes was also upregulated by A23187, a calcium ionophore; however, the upregulation level was less than 2-fold for each enzyme. Conversely, mRNA and protein expression of both enzymes was slightly reduced in cells pre-treated with 500 μM db-cAMP, which is known to increase cAMP.
[Discussion]
These results indicate during mast cell degranulation, representing an increase in intracellular calcium concentration, the expression of melatonin-producing enzymes is increased during resynthesis of the granules. However, IgE-mediated degranulation by allergic stimulation results in more production of melatonin than it is required for replacement, therefore contributing to the repair of damaged tissues.

Antioxidant potential of histidine-rich glycoprotein contributes to improvement of sepsis pathophysiology

高ヒスチジン糖タンパク質の抗酸化能は敗血症病態改善に寄与する

○和氣 秀徳[1]、森 秀治[2]、西堀 正洋[3]、ハティポール オメル ファルク[1]、西中 崇[1]、高橋 英夫[1]

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

Histidine-rich glycoprotein (HRG) has been reported to have regulatory effects on blood coagulation and fibrinolytic systems. In addition to these effects, we found that HRG is a factor that is specifically downregulated in sepsis, and that HRG contributes to the amelioration of sepsis via its effects on maintaining neutrophil morphology and function and protecting vascular endothelial cells. The involvement of ROS in the progression of septicemia has been reported, and in previous report, we have shown that HRG suppresses excess ROS production from neutrophils, but the direct effects of HRG on ROS and ROS-producing systems are not known. Therefore, in this study, we investigated the activity of HRG against ROS. HRG showed no effect on superoxide and oxygen peroxide among ROS, but on hydroxyl radicals, which are highly toxic in vivo, it was found that HRG suppressed the production of hydroxyl radicals by efficiently chelating the divalent iron ions used in the Fenton reaction during the production of hydroxyl radicals. With regard to peroxyl radicals involved in lipid peroxidation, which is important for cell damage, HRG exhibited antioxidant capacity even though it was not mediated by the Fenton reaction, suggesting that in this system, the antioxidant capacity was due to autoxidation.

Compound X is a novel ligand candidate for L-DOPA receptor GPR143

Compound X は L-DOPA 受容体 GPR143 のリガンド候補である

○井上 美優

横浜市立大・院医・分子薬理神経生物

We propose that L-3,4-dihydroxyphenylalanine(L-DOPA) by itself is a neurotransmitter. Recently, a G-protein coupled receptor GPR143, a gene product of ocular albinism 1, was identified as a receptor for L-DOPA. However, L-DOPA is an unstable compound, and GPR143 ligands other than L-DOPA have not been identified yet. In this study, we found compound X, a novel ligand for GPR143, and examined its pharmacological actions. Using zero-maze test, we found that Gpr143 gene-deficient ($Gpr143^{-/y}$) mice exhibited more anxiety-like behavior when compared to wild-type (WT) mice. Intraventricular administration of compound X caused anxiety-like behavior in WT mice. The effect of compound X was not observed in $Gpr143^{-/y}$ mice. These results indicate that compound X showed an antagonistic activity against GPR143. Compound X may be useful to clarify the role of L-DOPA/GPR143.

Cystathionine γ-lyase self-inactivates by generating supersulfides

超硫黄分子によるシスタチオニンγ−リアーゼの自己活性制御機構

○荒木 笙馬、土屋 幸弘、渡邊 泰男

昭和薬科大・薬・薬理学

〈Introduction〉 Cystathionine γ-lyase (CSE) is an enzyme responsible for the biosynthesis of cysteine from cystathionine. It also has the β-lyase activity toward cystine to generate cysteine hydropersulfide (Cys-SSH). Notably, the chemical reactivity of Cys-SSH is thought to be involved in the catalytic activity of proteins via protein polysulfidation. In the present study, we investigated Cys-SSH could regulate CSE enzyme activity through its polysulfidation.

〈Method〉 CSE enzyme activity (levels of enzymatically synthesized CysSSH) was determined with a fluorescent probe sulfane sulfur probe 4 (SSP4). Polysulfidated CSE was detected using modified biotin switch assay.

〈Results and discussion〉 *In vitro* incubation of CSE with Na_2S_4 induced the inhibition of the enzyme, accompanied by its polysulfidation. Treatment with dithiothreitol reversed the polysulfidation and the subsequent inhibition. Similarly, *in vitro* incubation of CSE with CSE-enzymatically synthesized Cys-SSH resulted in the inhibition of the enzyme. We generated Na_2S_4-insensitive CSE mutants in that its specific Cys resides were mutated with valine. Furthermore, the mutant displayed a reduction in CSE polysulfidation by Na_2S_4 relative to the wild-type enzyme. Interestingly, the enzyme activities of Na_2S_4-insensitive CSE mutant were higher than that of wild-type. Thus, CysSSH is endogenously generated and auto-inhibits CSE enzyme activity via polysulfidation of its specific cysteine residues.

Identification and Characterization of Human Colorectal Cancer Cluster Predominantly Expressing EP3 Prostanoid Receptor Subtype

EP3プロスタノイド受容体サブタイプを高発現するヒト大腸がんクラスターの同定と性質評価

○福島 圭穣、藤野 裕道

徳島大・院医歯薬

Colorectal cancer (CRC) is one of the common types of cancer in humans. Prostaglandin E_2 (PGE_2) is a well-known mediator of colorectal cancer through stimulation of four E-type prostanoid (EP) receptor subtypes: EP1, EP2, EP3, and EP4 receptors. All subtypes of EP receptors are involved in CRC promotion or malignancy. However, the characteristics of CRC that highly expresses EP receptor subtypes have not been clarified. In the present study, we classified CRC from a cancer genomic database and identified CRC clusters which highly express EP receptor subtypes. Most of these clusters predominantly expressed one subtype of EP receptor and showed different gene expression patterns. Among them, we focused on the cluster highly expressing the EP3 receptor (CL-EP3). As the result of characterization of gene expression, CL-EP3 was characterized as: epithelial mesenchymal transition (EMT)-induced progressed cancer with activation of transforming growth factor-β pathway, activation of hypoxia-inducible factor-1α, and suppression of runt-related transcription factor 3. Since we previously reported that EP3 receptor is involved in and induce colon cancer cell migration, EP3 receptor-expressing CRC may induce metastasis through these signaling pathways. Thus, the findings suggest the effectiveness of cancer clustering by gene expression of the EP receptor subtype to elucidate the mechanism of human CRC.

The recognition mechanism of the analgesic drug mirogabalin by recombinant human $\alpha_2\delta$1 protein

リコンビナントヒト$\alpha_2\delta$1タンパク質による鎮痛薬ミロガバリン認識機構

○香西 大輔[1,2,3]、沼本 修孝[4]、西川 幸希[5,6]、亀川 亜希子[3,5]、川崎 祥平[7]、廣明 洋子[1,2]、入江 克雅[1,8,9]、大嶋 篤典[1,8,10]、半沢 宏之[7]、島田 神生[11]、北野 裕[11]、藤吉 好則[3,5]

[1]名古屋大・細胞生理研セ、[2]バイオ産業情報化コンソーシアム、[3]東京医科歯科大・高等研院、[4]東京医科歯科大・難治疾患研、[5]CeSPIA、[6]東京農工大・生体分子解析学共研講、[7]第一三共RDノバーレ、[8]名古屋大・院創薬科学、[9]和歌山県立医科大・薬、[10]名古屋大・iGCORE、[11]第一三共

Mirogabalin is a novel gabapentinoid drug, following gabapentin and pregabalin, and given as a first-line agent for peripheral neuropathic pain. Mirogabalin is a γ-aminobutyric acid analog that binds to the voltage-gated calcium channel subunit $\alpha_2\delta$1. However, the mirogabalin recognition mechanism of $\alpha_2\delta$1 remains elusive. Here we analyzed a structure of recombinant human $\alpha_2\delta$1 bound to mirogabalin at 3.88 Å resolution by cryo-electron microscopy. Our structure and mutagenesis binding assays confirmed that the evidence shown by others for gabapentinoid binding to a conserved amino acid binding motif located in the extracellular dCache_1 domain distal pocket applies to mirogabalin. In addition to the conserved residues interacting with the carboxyl and amino groups of mirogabalin, our mutagenesis binding assays newly identified residues W205 and Y217 in the hydrophobic interaction region are critical for mirogabalin binding. The A215L mutation, which was introduced to increase the side chain length at the hydrophobic interaction site of mirogabalin based on our structure, predictably suppressed the binding of mirogabalin while promoting the binding of another ligand, L-Leu, with a smaller hydrophobic substituent than mirogabalin. Our study indicates the significance of hydrophobic interactions in $\alpha_2\delta$1 mirogabalin recognition.

Effects of the therapeutic drugs for Cystic Fibrosis in Caucasians on CFTR mutations found in Japanese patients.

日本人患者に見られるCFTR変異に対する白人の嚢胞性線維症の治療薬の効果について

○君島 莉央、相馬 光流、清水 正浩、大川 詩織、松澤 由佳、金子 すずな、深田 侑希、中尾 佳菜子、相馬 義郎

国際医療福祉大・院薬・分子病態治療学分野

Cystic fibrosis (CF) is an autosomal recessive disease in which mutations in the CFTR gene cause various symptoms through its channel malfunction. Among over 1900 gene mutations, the most common mutation is ΔF508 which causes a trafficking defect of CFTR to plasma membrane (classified as 'class II'). CF is quite rare in Japanese and listed in 'Intractable Diseases' by MHLW, Japan.

The most frequent point-mutations in Japanese patients are H1085R and L441P, both are class II same as ΔF508. Recently the FDA granted Vertex Inc. approval for "Trikafta", a combination of two expression correctors, elexacaftor and tezacaftor, and one channel function potentiator, ivacaftor. In Japan, at present, the CF treatment is limited to some symptomatic ones, and no radical treatment has not been approved yet. Trikafta could be effective against disease-associated mutations other than ΔF508.

In this presentation, we will discuss about the therapeutic effects of Tricafta against H1085R and L441P, based on our *in vitro* data.

Molecular dynamics simulation study on structural instability of CFTR mutants in Japanese CF patients and effects of CFTR correctors on them.

日本のCF患者におけるCFTR変異体の構造不安定性とCFTR補正因子の影響に関する分子動力学シミュレーション研究

○相馬 光流、君島 莉央、石塚 柊太、中尾 香菜子、相馬 義郎

国際医療福祉大・院薬・分子病態治療学研究室

Cystic fibrosis (CF) is a genetic disorder caused by abnormal function of CFTR. CFTR is expressed in the lumen lateral membrane of the transport epithelia of the whole body, such as the gastrointestinal and the respiratory epithelia, and plays a central role in the anion transport. CF is common in Caucasians but relatively rare in Asians. Deletion of phenylalanine at position 508 (ΔF508) is the most common CF-associated mutation and causes defects in CFTR trafficking to the plasma membrane (class II). The CFTR mutation profiles in Japanese are far different from those in Caucasians. Recently, some CFTR correctors rescuing the ΔF508-CFTR from the trafficking defect were developed for by Vertex. Inc. We have confirmed that the Vertex correctors for the ΔF508 mutant could also rescue Japanese CF-associated mutants, H1085R and L441P.
In this work, we study the molecular fluctuation of CFTR using molecular dynamics (MD) simulations because the molecular instability might lead to the protein degradation. We will discuss about the mechanisms of the Japanese CF mutations causing the trafficking defect and how the Vertex correctors rescue them.
(COI:No)

Trans-inhibitory effect of dotinurad, a uricosuric agent on uric acid reabsorptive transporter URAT1

尿酸再吸収トランスポーターURAT1に対する尿酸降下剤ドチヌラドのトランス阻害作用について

○藤田 一輝、朱 秋楠、荒川 大、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

URAT1 is responsible for renal reabsorption of uric acid (UA) and is a target of uricosuric agents. Newly developed uricosuric agent dotinurad selectively inhibits URAT1. Interestingly, in vitro experimentally obtained Ki of dotinurad was larger than in vivo Ki estimated by model analysis of clinical uricosuric effect (Pharmacol Res Perspect. 7(6):e00533, 2019). In the present study, we precisely analysed inhibition mechanism of URAT1 by dotinurad. *Xenopus* oocytes that were injected with cRNA of URAT1 were used for analysis of URAT1 activity. When dotinurad was preincubated with oocytes, UA uptake were decreased more strongly than without preincubation. Furthermore, when dotinurad was directly injected into oocytes and immediately measured UA uptake without dotinurad in the uptake medium, UA uptake was decreased, while such pre-injection effect was not observed by conventional URAT1 inhibitor benzbromarone. These results demonstrated that dotinurad specifically affects URAT1 from intracellular side (*trans*-inhibition) in addition to *cis*-inhibition. Accordingly, clinical effect of dotinurad is explained by inhibition of URAT1 from both tubular lumen side (*cis*-inhibition) and intracellular side (*trans*-inhibition) and such dual mechanism may explain apparent difference of URAT1 inhibition potency between in *vitro* and in *vivo*.

Physiological and Anatomical Properties of Utricular Hair Cells and Afferents in *Gpr156*$^{del/del}$ Mice Lacking a Mirror-Image Hair Cell Organization

The line of polarity reversalを欠落したGpr156ノックアウトマウスにおける卵形嚢有毛細胞と求心性神経の生理学的または形態学的特性の解析

○小野 和也[1,2]、タチーニ バジル[3]、イトック ルーズアン[2]、日比野 浩[1]

[1]大阪大・医・薬理学講座　統合薬理、[2]シカゴ大学、[3]ジャクソン研究所

In otolith organs, hair bundles of hair cells have varying orientations that reverse along the line of polarity reversal (LPR) located within or at the edge of the central striolar zone. Despite such unique anatomical feature, the significance of simultaneous excitatory and inhibitory inputs upon translational stimuli remains to be understood. GPR156 is a G protein-coupled receptor (GPCR) belonging to metabotropic glutamate receptor subfamily. Recently, Kindt et al. has shown that GPR156 uniformly expressed in the hair cells of five vestibular organs becomes polarized and reverses hair bundle orientation under the control of Emx2, a transcription factor, establishing mirror-image hair cell organization in the otolith organs. (Nat Commun 2021). *Gpr156*$^{del/del}$ otolith organs lose the LPR without any other clear anatomical defects. In this study, we examined whether the loss of LPR in *Gpr156*$^{del/del}$ mice affects physiological and anatomical properties of hair cells and primary afferent neurons.

We found that the transducer currents of type I and II hair cells in null and het animals had comparable properties (operating range, sensitivity, adaptation time course and extent).

We also determined whether zone-specific firing pattern is affected by loss of LPR. In wildtype animals, afferent neurons innervating the striola and extrastriola including LES tend to fire in transient and sustained fashion, respectively, in response to depolarizing current steps. Our results show that current steps applied to LES calyces of both *Gpr156*$^{del/+}$ and *Gpr156*$^{del/del}$ utricles elicited multiple spikes (sustained responses), suggesting unaltered firing pattern by loss of *Gpr156*.

Together, we conclude that some physiological properties are conserved in hair cells and afferent terminals of the LES in utricles without bundle orientation reversal as a result of deletion of *Gpr156*.

Different reactivity of TRPM8 and TRPA1 to menthol in dogs

イヌTRPM8とTRPA1 のメントールに対する反応性の違い

○山口 卓哉[1]、柳川 日向子[1]、松岡 彩那[1]、内田 邦敏[2]、山﨑 純[1]

[1]日本大・生物資源科学部・獣医薬理学研究室、[2]静岡県立大・食品栄養科学部・生体機能学研究室

Although menthol generally provides a pleasant cooling sensation in humans, at high concentrations it can cause discomfort and/or pain. This effect is attributable to two cation channels, TRPM8 and TRPA1, that exhibit different dose dependency in humans. Given that the dose dependency of these receptors to menthol might differ in other mammalian species, we examined the reactivity of canine TRPM8 and TRPA1 to menthol by using recombinantly expressed channels. HEK293T cells were transfected with canine TRPM8 or TRPA1 and subjected to calcium imaging. Canine TRPA1 was confirmed to be activated by the selective TRPA1 agonist allyl isothiocyanate in a dose-dependent manner, which was inhibited by the TRPA1 antagonist HC-030031. Canine TRPA1 was also activated by menthol in a dose-dependent manner, even at high concentrations (up to 3 mM), in contrast with TRPA1 in rodents, which was inhibited by a high concentration of menthol. Canine TRPA1 showed a much higher value at EC_{50} for menthol response compared with canine TRPM8 (178.9 μM and 5.3 μM, respectively). The activation of TRPA1 by menthol was inhibited by HC-030031 but it was not inhibited by the TRPM8 antagonist RQ-00203078. Our results suggest that menthol activates TRPM8 at low concentrations and may induce a pleasant cooling sensation, whereas at high concentrations it activates TRPA1 and may cause discomfort in dogs as in humans. In addition, this mechanism is suggested to be conserved in some carnivora and primates.

Functional and structural basis of hERG facilitation by its blockers

hERGの阻害剤による促進作用の機能的・構造的基盤

○古谷 和春、河野 諒太朗、喜多 紗斗美

徳島文理大・薬

Certain human Ether-à-go-go-Related Gene (hERG) blockers can facilitate hERG activation to increase hERG currents, which may reduce proarrhythmic potential. However, the molecular mechanism involved in the facilitation effect of hERG blockers remains unclear. Here, we demonstrate that 1) nifekalant accesses the receptor site within the pore of the open or inactivated channels at depolarized potentials, 2) upon return to the resting potentials, channels close and trap nifekalant inside, 3) trapped nifekalant biases the open-closed equilibrium towards the open state, and 4) the kinetics of drug escape from the channel are faster than channel closing rates at potentials where facilitation of hERG current is observed, thereby drug unbinding reveals channels that have been biased towards the open state. Simulations with a Markov model of such nifekalant-hERG interaction successfully reproduce key characteristics of hERG facilitation. We also present a potential structural model for hERG channel facilitation through drug interactions with the hydrophobic pocket of the hERG pore domain. This pattern of interaction is consistent with experimental data suggesting facilitating drugs may act as a wedge to bias hERG channel equilibrium towards the open state and increase hERG current amplitude in response to low-voltage depolarization.

Simvastatin attenuates cardiac fibrosis during the development of chronic heart failure via an inhibition of Hsp90-regulated cell signaling.

シンバスタチンはHsp90制御性細胞内情報伝達の抑制を介して慢性心不全進展過程の心線維化を軽減する.

○丸ノ内 徹郎、矢野 絵美、田野中 浩一

東京薬科大・薬

Hsp90 is a molecular chaperone that contributes to the regulation of various cell signaling pathways. In our previous study, we showed that an inhibition of Hsp90 prevented cardiac fibrosis and preserved cardiac function during the development of chronic heart failure (CHF). Simvastatin, an inhibitor of HMG-CoA reductase, has been shown to inhibit the chaperone activity of Hsp90. However, it is unclear whether simvastatin can prevent myocardial fibrosis by inhibiting Hsp90. Therefore, we examined effects of simvastatin in the models of CHF. The results showed that treatment with simvastatin prevented cardiac fibrosis. Furthermore, simvastatin treatment inhibited the c-Raf-Erk1/2 pathway, which contributes to cardiac fibrosis. *In vitro* experiment, the interaction of Hsp90 with c-Raf in cultured cardiac fibroblasts was decreased by the presence of simvastatin, and the expression level of c-Raf was also reduced. Furthermore, simvastatin inhibited the fibroblast proliferation and migration. Simvastatin also attenuated collagen production by inhibiting the differentiation of cardiac fibroblasts into myofibroblasts. These results suggest that an inhibition of Hsp90 is partially responsible for the inhibitory effect of simvastatin on cardiac fibrosis.

The BRG1/p300 Complex Increases the Acetylation Levels of the H3K122 in the Development of Heart Failure

心不全の進展においてBRG1/p300複合体はH3K122のアセチル化を増加した

○船本 雅文[1,2,3]、砂川 陽一[2,3]、刀坂 泰史[2,3]、清水 果奈[2,3]、長谷川 浩二[2,3]、森本 達也[2,3]

[1]徳島大・院医歯薬・薬理学分野、[2]国立病院機構京都医療セ・臨床研究セ・展開医療研、[3]静岡県立大・薬・分子病態

Background: Epigenetic mechanisms such as histone post-translational modifications are involved in heart failure (HF). Although the acetylation of tail domains, such as H3K9, has been extensively studied, that of H3K122, the globular domain, has received much less attention. The H3K122 acetylation directly activates transcription by destabilizing histone-DNA binding. However, the participation of these acetylated domains in the development of HF remains unknown.

Methods and Results: Phenylephrine increased the acetylation levels of H3K9 and H3K122 in Cultured cardiomyocytes. The acetylation levels of H3K9 and H3K122 on the hypertrophic reaction gene promoters such as BNP and b-MHC were increased in cardiomyocyte hypertrophy. In Dahl-salt sensitive rats, a heart failure model, *in vivo* ChIP assays revealed that the acetylation of H3K9 on the promoters of BNP and b-MHC was increased in left ventricular hypertrophy (LVH), while that of H3K122 was increased in HF. On the other hand, there was no difference in the amount of transcriptional coactivator p300 recruitment in LVH and HF. Interestingly, IP -WB showed that binding of p300 with BRG1, a key component of the SWI/SNF complex, was enhanced in HF. The recruitment of BRG1 was increased in HF compared to LVH. Moreover, PFI-3, a BRG1 inhibitor, suppressed transverse aortic constriction-induced cardiac hypertrophy and the development of HF.

Conclusion: This study shows that the acetylation of H3K122 is enhanced via the interaction of p300 with BRG1 in heart failure.

High-throughput screening for agonists of ROS production in live human vascular endothelial cells

血管内皮細胞血管内皮細胞におけるROS産生を誘導する刺激の新規ハイスループットスクリーニング法の確立

○笹原 智也[1,2]、星 美奈子[1,3]

[1]神戸医療産業都市推進機構・先端医療研究セ・神経変性疾患研、[2]TAOヘルスライフファーマ・神戸研・研究開発、[3]京都大・医・形態形成機構

Reactive oxygen species (ROS) are essential physiological molecules, and ROS production is strictly regulated. In many diseases, the change of physiological function and the agonist released induce the excessive increase of ROS production, and then cell function is impaired. In cardiovascular diseases, such as hypertension, diabetes, and Alzheimer's vascular lesions, the ROS production increases in vascular endothelial cells and eventually disturbs the vascular function. To better understand the role of ROS in disease, it is essential to elucidate what agonist increases the ROS production and where the ROS production occurs. Here, we show a novel high-throughput screening (HTS) protocol of what agonists induce ROS production and where it occurs in live human vascular endothelial cells using a fluorescence probe and an image cytometer. By using this HTS protocol, we found that Alzheimer‘s brain-derived assemblies, amylospheroids, increase the mitochondrial ROS production because the mitochondrial ROS inhibitors (YCG-063 and mito-tempol), but not NADPH oxidase inhibitors (VAS2870 and apocynin), blocked the ROS production. By elucidating whether ROS production occurs in mitochondria or plasma membrane of live cells, this protocol will lead to a better understanding of the role of ROS in disease.

Expression and function of basement membrane-derived matricryptins in thickening of mitral valve in rats

ラット僧房弁肥厚における基底膜由来matricryptinsの発現および機能の検討

○岡田 宗善、佐野 功汰、鈴木 竜之介、兒玉 朋子、大谷 紘資、山脇 英之

北里大・獣医

Myxomatous mitral valve disease, the most common form of canine heart disease, causes mitral regurgitation due to valvular degeneration (proliferation and thickening of spongiosa), similar to mitral valve prolapse in humans. The bioactive fragments cleaved from the fibrous components are called matricryptins. Arresten (α 1 chain) and canstatin (α 2 chain), the cleaved fragments of type IV collagen, are known as basement membrane-derived matricryptins which are expressed in the cardiac tissue in rats. Although the expression of these matricryptins may be altered during mitral valve degeneration, it has not been clarified. In this study, we investigated the expression of the matricryptins during mitral valve thickening in rats and its role in valvular interstitial cells (VICs). The expression of canstatin was increased in the thickened mitral valve in the old rats (>36-weeks-old). Stimulation of cultured rat mitral valve with transforming growth factor (TGF)- β increased the thickness of mitral valve and the expression of arresten. Both canstatin and arresten affected TGF- β -induced activation of VICs (increase of α -smooth muscle actin expression). These results suggest that the expression of canstatin and arresten changes with thickening of the rat mitral valve and that they are involved in the activation of VICs.

The regulation mechanisms of mitochondrial Ca^{2+} signaling mediated by cardiac Sigma-1 receptor

心筋Sigma−1受容体を介したミトコンドリアCa^{2+}シグナル調節機構

○田頭 秀章[1,2]、篠田 康晴[2]、沼田 朋大[1]、福永 浩司[3]

[1]秋田大・院医・器官・統合生理学、[2]東北大・院薬・薬理学、[3]東北大・院薬・先進脳創薬講座

Cardiovascular disease (CVD) is a leading cause of death worldwide. We previously reported that the Sigma-1 receptor (Sigmar1) is down-regulated in mice with cardiac dysfunction. Recent study suggested that Sigmar1 deficient mice display cardiac dysfunction via impairment of mitochondrial function. However, the mechanism of mitochondrial quality control mediated by Sigmar1 has not been investigated in detail. In this study, we investigated the role of Sigmar1 for ER-mitochondrial tethering and mitochondrial Ca^{2+} signaling using a Sigmar1-knockdown cardiomyocytes. We found that disruption of ER-mitochondrial tethering and reduction of ER-mitochondrial Ca^{2+} transport was induced by Sigmar1 knockdown in cardiomyocytes. We also demonstrated that Endothelin-1-induced cardiomyocyte hypertrophy is aggravated associated with induction of mitophagy in Sigmar1 knockdown cardiomyocytes. These data suggest that reduction of cardiac Sigmar1 is involved in myocyte hypertrophy by maintaining of intracellular Ca^{2+} signaling mediated by regulation of ER-mitochondrial tethering.

Species difference in the intracellular Ca^{2+}-mediated mechanisms in the pacemaker depolarization of mouse and guinea pig sinus node

洞房結節自動能における細胞内Ca^{2+}を介する機序の種差：マウスとモルモットの比較

○尾高 椋介、濵口 正悟、行方 衣由紀、田中 光

東邦大・薬・薬物

In general, smaller animals have higher heart rates, suggesting that there are differences in the mechanisms of pacemaker depolarization. Recently, it has been postulated that the pacemaker depolarization is influenced by intracellular Ca^{2+}. This study intended to clarify the intracellular Ca^{2+}-mediated mechanisms involved in the pacemaker depolarization of the mouse and guinea pig sinus node.

Microelectrode recordings revealed that the sinus node of the mouse, which had a higher beating rate, had a steeper slope of the pacemaker depolarization than that of the guinea pig. Intracellular Ca^{2+} interfering agents, BAPTA and ryanodine, significantly decreased the slope in both species. In contrast, SEA0400, a specific inhibitor of the Na^{+}-Ca^{2+} exchanger (NCX), as well as change to low Na^{+} extracellular solution, significantly decreased the slope in the mouse, but not in the guinea pig. Confocal microscopy revealed the presence of spontaneous Ca^{2+} oscillations during the interval between Ca^{2+} transients; Ca^{2+} oscillations were more pronounced in the mouse than in the guinea pig.

These results suggested that, although intracellular Ca^{2+}-mediated mechanisms were involved in the pacemaker depolarization of the sinus node in both species, the NCX current was involved in the mouse but not in the guinea pig.

Phenotypic analysis in cardiac-specific *Tric-b*-deficient mice

心臓特異的TRIC-B欠損マウスにおける表現型解析

○山崎 大樹[1]、松下 幸平[2]、徳竹 美佳[1]、中條 かおり[1]

[1]国立医薬品食品衛研・安全性生物試験研究センター・薬理部、[2]国立医薬品食品衛研・安全性生物試験研究センター・病理部

Ryanodine receptors and IP_3 receptor are Ca^{2+} release channels which expressed on the endoplasmic reticulum (ER) membrane. When Ca^{2+} is released through these channels, negative charges are generated in the ER lumen. Since the negative charge inhibits further Ca^{2+} release, it has been suggested that there are counter ion channels that transport counter ions into the ER lumen to neutralize the negative charge. We have been demonstrated that TRIC (trimeric intracellular cation) channel subtypes (A and B) played critical roles in this process. TRIC-A is expressed in excitable tissues such as skeletal muscle and heart. TRIC-B is expressed universally throughout the body. In systemic *Tric-b*-deficient (KO) mice, type II alveolar epithelial cells are impaired in surfactant production and secretion, resulting in impaired alveolus formation and neonatal lethality. It has also been shown that osteogenesis imperfecta occurs due to impaired collagen secretion in osteoblasts. Since *Tric-a* and *b* double KO mice show embryonic lethal, both subtypes may play important functions in the heart. Since *Tric-b*-KO mice are neonatally lethal, we have generated cardiac-specific *Tric-b*-KO mice and analyzed their function. Here, we examined the effects of β-adrenergic stimulation and cardiotoxicity on mice, as well as the effects of drug-induced arrhythmia.

Sex and arterial site differences in vasorelaxation via protease-activated receptor 2 in metabolic syndrome

メタボリックシンドロームにおけるプロテアーゼ活性化型受容体2を介した血管弛緩反応の雌雄差及び動脈部位差における検討

麓（丸山）加菜[1]、懐 理紗[1]、McGuire John J.[2]、篠塚 和正[1]、籠田 智美[1,3]

[1]武庫川女子大・薬・薬理2、[2]ウエスタン・オンタリオ大、[3]武庫川女子大・バイオサイエンス研

Activation of protease-activated receptor 2 (PAR2) on vascular endothelial cells causes vasorelaxation. Nitric oxide (NO)-mediated vasorelaxation of the aorta is impaired in male SHRSP.Z-*Lepr*^{fa}/IzmDmcr (SP.ZF) rats with metabolic syndrome (MetS), but PAR2-mediated vasorelaxation is preserved. In the current study, we investigated whether PAR2-mediated vasorelaxation in the thoracic aorta and superior mesenteric artery (MA) differed by sex and arterial site in SP.ZF rats, and SHR.Cg-*Lepr*^{cp}/NDmcr (CP) rats at 23–26 weeks of age, two different models of MetS.

Vasorelaxation was examined using the organ bath method. In isolated aortas from SP.ZF rats, vasorelaxations evoked by 2fLIGRLO, a PAR2 agonist, and by acetylcholine (ACh) were greater in females than in males, but in the case of CP rats, only ACh-induced relaxation was greater in females. In MA, 2fLIGRLO-induced vasorelaxation was smaller in females than in males in both strains. However, ACh-induced relaxations did not differ between sexes. Nitroprusside-induced relaxation did not differ significantly between sexes or arterial sites. The findings demonstrate the presence of sex- and arterial site-dependent differences in PAR2-mediated vasorelaxations in MetS. The results indicate less PAR2-mediated relaxation in MAs of female rats under the condition of a presumably maintained NO-mediated vasorelaxation pathway. Further studies are needed to elucidate the pathophysiological significance of lower capacity for vasorelaxation via PAR2 in females with MetS.

Analysis of vasoconstrictor effects of local anesthesia, mepivacaine, via α receptors and V_{1A}receptor.

局所麻酔薬メピバカインの末梢における血管収縮作用効果の検討

○池田 哲朗[1]、齋藤 良介[1,2]、田中 夏幹[1]、益見 厚子[2]

[1]青森大・薬・病態分子薬理学、[2]青森大・薬・分子薬理学

Several studies suggested that the amide-type local anesthetic mepivacaine has a longer duration of local anesthetic effect than the same amide-type lidocaine, and has a vasoconstrictor action. However, the mechanism of action on α_2 and α_1 adrenoceptors involved in peripheral vasoconstriction remains unclear.

0.25% mepivacaine with various ligands such as α adorenoceptors antagonists is randomly and blindly injected intracutaneously at a dose of 0.1mL into the back of shaved Hartly male guinea pigs (weight 300-350g). After marking the area around the wheal and confirming that there is a normal skin contraction reaction outside the wheal, six pricks were aplied inside each wheal. The test of six pinpricks was applied at intervals of about 3 to 5 seconds every 5 minutes. The number of times that did not respond to stimulus was measured and the sum (maximum value:132) served as an anesthetic score which indicates the degree of local anesthesia.

Mepivacaine dose-dependently prolonged the duration of anesthesia and increased local anesthesia scores. Therefore, 0.25% mepivacaine was used and compared with 0.25% lidocaine and 0.25% xylocaine. The anesthetic duration of 0.25% mepivacaine was longer than that of lidocaine and shorter than that of xylocaine.

0.25% mepivacaine were combined with 1 μM yohimbine, 1 μM prazosin, 1 μM JP1302, 10 μM BRL44408, 1 μM SR49059 (selective V_{1A} receptor blocker V:vasopressin), 10 μM indoramin, 0.5 μM BMY7378, 5 μM cyclazosin, 1 nM silodosin respectively. After mixing, the effect of various antagosits on mepivacaine were examined. As a result, antagonists other than 10 μM BRL44408 and 1 nM silodosin decreased the duration of mepivacaine anesthesia. These results indicated that mepivacaine's peripheral vasoconstrictor activity is mediated at least by α_{2C}, α_{1B}, α_{1D} and V_{1A}receptors.

Do female mice acquire a preference for the unattractive male mouse encountered after cocaine administration?

オスのマウスとの遭遇を伴うコカイン繰り返し投与はメスマウスのそのオスマウスへの嗜好性を高めるか？

○大西 克典、河原 幸江、大西 陽子、西 昭徳

久留米大・医

Occasional incidents of drug addiction among celebrities have been reported, and sometimes the presence of the opposite sex flickers. Even trafficking of women sometimes involves the use of illegal drugs. Whether drugs induce not only drug dependence but also an associated preference for the opposite sex is an important question in understanding and solving such cases.

Previously, we have established a female male preference test (FMPT), in which four male mice are compared to distinguish between attractive and unattractive male mice. Using this established system, we examined whether conditioning female mice with an addictive drug would increase their preference for the unattractive male mouse that was with them at the time.

Briefly, the female mice were administered drugs (cocaine 7.5 mg/kg ip or morphine 15 mg/kg sci) and then allowed to meet an unattractive male mouse defined by FMPT for 15 minutes for 3 consecutive days, and were examined by FMPT on the fourth day.

In the results, morphine treated female mice showed significant higher preference to the same male mouse as saline treated control female mice, and the mouse wasn‘t the conditioned unattractive male mouse, moreover, cocaine treated female mice didn‘t show significantly different preference to specific male mice including the conditioned unattractive male mouse, indicating that those drugs could have no effect to increase the preference to the conditioned unattractive male mouse.

However, since sexual activity might also be necessary for the attraction, the effects of sexual activity under cocaine administration into female mice will also be examined and reported.

Molecular mechanism of schizophrenia-like behavioral deficits induced by methylglyoxal detoxification impairments

メチルグリオキサール解毒障害による統合失調症様行動異常発現の分子機序解明

○鳥海 和也[1]、小池 伸[2]、段 孝[3]、鈴木 一浩[1,4]、宮下 光弘[1,5]、堀内 泰江[1]、小笠原 裕樹[2]、宮田 敏男[3]、糸川 昌成[1]、新井 誠[1]

[1]都医学研・精神行動医学・統失PJ、[2]明薬大・薬・分析化学、[3]東北大・院医・分子治療学、[4]信州大・医・精神医学、[5]都医学研・社会健康医学

Methylglyoxal (MG) is a reactive and cytotoxic alpha-dicarbonyl product of glycolysis. Various detoxification systems work together *in vivo* to remove highly toxic MGs, including a glyoxalase system by Glyoxalase 1 (GLO1) and GLO2, and the scavenging system by vitamin B6 (VB6). We found that VB6 levels in peripheral blood of the schizophrenia patients with GLO1 dysfunction are significantly lower than that of healthy controls. However, the effects of the MG detoxification deficits on the pathophysiology of schizophrenia remains poorly understood. Here, we generated a new mouse model of schizophrenia with impaired MG detoxification by feeding *Glo1* knockout mice VB6-deficent diets (KO/VB6(-)). KO/VB6(-) mice accumulated MG in the prefrontal cortex (PFC), hippocampus, and striatum, and displayed behavioral deficits, such as prepulse inhibition (PPI) deficit. Furthermore, we demonstrated abnormal mitochondrial respiratory function and subsequently enhanced oxidative stress in the PFC of KO/VB6(-) mice in the PFC. Finally, administration of an antioxidant resveratrol improved PPI impairment as well as oxidative stress. These results suggest that the combination of GLO1 dysfunction and VB6 deficiency results in mitochondrial dysfunction and increased oxidative stress in PFC, resulting in schizophrenia-like behavioral disorders.

Axo-axonic cells in the amygdala regulate associative fear learning.

扁桃体 Axo-axonic 細胞は恐怖連合学習を制御する

○中嶋 美紀[1]、池谷 裕二[1,2]、森川 勝太[1,2]

[1]東京大・院薬・薬品作用、[2]東京大・Beyond AI研究推進機構

Activity and plasticity of excitatory neurons are tightly regulated by local inhibitory neurons in a spatiotemporal specific manner. Axo-axonic cells (AACs) are a unique type of inhibitory neurons that express parvalbumin and innervate the axon initial segment (AIS) of excitatory neurons. While their anatomical features have been identified, the genetical marker and functional role remain unclear. Here, we show that vasoactive intestinal peptide receptor 2 (Vipr2)-expressing inhibitory neurons in the basolateral amygdala (BLA) exhibit the anatomical and electrophysiological properties typical of AACs. Furthermore, using an AACs-specific labeling approach, we conducted in vivo fiber photometry recording and functional inhibition experiments in a cued fear conditioning test. The activity of AACs was increased for both conditioned stimulus (CS: tone) and unconditioned stimulus (US: foot-shock), and inhibition of GABA transmission of AACs impaired cued fear conditioning. These results suggest that AACs are important for memory acquisition. Finally, we employed projection-specific monosynaptic rabies virus tracing to identify the direct monosynaptic inputs cells to AACs in the BLA. This study provides new insights into the detailed functional roles of AACs in the BLA.

Blockade of D-serine signaling and adult hippocampal neurogenesis attenuates remote contextual fear memory

D–セリンシグナルと海馬神経新生の阻害による遠隔恐怖記憶の抑制

○森 寿[1,2,3]、倪 献策[1,2]、井上 蘭[1,3]

[1]富山大・院医薬、[2]富山大・大学院生命融合科学教育部・分子神経科学講座、[3]富山大・アイドリング脳科学研究センター

Memory retrieval can trigger destabilization followed by reconsolidation for maintaining or enhancing original fear memory. Therefore, blockade of reconsolidation could weaken the original fear memory. The *N*-methyl-D-aspartate (NMDA) receptor and hippocampal neurogenesis play crucial roles in hippocampus-dependent memory processes, including reconsolidation. In this study, first, NMDA receptor signaling was downregulated by the genetic reduction of its co-agonist, D-serine, and the neurogenesis was ablated by focal X-ray irradiation on the hippocampus. We found that a progressive decrease in freezing following each retrieval, leading to an attenuation of remote contextual fear memory on day 28. Second, after conditioning, pharmacological approaches to simultaneously block D-serine signaling and inhibit neurogenesis, resulting in a similar suppressive effect on the remote fear memory. The present findings provide insights into the role of D-serine-mediated NMDA receptor signaling and neurogenesis in memory retrieval and the maintenance of remote fear memory, and provide a new strategy to improve exposure-based therapy for post-traumatic stress disorder (PTSD) treatment.

Enhancement neuronal activity in the basolateral amygdala in mice with the preference of nicotine intake

ニコチン摂取行動に関連した扁桃体基底外側部における神経活動の増強

○藤井 拓磨、泉尾 直孝、浅野 昴志、新田 淳美

富山大・院医薬・薬物治療学研究室

[Introduction] Despite the health damage risks of tobacco, success rate of smoking cessation is quite low. Tobacco dependence has aspects of substance and behavioral addiction. The latter aspect is supported by the limited efficacy of smoking cessation by nicotine replacement therapy such as nicotine gum or patches. In this study, to clarify the mechanism of behavioral addiction with smoking, we established the model of nicotine intake behaviors in mice corresponding to behavioral addition in human smokers, and examined the brain region associated with nicotine intake behaviors. [Methods] Two drinking bottles were placed on the housing cage of mice (C57BL/6J, 8-9 weeks, male). After the habituation period, mice were exposed to the water containing nicotine (75μg/ml) filled in both bottles for one week to learn nicotine intake behavior (behavioral priming). Then, to measure the preference to nicotinic solution, bottles containing vehicle or nicotinic solution were presented to mice. [Results] Mice which experienced behavioral priming much more preferred to the nicotinic solution than those without behavioral priming. Immunohistochemistry to FosB, a marker of neuronal activity, revealed that larger number of FosB-positive neurons were observed in the basolateral amygdala (BLA) in mice without the preference to the nicotine solution, than those with preference. [Conclusion] Enhanced neuronal activity in the BLA is suggested to be associated with behavioral addiction of tobacco dependence.

Evaluation of the circadian rhythms of activity in mice implanted with human stomach cancer cell lines.

ヒト胃がん細胞株を移植したマウスに見られる活動の概日リズムの評価

○上野 晋[1]、後藤 元秀[1]、野中 美希[2]、丸山 崇[3]、石塚 恒年[1]、長谷川 渉[1]、溝上 峻[1]、上田 陽一[3]、上園 保仁[2]
[1]産業医大・産生研・職業性中毒、[2]慈恵医大・医・疼痛制御研究、[3]産業医大・医・第１生理

Objective: Circadian rhythm is endogenous 24-hr oscillations usually entrained to the daily environmental cycle of 12/12 h light/dark (LD). It has been reported that disruption of circadian rhythm causes delirium, which is also often developed in patients with advanced cancer. However, the mechanisms by which the progression of cancer disrupts circadian rhythms and ultimately leads to the development of delirium has not been yet understood. We have recently established cancer-induced cachexia model mice by implantation of human stomach cancer cell line 85As2. In this study, we investigated the circadian rhythms of locomotor activity in 85As2-implanted mice.
Methods: 85As2 cells were implanted into 8-week-old male BALB/c nude mice ($2x10^5$ cells/mouse). Home-cage activity was measured after 2 weeks from implantation under LD. Mice were then placed in constant darkness (DD) or light (LL), and finally returned to LD cycle. The day-night variation were analyzed using Actogram.
Results: The active phase of 85As2-implanted mice gradually shifted from dark to light between 2 to 4 weeks after implantation, which was almost reversed after 4 weeks. The free-running period under DD or LL condition was significantly shorter compared to the control. Even after returning to LD, the active phase remained reversed in 85As2-implanted mice.
Summary: 85As2-implanted mice demonstrated the reverse of day-night variation without synchronization of photoperiod, and shortened free-running period, suggesting that 85As2-implantation may directly affect the circadian rhythm formation.

Maternal obesity impairs social behavior in female offspring by decreasing central estrogen function

妊娠中の肥満は雌仔マウスの中枢エストロゲン機能を低下させることで社会性行動を障害する

○鎌田 知紘、植田 大暉、米持 奈央美、池田 弘子

星薬科大・薬・薬物治療

Maternal obesity is reported to increase risk for metabolic diseases in offspring. Furthermore, recent research suggests that maternal obesity also affects the central nervous system in offspring, but a mechanism is unclear. The present study examined the effects of maternal obesity on central nervous system function in offspring. Female 8-week-old C57BL/6J mice were fed with a high-fat diet (HFD) or a regular diet (RD) for 4 weeks and mated with male 8-week-old C57BL/6J mice. The experiments were conducted when offspring reached 8 weeks of age. In the social interaction test, interaction time with a stranger mouse in female offspring of mice fed with HFD was reduced compared to female offspring of mice fed with RD; no such effect was observed in male offspring. These results suggest that only female offspring of obese mice show impairment in social behavior. The mRNA levels of estrogen receptor alpha in the amygdala, which regulates social behavior, were reduced in female offspring of mice fed with HFD, while plasma estradiol levels were unchanged. In addition, the estrogen receptor antagonist fulvestrant reduced interaction time with a stranger mouse. Taken together, our results suggest that maternal obesity impairs social behavior in female offspring by decreasing central estrogen function.

The role of sign tracking in the formation of process addiction

プロセス依存の形成におけるサイントラッキングの役割

○太田 宏之、石塚 俊晶

防衛医科大・医学科・薬理

Current treatment for process addiction, such as gambling disorder and game addiction, is primarily psychotherapy, and effective pharmacotherapy has not been established. One reason is the lack of appropriate behavioral test batteries for mice to identify the causes of addiction and conduct pharmacological tests. The scarcity of high-value rewards is generally considered the cause of addiction, and experimental behavioral tasks have been proposed accordingly. In recent years, however, it has become clear that signs that foretell rewards are involved in the formation of gambling addiction. In the present study, we developed a 2-hole nose poke-selective operant task for mice in which two retractable levers were used as predictive signs for the reward. As a result, mice showed adherence to the option with predictable rather than unpredictable reward signs. Adherence to the predictable reward signs continued even after the reward probability decreased. We also found that the suboptimal behavior formation was correlated with the frequency of sign tracking. This study allows us to study the role of sign tracking in the shape of process addiction using genetically engineered mice and drug administration models.

Reactivation of hippocampal place cells after experiencing multiple environments

複数の環境探索後における海馬場所細胞の再活性化

○横井 太紀[1]、鹿野 悠[2]、柳下 晴也[2,3]、池谷 裕二[2,4]、佐々木 拓哉[2,3]

[1]東北大・薬・薬理、[2]東京大・院薬・薬品作用、[3]東北大・院薬・薬理、[4]Beyond AI 研究所

The hippocampus plays important roles in learning and memory. In the hippocampus, spatial information is encoded by place cells during an experience and consolidation of spatial memory is supported by reactivation of place cells during rest/sleep periods. It remains to be unknown how multiple spatial experiences that are encoded by a subset of place cell ensembles are reactivated in subsequent rest/sleep periods. To address this issue, we recorded spike patterns of hippocampal CA1 and CA3 place cells in rats that sequentially experienced five different rooms. We confirmed that CA1 cells had larger numbers of place fields defined from individual rooms that CA3 cells, suggesting sparser spatial representations by CA3 cells. Overall, increases in reactivation rates of CA1 place cells from pre-rest to post-rest periods were correlated with the numbers of place fields, suggesting stronger reactivation in neurons that encoded more information during awake periods. We now analyze synchronous spike patterns of multiple neurons to clarify how experiencing multiple environments are encoded and consolidated by hippocampal neuronal ensembles.

Effect of μ-opioid receptor selective antagonist β-funaltrexamine on spontaneous behavior in mice

オピオイドμ受容体選択的拮抗薬β-funaltrexamineのマウス自発行動への影響

○北中 順惠[1]、北中 純一[2]、富田 和男[3]、五十嵐 健人[3]、田中 康一[2]、西山 信好[2]、佐藤 友昭[3]

[1]兵庫医科大・医、[2]兵庫医科大・薬・薬理、[3]鹿児島大・院医歯・応用薬理

In this presentation, we hypothesized that μ-opioid receptor antagonists could inhibit the natural reward as well as drug addiction such as opioids and stimulants. To evaluate natural reward, horizontal running wheels were applied to mice administered with a μ-opioid receptor antagonist β-funaltrexamine (β-FNA). In addition to the rotation of running wheels, horizontal locomotion, amounts of food intake and drinking, and access of food container were measured simultaneously by using a multi-configuration apparatus (0700/1900 light on/off). In naïve mice, horizontal locomotion increased day by day for three testing days. The increment was parallel to the increase in the rotation of running wheels but not to the number of accesses to food containers. The increase in the rotation of running wheels was sensitized, and the amount of food intake was unchanged in naïve mice. In mice group which was administered with a single injection of 5 mg/kg β-FNA, the locomotion and thus the rotation of the running wheels decreased especially in the first testing day. In contrast, no significant behavioral change was observed in mice under a continuous s.c. administration with osmotic mini-pumps (3.5 mg /kg of β-FNA for three days (71.5 h)). These results indicate that a transient increase in the levels of β-FNA reduced motivative behaviors in mice.

Pretreatment with CHIR-99021, a GSK-3 inhibitor, partially attenuates methamphetamine-induced stereotyped behavior in mice

GSK-3阻害薬CHIR-99021前処置は覚せい剤誘導常同行動を部分的に抑制する

○北中 純一[1]、北中 順惠[2]、富田 和男[3]、五十嵐 健人[3]、田中 康一[1]、佐藤 友昭[3]、西山 信好[1]

[1]兵庫医科大・薬・薬理、[2]兵庫医科大・医・薬理、[3]鹿児島大・院医歯・応用薬理

In mammals, glycogen synthase kinase-3 (GSK-3) is formed as the two isoforms termed GSK-3α and GSK-3β. GSK-3β is present in a high concentration in the abundance of tissues in the central nervous system, regulating a crucial role in neuronal signaling pathways. The research for involvement of GSK-3β signaling in drug abuse liability has been progressed based on the studies investigating molecular and cellular mechanism of action, but few reports have been made on animal research so far. In this presentation, we demonstrate that pretreatment of mice with CHIR-99021 (5, 10, 15 mg/kg, s.c.), a GSK-3 inhibitor, attenuated methamphetamine (METH)-induced stereotyped behavior (10 mg/kg of METH) in a dose-dependent fashion. Maximal inhibitory effect (ca. 50%) was observed at 10 and 15 mg/kg of CHIR-99021. CHIR-99021 dose-dependently attenuated the expression frequency of METH-induced stereotyped biting whereas CHIR-99021 increased the expression frequency of persistent locomotion. These observations are different results from those of other GSK-3 inhibitors such as SB216763 and AR-A014418, suggesting a possibility of CHIR-99021-specific effect of METH action

Protective Effect of Bcl-2-associated athanogene (BAG) 3 in Mouse Neuroblastoma N1E115 Cells

Bcl-2-associated athanogene (BAG) 3の神経保護作用

○東尾 里英子、猪俣 結衣、高橋 晋太郎、玉田 さち、夏堀 陽子、三部 篤

岩手医科大・薬・薬剤治療

Bcl-2-associated athanogene (BAG) 3 is known as a regulator of cell death as well as autophagic protein turnover in the heart. However, little information is present in functional role of BAG3 in neurons. We analyzed the functional role of BAG3 in N1E115 cells derived from mouse neuroblastoma. While overexpression of Bcl-xL, a member of anti-apoptotic factor, can prevent cell death against 30 nM staurosporine, no obvious protective effect was seen by overexpression of BAG3 in N1E115 cells. In contrast, combined overexpression of BAG3 with Bcl-xL can enhanced the effect by overexpression of Bcl-xL in N1E115 cells. Slight protective effect of BAG3 against ABT263, Bcl-2 inhibitor, was also observed in N1E115 cells. To address underlying mechanisms of protective effect of BAG3, overexpression as well as knockdown of BAG3 was performed. Although knockdown of BAG3 in N1E115 cells using si-RNA specifically targeted to BAG3 enhanced mitochondrial Bak1 protein levels, no alteration in Bak1 gene expression, and gene expression and protein levels of Bax, Bcl-2 was observed. Concomitant with increased Bak1, the proportion of TUNEL-positive N1E115 cells was increased, and overexpression of BAG3 led to suppression of the Bak1 level. Bafilomycin A1, an autophagy inhibitor, can inhibit the modification of Bak1 protein level by BAG3. These results suggest that BAG3 may be critical to protein level in Bak1 via modification of autophagy activity, and that autophagy regulation by BAG3 may play an important role in the apoptosis of neuronal cells.

The effect of a miR-96-5p inhibitor delivery to brain using microbubbles and ultrasound technology on neuroprotection and autophagy activation

マイクロバブル超音波技術を用いたmiR−96−5p機能抑制薬の脳内送達によるによる神経保護作用とオートファジー活性化

○木下 千智[1]、青山 晃治[1]、鈴木 亮[2]、松村 暢子[1]、小俣 大樹[2]、丸山 一雄[2]、中木 敏夫[3]

[1]帝京大・医・薬理、[2]帝京大・薬・薬物送達、[3]帝京大・医療技術・柔道整復

Glutathione (GSH) is an important antioxidant that plays a critical role in neuroprotection. GSH depletion in neuron induces oxidative stress promoting neuronal damage, which is regarded as a hallmark of the early stage in some neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. The neuronal GSH levels are mainly regulated by excitatory amino acid carrier 1 (EAAC1) and its inhibitory protein, glutamate transporter-associated protein 3-18 (GTRAP3-18). In this study, we found that GTRAP3-18 levels were increased by the up-regulation of the microRNA miR-96-5p, which has been reported to decrease EAAC1 levels in our previous study. We also discovered that neuro-oncological ventral antigen 1 (NOVA1) is an intermediate protein for GTRAP3-18 expression via miR-96-5p. Moreover, we show that the intra arterial administration of a miR-96-5p-inhibiting nucleic acid to living mice by a drug delivery system using microbubbles and ultrasound decreased the levels of GTRAP3-18 via NOVA1, while increased the levels of both EAAC1 and GSH in the mouse brain. Moreover, we found that the administration of a miR-96-5p inhibitor increased autophagy activation in the mouse hippocampus. These findings suggest that the delivery of a miR-96-5p inhibitor to the brain would efficiently increase the neuroprotective activity by increasing GSH levels via EAAC1, GTRAP3-18 and NOVA1.

3‘,4‘,7-Trihydroxyflavone inhibits NO production in LPS-activated MG6 microglial cells by suppressing the JNK–STAT1 pathway

3‘,4‘,7-Trihydroxyflavoneの神経炎症抑制作用におけるJNK-STAT1経路の関与

○赤石 樹泰、山本 昇平、阿部 和穂

武蔵野大・薬

We investigated the effects of the natural flavonoid 3‘,4‘,7-trihydroxyflavone on lipopolysaccharide (LPS)-induced neuroinflammatory responses in MG6 microglial cells. 3‘,4‘,7-Trihydroxyflavone inhibited LPS-induced nitric oxide (NO) production and the upregulation of inducible NO synthase (iNOS) in MG6 cells. 3‘,4‘,7-Trihydroxyflavone also suppressed LPS-induced phosphorylation of signal transducer and activator of transcription 1 (STAT1), which is crucial for iNOS expression. LPS stimulation induced rapid phosphorylation of c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase (ERK) in MG6 cells. 3‘,4‘,7-Trihydroxyflavone significantly inhibited the LPS-induced phosphorylation of JNK, but not that of ERK and p38 MAPK. The inhibitory effect of 3‘,4‘,7-trihydroxyflavone on NO production was mimicked by pharmacological inhibition of the JNK signaling pathway with SP600125. Furthermore, SP600125 significantly inhibited LPS-induced phosphorylation of STAT1 in MG6 cells. These results suggest that 3‘,4‘,7-trihydroxyflavone exerts anti-neuroinflammatory effects via inhibition of the JNK-STAT1 pathway in microglia.

Brain Transport of Non-Esterified Docosahexaenoic Acid Across the Blood–Brain Barrier is Decreased in Middle-Aged and Aged Mice

血液脳関門を介した非エステル型ドコサヘキサエン酸の脳移行は中高齢及び老齢マウスにおいて減少する

○岩尾 卓朗、高田 芙友子、片岡 泰文、横谷 みき、有留 尚孝、安永 美保、道具 伸也

福岡大・薬・応用薬剤学教室

Nutrients are actively taken up by the brain through various transporters at the blood–brain barrier (BBB). Orally supplied DHA must be transported from the circulating blood to the brain across the BBB through transport carriers including major facilitator superfamily domain-containing protein 2a (MFSD2A) for esterified DHA and fatty acid-binding protein 5 (FABP5) for non-esterified DHA. Although BBB integrity is known to be altered in aging, the impact of aging on DHA transport across the BBB is not well understood. Here, we used 2-, 8-, 12-, and 24-month-old male C57BL/6 mice to evaluate the brain uptake of [^{14}C]DHA, as non-esterified form, using an in situ transcardiac perfusion technique. Primary culture of rat brain endothelial cells (RBECs) were used to evaluate the effect of siRNA-mediated MFSD2A knockdown on cellular uptake of [^{14}C]DHA. We found that brain uptake of [^{14}C]DHA was inhibited by an excess amount of unlabeled DHA in 2-month-old mice. Transfection of MFSD2A siRNA into RBECs decreased the protein expression levels of MFSD2A and cellular uptake of [^{14}C]DHA. 12- and 24-month-old mice showed significant decrease in brain uptake of [^{14}C]DHA and MFSD2A protein expression in the brain microvasculature compared with 2-month-old mice, nevertheless FABP5 protein expression was up-regulated with age. Our findings suggested that MFSD2A is involved in non-esterified DHA transport at the BBB. The middle-aged and aged brain has decreased DHA transport across the BBB due to age-related down-regulation of MFSD2A rather than FABP5.

Neurotoxicity assessment of pyrethroids using multielectrode array recordings of human iPSC-derived neurons

ヒトiPS細胞由来神経細胞を用いた多点電極アレイシステムによるピレスロイド系農薬の神経毒性評価

○安彦 行人、山田 茂、諫田 泰成

国立衛研・薬理部

Neurotoxicity of environmental chemicals, such as pesticides, is a worldwide concern in human welfare. It is challenging to identify human neurotoxicity by animal tests, due to differences in species, costs, and labor. Human induced pluripotent stem cells (hiPSC)-based in vitro tests might be a valuable method to evaluate chemical neurotoxicity, but the standard procedure, such as cell lines and endpoints, has not been fully understood.

In this study, we examined the neurotoxicity of pyrethroid insecticides using the multielectrode array (MEA) recordings of hiPSC-derived neurons. We exposed the hiPSC-derived neurons XCL-1 to pyrethroid insecticides and performed MEA recordings using MED64-Presto.

Exposure with pyrethroids to hiPSC-derived neurons reduced neural network function, such as spikes per network burst and network burst duration in a dose-dependent manner. RT-PCR analysis revealed the expression of the pyrethroid-sensitive voltage-gated sodium channels (VGSCs) in hiPSC-derived neurons. The half-maximal inhibitory concentrations (IC50s) of the MEA parameters were lower than the 30% effective dosages (ED30s) of motor activity repression obtained from the animal experiments.

Taken together, MEA recordings in network activity of hiPSC-derived neurons could be an effective tool to screen compounds with neurotoxicity.

Low concentration of methotrexate causes toxicity against neuron during synapse formation

低濃度メトトレキサートがシナプス形成時期の神経に毒性を引き起こす

○山村 真伊、花村 健次、川辺 浩志

群馬大・院医・薬理

High-dose methotrexate (MTX) application is widely used for the treatment of acute lymphoblastic leukemia in children. MTX can cross blood-brain barrier to reach neurons when applied with a high dose. Indeed, high-dose MTX application could cause neurological side effects including cognitive impairment. In our previous reports, we established a high-throughput imaging system for the detection of the effects of various neurotoxic compounds using primary cultured neurons. Using this system, we studied the neurotoxic effects of various concentrations of MTX in the present study. For this purpose, primary cultured cerebral cortical cells were prepared from embryonic days 17 Wister rat. Cerebral cortical cells were cultured for 21 days on 96-well microplates. At 4 days in vitro (4 DIV), MTX was applied to the cultured cells in each well at the concentration of 0.01, 0.03, 0.1, 0.3, 1, 10, or 100 μM, respectively. At 21 DIV, cerebral cortical cells were fixed with paraformaldehyde and stained with DAPI and with neuronal and synaptic markers. To our surprise, low concentrations of MTX reduce the number of cultured cerebral cortical neurons in the well. Our finding indicates that MTX could be toxic at a concentration lower than what we expected.

The effect of opioid promoting social behavior is negatively regulated by the activity of dorsomedial part of periaqueductal grey

オピオイドがもたらす社会性向上作用は、中脳水道周囲灰白質背側部により負に制御される

○大波 壮一郎[1,2]、山川 英訓[1,2]、大石 直也[2]、小川 公一[1,2]

[1]塩野義製薬・創薬疾患研究所、[2]京都大・医・メディカルイノベーションセンター・SKプロジェクト

The opioid system has been reported to play a crucial role in modulating social behavior in humans and animals. However, brain regions which mediate the effect of opioids on promoting sociability and reason why the effect is suppressed depending on the dose are not determined yet. Opioid receptors are densely distributed in mPFC and NAc, which could promote sociability. On the other hand, PAG, a key region for analgesia of opioids, is reported to negatively affect sociability. Thus we hypothesized that sociability would be promoted by controlling these regions in a well-balanced manner. Firstly, we confirmed that morphine (Mor, s.c.) with a low dose increased social interaction behavior in mice. At that dose, the number of c-Fos-positive cells was significantly increased in mPFC and NAc. At a higher dose, dorsomedial (dm), but not dorsolateral/lateral, PAG was activated in addition to mPFC and NAc. At last, we found that the effect on social behavior induced by Mor (low dose, s.c.) was antagonized by topical administration of high dose Mor to dmPAG. These results suggest that the dmPAG works as a gate of social behavior promoted by Mor-induced activation of mPFC and NAc. A new opioid drug with an appropriate action that does not activate dmPAG may be a new therapeutic strategy for the deficit of sociability in mental disorders.

Pyrylium based derivatization imaging mass spectrometer revealed the localization of L-DOPA

誘導体化イメージング質量分析による脳内カテコールアミン類の一斉可視化とパーキンソン病症状改善効果の検討

○平 修[1]、鹿野 仁美[1]、池田 明夏里[2]、寺内 勉[2]、横山 順[2]

[1]福島大学・農学群・食農学類、[2]大陽日酸（株）・SI事業部

Simultaneous imaging of l-dihydroxyphenylalanine (l-DOPA), dopamine (DA) and norepinephrine (NE) in the catecholamine metabolic pathway is particularly useful because l-DOPA is a neurophysiologically important metabolic intermediate.

In this study, we found that 2,4,6-trimethylpyrillium tetrafluoroborate (TMPy) can selectively and efficiently react with target catecholamine molecules. Specifically, simultaneous visualization of DA and NE as metabolites of l-DOPA with high steric hinderance was achieved by derivatized-imaging mass spectrometry (IMS). Interestingly, l-DOPA showed strong localization in the brainstem, in contrast to the pattern of DA and NE, which co-localized with tyrosine hydroxylase (TH).

In addition, to identify whether the detected molecules were endogenous or exogenous l-DOPA, mice were injected with l-DOPA deuterated in three positions (D_3-l-DOPA), which was identifiable by a mass shift of 3Da. TMPy-labeled l-DOPA, DA and NE were detected at *m/z* 302.1, 258.1 and 274.1, while their D_3 versions were detected at 305.0, 261.1 and 277.1 in mouse brain, respectively. l-DOPA and D_3-l-DOPA were localized in the BS. DA and NE, and D_3-DA and D_3-NE, all of which are metabolites of L-DOPA and D_3-l-DOPA, were localized in the striatum (STR) and locus coeruleus (LC). These findings suggest a mechanism in the brainstem that allows l-DOPA to accumulate without being metabolized to monoamines downstream of the metabolic pathway.

High-content analysis using drebrin immunocytochemical images of cultured rat hippocampal neurons

ラット海馬培養細胞のドレブリン染色画像を使ったハイコンテント分析法

○間瀬 省吾[1,2]、光岡 俊成[3]、小金澤 紀子[2]、山崎 博幸[2,4]、加藤 祐一[1]、筒井 泉雄[1]、川辺 浩志[2]、白尾 智明[2]、関野 祐子[1]

[1]東京大・院農学生命科学、[2]群馬大・院医、[3]北海道科学大・薬、[4]群馬医療福祉大・社会福祉

Drebrin is a major F-actin binding protein in dendritic spines that is critically involved in their morphological plasticity. Subcellular localization of drebrin is dependent on NMDAR activity. Drebrin's change in dendritic spines is an indicator of toxic effect of compounds to the brain and can be used for prediction of brain dysfunction prior to the neuronal cell death. In the present study, we have developed new drebrin clusters analysis method applied the confocal high-content screening method. Hippocampal neurons prepared from embryonic rats (SKY neuron, AlzMed, Inc., Tokyo) were incubated in 96-well microplates. After 21 days, the cultured neurons were treated with 10, 100 μM glutamate. After the treatments, they were fixed and processed for immunocytochemistry to visualize drebrin, MAP2 and cell nucleus. After automated image acquisitions, neuron number, dendrite length, drebrin clusters were quantified with original algorithm. The high-throughput immunocytochemical assay demonstrated that glutamate treatment decreased drebrin cluster density. The ratio of decrease in the density was concentration dependent and found to be dependent on the number of surrounding neurons. Our method is sensitive enough to detect the interaction with NMDAR and is useful for drug screening studies for synaptic dysfunction.

Single-cell analysis of electrical activities in human iPS cell-derived neural networks using 236,880-electrode CMOS-MEA

236,880電極CMOS-MEAを用いたヒトiPS細胞由来ニューラルネットワークの電気活動のシングル細胞解析

○松田 直毅、韓 笑波、鈴木 郁郎

東北工業大

In vitro microelectrode array (MEA) assessment using human induced pluripotent stem cell (iPSC)-derived neurons holds promise as a method of seizure and toxicity evaluation. However it is difficult to detect the response of drugs with different mechanisms of action with a single parameter, and the analysis method has become an issue. One effective way to solve this problem is to obtain more detailed information on neural network activity. Therefore, in this study, we cultured human iPS cell-derived cortical neurons on a 236,880-electrode CMOS-MEA and obtained precise single-neuron electrical activity.

As a result of acquiring spontaneous activity after 6 weeks of culture, an average of 296 ± 47 neurons (n = 9 wells) were identified, and network bursting was observed. There, 4-AP 10-30 μM, PTX 1-10 μM, AP5 25 μM, and CNQX 30 μM were administered, and in addition to conventional network burst analysis, we analyzed the burst activity of single-neuron and the synaptic connections between neurons that form networks. As a result of burst analysis, parameters with statistically significant changes were different between network burst and single burst, and the number of concentrations with significant differences also increased with single burst. In addition, in synaptic connections analysis, 4-AP showed no significant change, whereas PTX showed enhanced synaptic connections. CMOS-MEA, which can accurately acquire the electrical activity of single neurons, can increase the number of parameters that can be used to evaluate the effects of drugs, so it is effective as a method for predicting toxicity and the mechanism of action of compounds.

Neuroprotective Effect of an Inhibitor of Hypoxia-inducible Factor-Prolyl Hydroxylase in a Cell Culture Model of Parkinson's disease

パーキンソン病細胞モデルにおけるHypoxia-inducible Factor-Prolyl Hydroxylase阻害剤の神経保護効果

○藤牧 綾香、大内 一輝、村上 貴規、滝沢 進之佑、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

Oxidative stress is associated with the progression of neurodegenerative diseases such as Parkinson's disease (PD). In the present study, to examine whether a hypoxia-inducible factor (HIF)-prolyl hydroxylase inhibitor has neuroprotective effect against α-synuclein (syn)-induced neurotoxicity in a cellular model, we used FG-4592, also known as roxadustat. To investigate the effect of FG-4592 against, we evaluated the α-syn protein level. mRNA levels of oxidative stress response genes whose transcription was regulated by HIF-1α were analyzed. Immunofluorescence staining with redox-sensitive dyes was also performed to examine the α-syn-induced oxidative stress. Previously, we succeeded in generating a new α-syn stably expressing cell line. In this cell line, we found that oxidative stress induced by α-syn caused cell death. FG-4592 exhibited significant neuroprotective effects against α-syn-related neurotoxicity. However, FG-4592 did not affect α-syn protein levels. FG-4592 induced heme oxygenase-1 (HO-1) expression levels in a concentration-dependent manner, suggesting that FG-4592 reduced oxidative stresses *via* the induction of HO-1. Thus, FG-4592 prevents α-syn-induced neurotoxicity through the reduction of oxidative stress by induction of HO-1.

PAI-1 induction is a critical event for the onset of lipopolysaccharide-induced acute kidney injury.

PAI-1の発現誘導はlipopolysaccharideによる急性腎障害発症に重要である

○田中 恒輝[1]、尾花 理徳[1,2]、坂井 響[1]、禿 宏保[1]、山本 彩葉[1]、田中 翔大[1]、岡田 欣晃[1]、藤尾 慈[1,2]

[1]大阪大・院薬・臨床薬効、[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】

A systemic inflammatory response caused by sepsis leads to widespread organ dysfunction, including acute kidney injury. However, the pathogenetic mechanisms of acute kidney injury（AKI） in sepsis remain fully elucidated. Recently, it has been reported that plasminogen activator inhibitor-1 (PAI-1) which is induced by interleukin (IL)-6, plays a central role in thrombogenesis in septic patients. The aim of this study is to elucidate the involvement of PAI-1 in septic AKI using lipopolysaccharide (LPS)-induce AKI model.

【Methods/Results】

C57BL/6J mice were intraperitoneally treated with LPS. LPS administration elevated IL-6 expression in sera. Quantitative PCR demonstrated that the mRNA expression of *Pai-1* was increased in kidneys 6 hours after LPS treatment. Administration of IL-6 to LPS model mice further increased PAI-1 expression compared to LPS alone, accompanied by renal impairment. Finally, to investigate whether PAI-1 is involved in kidney injury, TM5441, a PAI-1 inhibitor, was used. Administration of TM5441 suppressed urinary albumin/creatinine ratio, a kidney injury marker, and *Lcn2* mRNA expression, a tubular injury marker.

【Conclusion】

PAI-1 induction, which is potentiated by IL-6, contributes to the pathogenesis of LPS-induced AKI.

Activation of FSGS-associated N-terminus-mutant TRPC6 channels by mechanical and receptor stimulations shows abnormal filtration barrier function of mouse podocytes

FSGSの原因となる腎糸球体ポドサイトTRPC6チャネルN端変異は受容体・機械刺激応答およびタンパク濾過障壁機能に影響を及ぼす

○市川 純[1]、中川 緑[2]、井上 隆司[2]

[1]佐野日本大学短期大学・総合キャリア教育学科・栄養士フィールド、[2]福岡大・医・生理学教室

Neurohormonal and mechanical responses exquisitely regulate various renal glomerular functions. Previously, we found that mechanical stimulation reduces the activity of receptor -activated canonical transient receptor potential 6 (TRPC6) channel in podocytes to enhance the glomerular barrier function. To explore its pathological implications, we carried out the intracellular Ca^{2+} imaging, whole-cell patch clamp and albumin-permeation assay using immortalized mouse podocytes stably expressing wild-type (wt) TRPC6 or its N-terminus mutants associated with focal segmental glomerulosclerosis (FSGS) (P111Q, M131T and N142S). Application of a membrane-expanding agent 2,4,6-trinitrophenol (TNP) immediately suppressed wt Ca^{2+} responses evoked by angiotensin II (Ang II). However, these responses were reversed in P111Q and entirely absent in M131T and N142S. Simultaneous stimulation with Ang II and TNP in wt reduced FITC-labelled albumin leak, while this was weakened in FSGS mutants. Pretreatment with a TRPC6-specific inhibitor SAR7334 enhanced the leaks, the extent being greater in the mutants than wt. These results strongly suggest that FSGS-associated N-terminus TRPC6 mutations may impair the filtration barrier function through the altered efficiency of mechanical stress in suppressing receptor-activated TRPC6 channel activities.

Effect of hyperphosphatemia on impairment of vascular function in adenine-induced renal injury in rats

アデニン腎障害ラットの血管機能障害に対する高リン血症の影響

○小渕 修平、上田 紗夢、秋山 直樹、西畑 佑哉、上田 晴康

兵庫医科大・薬・薬理学

We reported that vascular function in adenine-induced renal injury rats was impaired mediated by increase in plasma indoxyl sulfate concentration. It is known that hyperphosphatemia leads to calcification and atherosclerosis. In the present study, we examined effect of hyperphosphatemia on vascular dysfunction in adenine-induced renal injury in anesthetized rats. Renal injury was induced by feeding 0.75% adenine diet for 4 weeks. Lanthanum carbonate was treated with gavage after 2 weeks induced adenine. Treatment with lanthanum carbonate significantly decreased in plasma phosphorus concentration in adenine rats. N-nitro-L-arginine (L-NA) potentiated the ACh-induced depressor response in normal-diet. However, L-NA failed to potentiate the response in adenine rats or lanthanum-treated rats. Sodium nitroprusside (SNP)-induced depressor response in adenine rats was significantly smaller than that in normal rats. Lanthanum carbonate tended to recover impairment of SNP-induced depressor responses in adenine rats. These findings suggest that hyperphosphatemia is partially related to impairment of smooth muscle function rather than that of endothelial function in adenine rats.

Pharmacological inhibition of protein arginine methyltransferase 5 suppresses TGF-β-induced fibrotic responses in cultured kidney fibroblasts

アルギニンメチル化酵素PRMT5選択的阻害剤はTGF−β刺激による腎臓線維化を抑制した

○茂木 飛佑馬[1]、刀坂 泰史[1,2,3]、砂川 陽一[1,2,3]、浜辺 俊秀[1,2,3]、小見山 麻紀[2]、長谷川 浩二[1,2,3]、森本 達也[1,2,3]

[1]静県大・薬・分子病態、[2]京都医セ・展開医療、[3]静県総病院・臨床研究部

Introduction: Although renal fibrosis is observed in chronic kidney disease, there is still no effective treatment for it. Our previous study has shown that protein arginine methyltransferase 5 (PRMT5) is essential for transforming growth factor-β (TGF-β)-induced transcription of fibrotic genes in cardiac fibroblasts. In this study, we aimed to investigate the function of PRMT5 in renal fibrosis.

Methods: NRK-49f kidney fibroblast cells were stimulated with TGF-β for 48 hours. PRMT5 expression levels were examined by qPCR and Western blotting (WB). Next, NRK-49f cells were transfected with siRNA of PRMT5 followed by TGF-β stimulation and mRNA levels of myofibroblast marker α-smooth muscle actin (α-SMA) and fibrotic genes (Col3a1, CTGF) were investigated by qPCR. Finally, NRK-49f cells were treated with EPZ015666, a selective inhibitor of PRMT5 for 2 hours and then stimulated with TGF-β. The expression levels of α-SMA were examined by qPCR and WB.

Results: qPCR and WB revealed that the expression levels of PRMT5 were upregulated by TGF-β stimulation. The expressions of α-SMA and the fibrosis-related genes were upregulated by TGF-β stimulation, whereas knockdown of PRMT5 suppressed the upregulation of these genes. TGF-β-induced increase in α-SMA was suppressed by treatment with EPZ015666.

Conclusion: Knockdown and pharmacological inhibition of PRMT5 suppressed TGF-β-induced myofibroblast differentiation in kidney fibroblasts. These results suggest that PRMT5 is an essential molecule for TGF-β-induced fibrotic response in kidney fibroblasts.

Protective effects of losartan on bladder dysfunction in spontaneously hypertensive rats

自然発症高血圧ラットの膀胱機能障害に対するロサルタンの効果

○清水 翔吾[1]、長尾 佳樹[2]、倉林 睦[3]、清水 孝洋[1]、東 洋一郎[1]、Zou Suo[1]、齊藤 源顕[1]

[1]高知大・医・薬理、[2]高知大・医・小児思春期、[3]高知大・医・病理

Purpose: Our previous report showed aging caused bladder dysfunction in spontaneously hypertensive rats (SHRs). In this study, we investigated the protective effects of an antihypertensive drug, angiotensin II type 1 receptor blocker losartan on bladder dysfunction in aged SHRs.

Materials and Methods: Thirty-six-week-old male SHRs were orally treated with losartan (0, 3 or 10 mg/kg) once daily for 18 weeks. Vehicle-treated Wistar Kyoto rats (WKYs) were used as normotensive controls.

Results: Vehicle-treated SHRs had significantly higher detrusor thickness, bladder arterial wall thickness compared to vehicle-treated WKYs. Moreover, SHRs showed significantly higher single voided volume (SVV), post-voiding residual urine volume (RV), bladder capacity (BC), and intercontraction interval (ICI) and lower voiding efficiency compared to vehicle-treated WKYs. A low dose of losartan decreased RV, BC and ICI but not mean blood pressure in SHRs. A high dose of losartan ameliorated changes in mean blood pressure, detrusor thickness, bladder arterial wall thickness, SVV, RV, BC and ICI in SHRs.

Conclusion: Treatment with losartan ameliorated bladder dysfunction in aged SHRs.

Inhibition of histone demethylation augments NAD synthesis through the Preiss-Handler pathway in cultured human proximal tubular epithelial cell line

ヒストン脱メチル化の阻害は、培養ヒト近位尿細管上皮細胞において、Preiss-Handler経路を介してNAD合成を増強する

○ハサン アリフ[1,2]、丸茂 丈史[2]、小原 真美[1]、佐藤 幸子[1]、近藤 ゆき子[1]、村瀬 真一[2]、平 英一[1]

[1]岩手医科大・医・薬理学講座情報伝達医学分野、[2]国際医療福祉大・医・薬理学

Boosting nicotinamide adenine dinucleotide (NAD) production shows beneficial effects against diabetic kidney disease (DKD). However, the molecules that possess the therapeutic potential for increasing intracellular NAD-pool are not available. On the other hand, epigenetic modifiers are gradually gaining much interest in treating diseases such as DKD. KDM1A demethylates Lys 4 and Lys 9 of histone H3. We hypothesize that a selective KDM1A inhibitor, ORY-1001 may augment intracellular NAD synthesis in the DKD model. We induced hyperglycemic milieu in human proximal tubular epithelial HK2 cell-line using high glucose (4.5 g/L) in the presence or absence of ORY-1001 and compared the effect with their low glucose (1.0 g/L) containing counterparts. As anticipated, high glucose significantly reduced cellular NAD, while increasing NADH contents. The condition was restored by ORY-1001. To explore the underlying mechanisms, we thoroughly analyzed the mRNA expression profiles of the genes that are involved in NAD synthesis and metabolism. Consistent with the NAD production, NAPRT1 expression of the Preiss-Handler pathway was reduced by the high glucose, and treatment with ORY-1001 restored the expression. Moreover, a similar expression profile was found for PPARGC1a expression, a downstream effector of intracellular-NAD. Based on these findings, we presume that the KDM1A-inhibitor ORY-1001 has potential therapeutic benefits against DKD through augmenting NAD synthesis through upregulation of NAPRT1 of the Preiss-Handler pathway of NAD synthesis.

Development of a SARS-CoV-2 infection model using human iPSC-derived intestinal epithelium

ヒトiPS細胞由来小腸上皮細胞を用いたSARS−CoV−2感染モデルの開発

○山田 茂[1]、野田 隆政[2,3,4,5]、岡部 かおり[2]、柳田 翔太[1]、西田 基宏[6,7]、諫田 泰成[1]

[1]国立医薬品食品衛研、[2]国立精神・神経研、[3]国立精神・神経研、[4]国立精神・神経研、[5]東京慈恵会医科大、[6]九州大、[7]生理学研

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly widespread and led to global health crises. COVID-19 causes well-known respiratory failure and gastrointestinal symptoms, such as diarrhea, nausea, and vomiting. Thus, human gastrointestinal cell model is urgently needed for COVID-19 research, nevertheless, primary human cells can be difficult to obtain. In the present study, we used small intestinal epithelial like cells (SIECs) from human induced pluripotent stem cells (iPSCs) for SARS-CoV-2 infection and drug testing. We observed that iPSC-SIECs, such as absorptive cells and paneth cells, were infected with SARS-CoV-2. SARS-CoV-2 infection decreased transepithelial electrical resistance (TEER), an indicator of epithelial integrity. In addition, SARS-CoV-2 infection increased expression levels of proinflammatory genes, which are elevated in patients with COVID-19. We further observed that remdesivir treatment suppressed SARS-CoV-2 infection to SIECs. These data suggest that human iPSC-derived SIECs provide a useful *in vitro* model for elucidation of COVID-19 pathology.

Allyl isothiocyanate-induced acute inflammation in the gastric mucosa leads to the impaired gastric motility in rodents: involvement of substance P and calcitonin gene-related peptide, but not mast cells

アリルイソチオシアネート誘起急性胃粘膜炎症は実験動物の胃運動減弱を引き起こす ーサブスタンスPおよびカルシトニン遺伝子関連ペプチドの関与ー

○田嶋 公人、大重 茉里奈、藤井 瑶子、堀江 俊治

城西国際大・薬

We investigated the mechanism of acute inflammation induced by allyl isothiocyanate (AITC), a TRPA1 activator, through measurement of vascular permeability and gastric mucosal blood flow (GMBF) in rat stomachs to provide the rodent model of impaired gastric motility. Male SD rats were used after 18 h-fasting. Stomachs were mounted on the *ex-vivo* chambers in anesthetized rats. Vascular permeability (extravasated Evans blue) and GMBF (a leaser doppler) were measured in response to mucosal application of AITC. AITC obviously increased vascular permeability and GMBF. Gastric mucosal swelling was observed after application of AITC without hemorrhagic lesions. The both vascular permeability and GMBF in response to AITC were significantly attenuated by the pretreatment of a substance P receptor antagonist aprepitant, whereas GMBF was alone significantly decreased by the pretreatment of a TRPA1 blocker A-967079 and a calcitonin gene-related peptide (CGRP) receptor antagonist BIBN 4096. However, vascular permeability and GMBF in response to AITC hardly affected by the pretreatment of a mast cell stabilizer cromoglycate. These results suggest that AITC-induced acute inflammation with no mucosal damage is dependent on substance P and CGRP released from TRPA1-expressing nerves in rat stomachs, but not mast cells. Those inflammation could lead to the impaired motility in rodents.

A dextran sodium sulfate-induced chronic colitis murine model of intestinal fibrosis

デキストラン硫酸ナトリウム誘起慢性大腸炎の線維化モデルの検討

○佐々木 礼一郎[1]、池田 一生[2]、天ヶ瀬 紀久子[1,2]

[1]立命館大・院薬・病態薬理学研究室、[2]立命館大・薬・病態薬理学研究室

Chronic inflammatory disease such as Ulcerative Colitis (UC) and Crohn's Disease (CD) causes excessive fibrosis and strictures in the intestine. An intestinal fibrosis model using dextran sodium sulfate (DSS)-induced UC in mice was investigated to determine new treatment methods for UC. DSS containing drinking water was given to C57BL/6N mice for 7 days, followed by 14 days of water (repeated three times). Animal body weight, blood in the feces, fecal consistency, and disease activity index (DAI) were evaluated. To evaluate inflammation, myeloperoxidase (MPO) activity was determined and hematoxylin and eosin (H&E) staining was performed. Masson's trichrome, Picrosirius red staining, immunohistochemistry (IHC) and western blotting were used to evaluate the degree of fibrosis. The DSS group had higher DAI scores, a reduction in goblet cells, infiltration of lymphocytes, and higher MPO activity than the control group. Increased collagen fiber deposition in the colon and increased type I collagen in the lamina propria and submucosal tissue was evident in the DSS group, with myofibroblasts in the lamina propria. Heat shock protein-47 and tissue inhibitor of metalloproteinases-1 were detected. Repeated DSS intake exacerbates colitis with lymphocyte infiltration and fibrosis, with an increase in type I collagen in the lamina propria and submucosal tissue.

Thromboxane A_2 receptor signaling in macrophages attenuates acetaminophen-induced liver injury

マクロファージにおけるトロンボキサンA_2受容体シグナルはアセトアミノフェン誘導肝障害を軽減する

○田邉 美奈[1]、伊藤 義也[1]、長田 真由子[1]、山下 敦[1]、古江 明子[1]、細野 加奈子[1]、畑中 公[1]、馬嶋 正隆[2]、天野 英樹[1]
[1]北里大・院医療・分子薬理学、[2]神奈川工科大・健康医療

Objective: Over dosage of acetaminophen (APAP) administration causes severe acute liver failure. Accumulating evidence suggests that macrophages contribute to APAP-induced liver injury; however, underlying mechanisms of involvement of macrophages remain unknown. We recently reported that thromboxane A_2 (TXA_2) improves chemical-induced liver injury by accumulating macrophages. Here, we examined the role of TXA_2 in macrophages during APAP-induced liver injury.

Methods and Results: APAP (300 mg/kg, ip) was administered to macrophage-specific thromboxane prostanoid receptor (TP) deficient mice (mTPKO) and control mice (Cont). Compared with Cont, mTPKO exhibited severe liver injury as indicated by increased levels of ALT and necrotic area and decreased expression of PCNA, a marker of hepatocyte proliferation at 48 h post-APAP treatment. There was no statistical difference in hepatic GSH levels between the two genotypes. TP and TXA_2 synthase were expressed in CD68-positive cells in the liver. Immunofluorescence revealed CD68-positive cells accumulated extensively into the necrotic regions of livers from Cont as compared with mTPKO. The expression of mRNA encoding pro-inflammatory mediators including TNF-α, IL-1β and IL-6 in mTPKO were higher than in Cont, whereas HGF levels in mTPKO were lower than in Cont.

Conclusions: TP receptor signaling in macrophages attenuated APAP-induced liver injury by reducing inflammatory cytokines and promoted liver repair by increasing macrophages in the necrotic regions and HGF production.

Orphan G protein-coupled receptor GPR35 contributes to the pathogenesis of dextran sulfate sodium-induced colitis in mice.

オーファンGタンパク質共役型受容体GPR35のデキストラン硫酸ナトリウム誘起マウス大腸炎の病態における役割

○岸 采花[1]、橘 佑輔[1]、村瀬 由依[1]、徳山 珱雅[1]、斉藤 美知子[2]、安田 浩之[1]、松本 健二郎[1]、加藤 伸一[1]

[1]京都薬科大・病態薬科・薬物治療、[2]京都薬科大・バイオサイエンス研究セ

Orphan G protein-coupled receptor GPR35, which is activated by lysophosphatidic acid and kynurenic acid, is highly expressed in gastrointestinal tracts. Clinical findings indicate that this receptor has been implicated in the onset of inflammatory bowel disease (IBD), but its role in physiological and pathological processes in the colon remains undefined. The present study investigated the role of GPR35 in the pathogenesis of exmerimentally-induced colitis in mice.GPR35-deficient (GPR35KO) mice were generated by CRISPR-Cas9-mediated genome editing on C57BL/6 background. Experimental colitis was induced in GPR35KO and wild-type (WT) mice by the treatment with dextran sulfate sodium (DSS) for 7 days. Lodoxamide, which has a GPR35 activating effect, was administered i.p. once daily for 7 days. DSS treatment produced body weight loss with diarrhea and blood feces, and severe colitis characterized by shortening colon length and histological injury 7 days later. The severity of colitis with systemic symptoms was significantly augmented in GPR35KO mice compared with WT mice. In contrast, daily administration of lodoxamide significantly reduced the severity of DSS-induced colitis in WT mice. However, the protective effect of lodoxamide was not observed in GPR35KO mice. These findings suggest that GPR35 plays an anti-inflammatory role in DSS-induced colitis. Thus, GPR35 may be a promising target for treatment and prevention of inflammatory bowel disease.

Protective role of Ca^{2+}-permeable TRPV6 in dextran sulfate sodium-induced colitis in mice

Ca^{2+}高選択性TRPV6のデキストラン硫酸ナトリウム誘起マウス大腸炎の病態における役割

○森 風帆[1]、高山 麻由[1]、斉藤 美知子[2]、安田 浩之[1]、松本 健二郎[1]、加藤 伸一[1]

[1]京都薬科大・病態薬科・薬物治療、[2]京都薬大・バイオサイエンス研究セ

Transient receptor potential vanilloid 6 (TRPV6), which is a highly Ca^{2+}-selective ion channel, is expressed in gastrointestinal epithelium and implicated in maintaining Ca^{2+} homeostasis via transcellular Ca^{2+} transport. However, the local pathophysiological roles of TRPV6 in the gastrointestinal tract remains undefined. In the current study, we investigated the role of TRPV6 in the pathogenesis of experimentally-induced colitis in mice. TRPV6-deficient (TRPV6KO) mice were generated by CRISPR-Cas9-mediated genome editing on C57BL/6 background. Experimental colitis was induced in TRPV6KO and wild-type (WT) mice by the treatment with dextran sulfate sodium (DSS) for 7 days. Intestinal permeability was evaluated by FITC-dextran methods. DSS treatment produced body weight loss with diarrhea and blood feces, and severe colitis characterized by shortening colon length and histological injury 7 days later. The severity of colitis with systemic symptoms was significantly augmented in TRPV6KO mice compared with WT mice. Intestinal permeability was increased in TRPV6KO compared with WT mice. The expression of E-cadherin and occludin in the colon was reduced in TRPV6KO mice compared with WT mice. These findings suggest that TRPV6 plays a protective role in the pathogenesis of DSS-induced colitis via maintaining colonic barrier functions. Thus, TRPV6 may be a novel target for the treatment of gastrointestinal diseases.

Functional expression of calcium-sensitive receptors in activated hepatic stellate cells

活性化型肝星細胞におけるカルシウム感受性受容体（CASR）の機能発現

○近藤 るびい、川田 成紀、鈴木 良明、山村 寿男

名古屋市立大・院薬・細胞分子薬効解析学

Hepatic stellate cells (HSC) are liver-specific fibroblasts that play a critical role in the development of hepatic fibrosis. During liver injury, these cells transdifferentiate into the activated phenotype, resulting in enhanced cel proliferation and extracellular matrix production. The functions of activated HSCs require an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). However, the regulatory mechanisms underlying Ca^{2+} signaling in activated HSCs remain largely unknown. In the present study, the pathophysiological roles of calcium sensing receptors (CaSRs) were examined in human hepatic stellate cells LX-2. Expression analyses revealed that CaSR proteins were expressed in α-smooth muscle actin-positive LX-2 cells. Extracellular Ca^{2+} restoration (from 0 to 2.2 mM) increased $[Ca^{2+}]_{cyt}$ in LX-2 cells. The extracellular Ca^{2+}-induced increase in $[Ca^{2+}]_{cyt}$ was reduced by the CaSR antagonists, 10μM NPS2143 and Calhex 231.TGF-β1 caused the upregulation of myofibroblast markers, α-SMA and Col1α1, in LX-2 cells. This upregulation was markedly reduced by NS2143. The treatment with NS2143 and Calhex 231 significantly attenuated the proliferation of LX-2 cells. These results indicate that CaSRs are functionally expressed in hepatic stellate cells and contribute to extracellular matrix production and cell proliferation.

The role of Id4 in salivary gland and its involvement in the pathology of IgG4-related disease

唾液腺におけるId4の役割とIgG4関連疾患病態形成への関与

○木村 宗惟[1,2]、林 慶和[1,3]、矢野 恵奈[1]、佐伯 彩華[1]、安河内 篤[2]、森山 雅文[2]、中村 誠司[2]、自見 英治郎[1]、安河内（川久保） 友世[1]

[1]九州大・院歯・OBT研究セ、[2]九州大・院歯・口腔顎顔面病態学・顎顔面腫瘍制御学、[3]福岡歯科大・歯・生体構造学・機能構造

Id (inhibitor of DNA binding/differentiation), a group of dominant negative transcriptional regulators for basic helix-loop-helix transcription factors, consists of Id1-Id4. Previous studies showed Id proteins are involved in cell differentiation and proliferation, and those deficiency leads various pathological conditions. However, the physiological functions of Id4 have not been clarified. We thus investigated the role of Id4 in the salivary gland. In this study, we first analyzed the impact of Id4 on salivary glands using Id4 deficient (*Id4* KO) mice. The submandibular glands (SMG) weight and saliva secretion in *Id4* KO mice were significantly decreased compared to those in wild-type mice. Histological analysis revealed that increased expressions of various differentiation markers and significant mucus accumulation were observed in the SMG of *Id4* KO mice.
Subsequently, we investigated the possibility that Id4 is involved in human pathology, such as Sjögren syndromeand IgG4-RD in which saliva secretion often decreases. As a result, the expression level of Id4 was significantly decreased in the SMG tissue of IgG4-RD, and miRNA-mRNA integrated analysis using human samples revealed that Id4 might be downregulated by hsa-miR-486-5p in IgG4-RD salivary glands.
Taken together, we suggest that Id4 is essential for the homeostasis of salivary glands, and miR-486-5p, as well as Id4, might be associated with the pathophysiology of IgG4-RD.

Pharmacological analysis of salivary secretion mechanisms using rats with different strains of Aquaporin 5 levels.

AQP5レベルの異なるラット系統を用いた唾液分泌機構の薬理学的解析

○根津 顕弘、Akter MST Tahmina、谷村 明彦

北海道医療大・歯

Aquaporin 5 (AQP5) plays an important role for the transcellular fluid secretion in salivary gland cells. In this study, we compared acetylcholine (ACh)-induced salivary secretion and blood flow (BF) dynamics in submandibular gland (SMG) in rat strain with low levels of AQP5 protein (AQP5/low), Sprague-Dawley (SD) and Wistar/ST rats.The whole saliva secretion with high-dose of ACh (720~1440 nmol/min) in AQP5/low and Wistar/ST were~70% of that in SD. The level of AQP5 protein in Wistar/ST was same as that in AQP5/low, and was much lower than that in SD, suggesting that the AQP5 level determine the maximum rate of salivary secretions.Interestingly, the salivary secretions with low-dose of ACh (60~120 nmol/min) in Wistar/ST was two times higher than that of AQP5/low, and was comparable to that in SD. The ED_{50} values for ACh-induced salivary secretion in AQP5/low, Wistar/ST, and SD were 309, 102, and 134 nmol/min, respectively. These results suggest that ACh sensitivity in salivary secretion does not correlate with AQP5 levels. Monitoring of BF in SMG demonstrated that low-dose of ACh induced oscillatory changes in BF in all strains. The BF oscillations in Wistar/ST wereobservedmostlyabove the resting level, whereas that in AQP5/low were observed below the resting level. We also found that angiotensin II-mediated vasoconstriction reduced BF during ACh stimulations, resulting in a decrease insalivary secretionin AQP5/low.These results suggest that the regulation of salivary secretionwith physiological stimuli can be determined by BF rather than by the level of AQP5.

In vivo monitoring of increase in norepinephrine and serotonin in the interstitial fluids in rat submandibular glands by perfusion with imipramine

イミプラミンは唾液腺内ノルエピネフリン、セロトニン遊離量を増加する −唾液腺マイクロダイアリシス法を用いた検討−

○白勢 康介[1]、姜 卓義[1]、渡邊 真理子[1]、松田 光正[1]、伊藤 健二[1]、鈴木 武志[1]、小林 広幸[2]、吉川 正信[2]

[1]東海大・医・麻酔科学、[2]東海大・医・臨床薬理学

Previous studies demonstrated contents of monoamines in homogenate obtained from salivary glands. Monoamines in homogenate, however, includes that stored in the cells, as well as that released in the interstitial fluids. So far, direct monitoring of autonomic nervous activity within salivary glands has been difficult, and the relationship between neurotransmitter release in the salivary gland and salivary secretion has not been analyzed. Microdialysis was applied to salivary glands and successfully monitored multiple neurotransmitters simultaneously. The present study examined the effects of imipramine, a tricyclic antidepressant, on contents of monoamines in interstitial fluids within submandibular glands of rats. The results revealed the following: (1) that microdialysis allowed detection of norepinephrine and serotonin, but not epinephrine or dopamine; (2) that their concentrations in the dialysate were highly variable and unstable over the first 120 min after probe implantation, but reached a nearly stable level thereafter; and (3) that perfusion with imipramine significantly and dose-dependently increased norepinephrine and serotonin concentrations in the dialysate. These results indicate that the present microdialysis technique offers a powerful tool for detecting changes in sympathetic activity within the salivary glands.

Arginase 1 might regulate the exocrine secretion *via* metabolism

代謝を介したアルギナーゼ1の外分泌調節の可能性

○大野 雄太[1]、長瀬 春奈[1]、佐藤 慶太郎[2]、設楽 彰子[1]、中本 哲自[3]、柏俣 正典[1]

[1]朝日大・歯・歯科薬理、[2]明海大・歯・薬理、[3]朝日大・歯・インプラント

Sjogren's syndrome induces salivary and lacrimal hyposecretion, which leads to reduced quality of life. Although many studies have been conducted from the perspective of inflammation, the development of a causal treatment has not been achieved yet. We previously investigated the cause from the perspective of non-inflammation, and identified arginase 1 as a novel non-inflammatory regulator of exocrine function. However, the mechanism of arginase 1 regulating the function remains unknown, so we aimed to elucidate the mechanism.

We first confirmed the expression of arginase 1 in both salivary and lacrimal glands from BALB/c mice. Arginase 1 inhibitor, CB-1158, reduced the pilocarpine-induced saliva and tear secretion *in vivo*. The metabolome analysis of lacrimal glands revealed the altered concentration of amino acids related to energy metabolism. Furthermore, in an *ex vivo* perfusion system in which submandibular glands removed from the CB-1158-treated mice were cannulated with arteries, the saliva flow rate immediately after stimulation was unchanged compared to the control group but then decreased. We did not observe the saliva decrease in *ex vivo* experiments with perfusion of CB-1158 itself. These results indicated that arginase 1 might regulate paracellular fluid secretion *via* energy metabolism.

Examination of making a cylindrical defect model of rabbit meniscus

ウサギ半月板の円筒形欠損モデル作製検討

○岸浪 昌礼、鈴木 陽子、佐々木 麻衣、西堀 頼史、山崎 則之

㈱新薬リサーチセンター・非臨床研究部

【Background and Objective】

We have created a rabbit osteoarthritis model by partial resection of the lateral meniscus of the left hind limb knee joint. This time, we created a cylindrical defect model of the meniscus by biopsy trepan using a Japanese white rabbit, and compared the condition of the meniscus and the condyle joint 28 days after the operation.

【Method】

A hole was made in the center of the medial meniscus of the right knee joint of 4 Japanese white rabbits weighing 2.9 to 3.4 kg using biopsy trepan (ϕ 1.0 and 2.0 mm). The tibial condyle and meniscus were removed and visually observed.

【Result】

In the ϕ 1 mm cylindrical defect model, the meniscus remained punctured.

The ϕ 2 mm cylindrical defect model also had holes in the meniscus.

We compared the damage to the cartilage of the femoral condyle and tibial condyle with the partial meniscal resection model.

【Conclusion】

This model was also considered to be effective as a meniscus repair test.

Lactoferrin improves the reduction of bone formation in Dexamethasone-induced osteopenia mice

ラクトフェリンは、デキサメタゾン誘発性骨減少症マウスの骨形成の減少を改善する。

○青木 亮憲[1]、古川 恵[1]、東方 優大[2]、伊藤 芳久[1]、大野 恵[3]、出雲 信夫[2,4]

[1]横浜薬科大・薬・薬学教育セ、[2]横浜薬科大・薬・薬物治療学研、[3]（株）NRLファーマ、[4]横浜薬科大・薬・総合健康メディカル研究セ

【Objective.】

Steroids are used in clinical practice for a variety of conditions, including immunosuppression. However, steroid-induced osteoporosis due to decreased bone formation (De Nijs., 2008) is a problem (Suzuki et al., 2014). Lactoferrin (LF) is a protein found in breast milk and other sources and has been reported to promote osteoblast differentiation (Icriverzi et al., 2020). In this study, we investigated the therapeutic effect of LF on dexamethasone (DEX)-induced bone loss.

【method】

8-week-old ddy male mice were treated with DEX (2 mg/kg) for 8 weeks. 4 weeks after DEX administration, LF was administered by forced oral administration at a dose of 100 or 300 mg/kg for 6 days per week. 4 weeks after LF administration, femur and tibia were removed and bone strength measurements, CT measurements and mRNA The gene expression levels of bone metabolism markers were examined using RT-PCR.

【Results and Conclusions】

Bone strength after 8 weeks of DEX treatment was significantly lower than that of the untreated group, and LF administration ameliorated the decrease in bone strength. Similarly, CT results showed that LF administration significantly improved the DEX-induced decrease in bone mineral content and bone mineral density. RT-PCR results showed that the gene expression levels of osteogenic markers were significantly decreased by DEX administration and significantly improved by LF administration. These results suggest that LF improves the inhibition of bone loss by DEX through the involvement of the osteogenic system.

唾液腺と涙腺におけるCdc42は、上皮細胞の極性形成において同一の役割を果たすが、分泌機構においては反対の役割を担っている。

Cdc42 in salivary and lacrimal glands plays an identical role in epithelial cell polarity formation but an opposite role in the secretory mechanism.

○長瀬 春奈[1]、大野 雄太[1]、佐藤 慶太郎[2]、柏俣 正典[1]、設楽 彰子[1]

[1]朝日大・歯・薬理、[2]明海大・歯・薬理

Epithelial cells of exocrine glands responsible for saliva and tear secretion bear cell polarity. Cdc42, essential for the polarity of epithelial cells, is required for the formation and maintenance of luminal structures which is important site for the secretion. However, it is still unclear whether Cdc42 plays the identical role in different epithelial tissues *in vivo*. In this study, we generated exocrine epithelial cell-specific *Cdc42* conditional knockout (KO) mice and analyzed the difference of Cdc42 roles between salivary glands and lacrimal glands.
Morphological analysis showed that luminal structures changed to thick, short, bulging structures in the both *Cdc42* KO glands. Since these glands weight decreased, we analyzed inflammation with HE staining and apoptotic cell death with TUNEL staining. Both glands showed no inflammation nevertheless TUNEL (+) cells increased, suggesting disrupted cell polarity. In contrast, pilocarpine-stimulated saliva secretion decreased while tear secretion increased. Moreover, protein expression of AQP5, essential for exocrine fluid, also decreased in the salivary glands but conversely increased in lacrimal glands.
These findings suggest that Cdc42 in salivary glands and lacrimal glands plays an identical role in epithelial cell polarity formation but an opposite role in the secretory mechanism.

mPGES-1 promotes granulation tissue angiogenesis through regulatory T-cell accumulation

mPGES-1は制御性T細胞の集積により肉芽組織の血管新生を促進する

○兵頭 徹也[1,2]、天野 英樹[1]、伊藤 義也[1]、細野 加奈子[1]、畑中 公[1]、江島 耕二[3]、林 泉[4]、植松 智[5]、審良 静男[6]、武田 啓[2]、馬嶋 正隆[7]

[1]北里大・医・薬理学、[2]北里大・医・形美学、[3]北里大・医・免疫学、[4]日本薬科大・医療薬学科、[5]大阪市立大・院医・医・ゲノム免疫学、[6]大阪大・免・フロンティア研究センター、[7]神工大・健医学・臨工科病態治療研究室

Microsomal prostaglandin E synthase-1 (mPGES-1) is an enzyme responsible for the final step of prostaglandin E2 (PGE2) synthesis. PGE2 involves in wound-induced angiogenesis. Regulatory T cells (Tregs) regulate not only immune tolerance but also tissue repair and angiogenesis. Herein, we examined whether the mPGES-1/PGE2 axis contributes to wound-induced angiogenesis and granulation tissue formation through Treg accumulation. Polyurethane sponge disks were implanted into the dorsal subcutaneous tissues of the male mPGES-1-deficient (mPGES-1-/-) and C57BL/6 wild-type (WT) mice. Compared with WT mice, angiogenesis was suppressed in mPGES-1-/- mice, which was associated with attenuated forkhead box P3 (Foxp3) expression and Foxp3+ Treg accumulation. The numbers of double-positive cells for Foxp3/TGFβ and Foxp3/VEGF were lower in mPGES-1-/- mice than in WT mice. Deleting Tregs with neutralizing antibodies (Abs) against CD25 or folate receptor 4 (FR4) inhibited the Foxp3+ Treg angiogenesis and accumulation in WT mice but not in mPGES-1-/- mice. The topical application of PGE2 into the implanted sponge enhanced Treg angiogenesis and accumulation expressing TGFβ and VEGF in WT and mPGES-1-/- mice. These results suggest that mPGES-1-derived PGE2 promotes wound-induced angiogenesis, at least in part, by producing TGFβ and VEGF in accumulated Tregs. mPGES-1 induction would control angiogenesis with Treg recruitment in skin wounds.

P2X4 receptor signal enhances mast cell activation by Mas-relate G protein-coupled receptor b2 in a phosphatidylinositol-3-kinase-dependent manner

P2X4受容体シグナルはPI3K依存的にMrgprB2によるマスト細胞の活性化を促進する

○吉田 一貴、大林 晃右、伊藤 政明、松岡 功

高崎健康福祉大・薬

Mas-relate G protein-coupled receptor X2 (MRGPRX2) and its mouse ortholog Mrgprb2 are specifically expressed in mast cells and involved in pseudoallergic reactions. We previously reported that extracellular ATP augmented Mrgprb2-mediated mast cell activation via P2X4 receptors. In this study, we investigated the mechanism underlying the synergistic effects of co-stimulation of Mrgprb2 and P2X4 receptor on degranulation in mouse peritoneal mast cells (PMCs). Stimulation of Mrgprb2 with compound 48/80 induced degranulation accompanied by an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). ATP also induced a rapid increase in $[Ca^{2+}]i$. Co-stimulation with ATP and compound 48/80 promoted the sustained increase in $[Ca^{2+}]i$. This synergistic Ca^{2+} response was absent in PMCs prepared from P2X4 receptor deficient mice. In addition, both increased degranulation and synergistic Ca^{2+} response to co-stimulation with ATP and compound 48/80 were inhibited by the PI3K inhibitors wortmannin and AS605240. These results suggested that the P2X4 receptor signal promotes Mrgprb2-induced degranulation and Ca^{2+} response in a PI3K-dependent mechanism.

CDK8/19 inhibitors induce M2-like macrophage polarization.

CDK8/19阻害薬によるM2マクロファージ誘導の可能性

○水野 夏実、志賀 咲紀、柳川 芳毅

北海道医療大・薬

Macrophages polarize into anti-inflammatory macrophages (M2 macrophages) by interleukin (IL)-4, and these M2 macrophages express arginase-1. In addition, it has been reported that several cyclin-dependent kinase (CDK) 8/19 inhibitors promotes anti-inflammatory responses, and a number of CDK8/19 inhibitors have been developed. However, the effects of CDK8/19 inhibitors on arginase-1 expression in macrophages have not been clarified. In this study, we investigated the effects of CDK8/19 inhibitors on arginase-1 expression in murine macrophage cell line RAW264.7. The cells were pre-treated with BRD6989 or Senexin A, a CDK8/19 selective inhibitor, and then stimulated with IL-4. BRD6989 and Senexin A increased the IL-4-induced arginase-1 expression. Furthermore, we founded that p38 MAPK inhibitors suppressed the BRD6989-increased arginase-1 expression. On the other hands, BRD6989 and Senexin A increased the surface expression of CD206, a M2 macrophage marker, in RAW264.7 cells. In conclusion, we demonstrated for the first time that CDK8/19 inhibitors increased arginase-1 expression in macrophages via p38 MAPK activation. These findings suggest that CDK8/19 inhibition might induce anti-inflammatory M2-like macrophages.

Analysis of mechanism underlying the synergistic inflammatory cytokine production via P2X4 and EP_3 receptors in mast cells

マスト細胞におけるP2X4およびEP_3受容体を介した相乗的な炎症性サイトカイン産生メカニズムの解析

大林 昂右、吉田 一貴、朝比奈 愛理、内田 真耶子、伊藤 政明、○松岡 功

高崎健康福祉大・薬

Mast cells (MCs) produce a variety of chemokines and cytokines to induce allergic inflammation. Previously, we have shown that co-stimulation with ATP and prostaglandin (PG) E_2 synergistically increases the secretion of various inflammatory cytokines via P2X4 and EP_3 receptors, respectively. In the present study, we examined the mechanism underlying the synergistic cytokine production by co-stimulation of P2X4 and EP_3 receptors in mouse bone marrow-derived MCs (BMMCs). Stimulation of BMMCs with PGE_2 elicited rapid phosphorylation of ERK1/2, p38 MAP kinase, and Akt. In contrast, ATP alone had only weak effects on phosphorylation of these signaling molecules, and little affected PGE_2-induced responses. Although ATP and PGE_2 hardly induced NF-kB p65 phosphorylation, co-stimulation with them elicited an increase in NF-kB p65 phosphorylation. This effect was absent in BMMCs obtained from P2X4 receptor deficient mice. The cytokine production and NF-kB p65 phosphorylation induced by co-stimulation with ATP and PGE_2 were suppressed by inhibitors of NF-kB and phosphatidylinositol-3-kinase (PI3K). These results suggest that co-stimulation of P2X4 and EP_3 receptors enhances MC cytokine production by activating NF-kB signaling pathway in a PI3K-dependent manner

Expression and distribution of immune checkpoint molecules LAG-3 in amyotrophic lateral sclerosis

筋萎縮性側索硬化症モデルマウスにおける免疫チェックポイント分子LAG-3の発現と局在

○大島 基希[1]、青木 拓門[1]、青野 らん[1]、森﨑 祐太[1]、山中 宏二[2]、三澤 日出巳[1]

[1]慶應義塾大・薬・薬理学講座、[2]名古屋大・環境医学研究所　病態神経科学分野

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the selective death of motor neurons. As a mechanism of motor neurodegeneration, neuroinflammation by immune cells such as glial cells and other peripheral immune cells infiltrating in the central nervous system has been suggested. However, it is still unclear how the immune cell activation is regulated. We focused on immune checkpoint molecules (e.g. PD-1 and LAG-3) and analyzed their expression in ALS model mice ($SOD1^{G93A}$ mice). After the disease onset, when compared to WT mice, $SOD1^{G93A}$ mice showed an increased expression of membrane and soluble forms of LAG-3 in the spinal cord, but no change was detected in LAG-3 expression in peripheral tissues such as spleen and lymph nodes. We performed immunohistochemical analyses in the spinal cord of $SOD1^{G93A}$ mice and found that LAG-3 was expressed in microglia. Using the mouse microglial cell line BV2, we analyzed changes in LAG-3 expression upon differentiation into inflammatory (M1)/anti-inflammatory (M2) state. We found that the LAG-3 mRNA expression levels were upregulated in M1 microglia, but not in M2 microglia. Elucidation of LAG-3 function in microglia will lead to a better understanding of the involvement of the immune checkpoint molecule in the pathophysiology of ALS.

Acute stress exacerbates itch-related scratching in atopic dermatitis mice: possible involvement of allopregnanolone in the brain

アトピー性皮膚炎マウスにおいて急性ストレスは掻痒行動を増悪させる：脳内アロプレグナノロンの関与の可能性

○藤井 正徳、今林 怜矢、岸 美羽、山根 優、田中 里奈

京都薬科大

Itch is the most bothersome symptom of atopic dermatitis and is often exacerbated by stress. Allopregnanolone (ALLO), one of the neurosteroids in the brain, is shown to increase rapidly following acute stress, serving as a homeostatic response to stress. We have previously demonstrated that an intracisternal injection of ALLO markedly increases itch-related scratching behavior in atopic dermatitis model mice. The present study examines whether acute stress exacerbates itching in those mice by comparing scratching behavior under three different conditions: 1) conventional conditions (5 mice in the housing cage), 2) confinement stress (under isolated conditions in a small invisible chamber), and 3) confinement stress + forced swim stress (FSS) (under isolated conditions in the same chamber immediately following FSS). Scratching was significantly increased under confinement stress conditions compared to conventional conditions. FSS further exacerbated scratching behavior. Our results show that acute stress exacerbates itch-related scratching in atopic dermatitis mice. This exacerbation of scratching may involve ALLO transiently increased in the brain as a response to stress.

Involvement of neuropeptide; Gastrin-releasing peptide (GRP) and Galanin (GAL) in allergic rhinitis model mice.

アレルギー性鼻炎における神経ペプチドGastrin-releasing peptide（GRP)とGalanin（GAL)の関与

○木村 徹[1]、横井 秀格[2]、松本 祐磨[2]、川田 往嗣[2]、齋藤 康一郎[2]、櫻井 裕之[1]

[1]杏林大・医・薬理、[2]杏林大・医・耳鼻

Allergic rhinitis (AR) is caused by an allergic reaction at nasal epithelia against allergens such as pollen and house dust. The antihistamine drugs or leukotriene receptor antagonist are used for the AR therapy, however there are some patients, who do not respond to these drugs. Hence, it needs to develop newer drugs. In this study, we focused on neuropeptides known to be involved in an inflammatory process: Gastrin-releasing peptide (GRP) and Galanin (GAL).

AR model mice were constructed as follows. The ovalbumin together with the adjuvant, aluminum hydroxide was injected into mice intraperitoneally three times every other week as primary sensitization. Then ovalbumin was administrated intraperitoneally 14 consecutive days as secondary sensitization. Allergic reactions were evaluated by the number of rubbing sneezing episodes.

GRP and its receptor, GEPR, were expressed in nasal epithelial cells and the mast cells, and their expression was increased after AR induction. A GRPR antagonist suppressed AR symptoms. GAL and its receptor, GALR2 were expressed in nasal epithelial cells and B cells. A GALR2 antagonist suppressed the level of serum IgE, numbers of B cells. It seems that the signaling of GRP and GAL may be involved in the pathophysiology of AR and their inhibition would become the new therapeutic target for AR.

Effects of extracellular nucleotides on macrophage activation induced by stretch-mediated mechanical stimulation

機械的進展刺激によるマクロファージ活性化に及ぼす細胞外ヌクレオチドの影響

○伊藤 政明、長谷川 敦也、大熊 範和、吉田 一貴、松岡 功

高崎健康福祉大・薬

Although the immune cells experience mechanical forces and pressures throughout their life cycle, little is known about how such mechanical processes regulate the immune cell function. We previously reported that cyclical stretch (CS) stimulation of murine macrophage RAW264.7 (RAW) cells evoked release of nucleotides including ATP and also triggered elevation of mRNAs for various pro-inflammatory factors. In this study, we investigated the role of extracellular nucleotides on RAW cells activation induced by CS stimulation.

In RAW cells, CS stimulation evoked a marked release of ATP and also elevated mRNA and protein levels for monocyte chemoattractant protein-1 (MCP-1). Direct stimulation of RAW cells with extracellular nucleotides also triggered MCP-1 mRNA expression with a rank order efficacy: UTP, UDP >> ATP. RAW cells expressed functional P2 receptors including $P2Y_6$ receptor for uridine nucleotide. In the presence of $P2Y_6$ receptor antagonists, CS-induced MCP-1 expression was suppressed. In addition, $P2Y_6$ receptor gene knock-down with siRNA reduced CS-induced MCP-1 mRNA elevation without affecting ATP release.

These results suggest that $P2Y_6$ receptor activation via autocrine stimulation by released extracellular nucleotides may be involved in CS-induced MCP-1 expression in macrophages.

Changes in the expression of interferon-induced transmembrane protein-3 (IFITM3) in the brains of Alzheimer's disease model mice

アルツハイマー病モデルマウスにおける脳内IFITM3の発現変化

○LIU YUE[1]、溝口 博之[1,2]、祖父江 顕[3]、山中 宏二[3]、山田 清文[1]

[1]名古屋大・院医・医療薬学、[2]名古屋大・環境医学研究所・MIRAIC−未来の医学研究センター、[3]名古屋大・環境医学研究所・病態神経科学

Interferon-induced transmembrane protein-3 (IFITM3) belongs to the IFITM family, which comprises five and seven subtypes in humans and mice, respectively. IFITM proteins participate in various biological processes such as the immune response, including suppression of viral infection. While the immune response is associated with the pathology and development of Alzheimer's disease (AD), it is unknown whether this response is beneficial or harmful. Recently, IFITM3 was found to be a γ-secretase modulatory protein, a type of protein associated with the generation of amyloid b (Aβ). However, further research is needed to determine whether IFITM3 is associated with abnormal behaviors, including cognitive impairment in AD, and whether it may be a new molecular target for AD therapy. Since our final goal is to clarify the role of IFITM3 in an animal model of AD, in this study we used *App-KI* mice, which overproduce Aβ-42 without overexpressing amyloid precursor protein, to examine changes in IFITM3 expression. The cortex, dentate gyrus (DG), and CA3 regions of 4- and 8-month-old *App-KI* mice exhibited Aβ accumulation and also increased IFITM3 expression. Expressions of astrocytes and microglia were age-dependently increased around Aβ plaques. Notably, IFITM3 expression colocalized with astrocytes, which were situated near Aβ in all brain regions of 8-month-old *App-KI* mice. These results suggest that IFITM3 is increased in astrocytes and is accompanied by Aβ accumulation.

Cigarette smoke extract derived from heated tobacco products promotes cancer stem cell properties of lung cancer cell lines.

加熱式タバコ由来抽出液は肺癌細胞株の癌幹細胞性を促進する

○平田 尚也[1]、堀之内 孝広[2]、諫田 泰成[1]

[1]国衛研・薬理、[2]北海道大・院医・細胞薬理

Cigarette smoking is a risk factor for carcinogenesis and the development of several types of cancer, including lung cancer. Cigarette smoke is considered to contain over 5,000 chemicals, including carcinogens. Heated tobacco products (HTPs) have been recently reported to reduce levels of toxic chemicals, such as nicotine and tobacco-specific N-nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), compared to burned cigarettes. We have previously reported that nicotine and NNK induce the proliferation of cancer stem cells (CSCs), which contribute to tumorigenicity, drug resistance and recurrence. To evaluated whether HTPs are involved in cancer development, we examined the effects of cigarette smoke extract (CSE) derived from HTPs on lung CSCs. We found that CSE induced the proliferation of lung CSCs and increased the expression levels of stem cell markers. Since CSCs exhibit epithelial-mesenchymal transition (EMT), we analyzed the expression of EMT markers. CSE induced expression of EMT markers, such as Twist and Snail. These results suggest that HTPs can promote CSC properties in vitro and may result in cancer development.

Doxorubicin did not affect the lysosomal acidification

抗ガン剤ドキソルビシンのオートファジー障害はリソソーム機能障害によるものでは無い

○佐藤 岳哉[1,3]、戸田 法子[1,2]、斎藤 将樹[3]、山内 正憲[2]、阿部 高明[1]

[1]東北大・院医・病態液性制御学分野、[2]東北大・院医・麻酔科学・周術期医学、[3]東北大・院医・分子薬理学分野

Doxorubicin (Dox) has been used as an effective antitumor agent against various types of cancers. However, its application is limited owing to severe cardiomyopathy. Many researchers have attempted to clarify the mechanism of DOX-induced cardiomyopathy; however, its underlying mechanisms remain unclear. We showed that Dox impaired autophagic flux. In this study, we focused on the effect of Dox on lysosomes, a prerequisite organelle for autophagy that degrades autophagic cargoes. We speculated that Dox might inhibit autophagy by inhibiting the lysosomal function. To evaluate the effect of Dox on lysosomal acidification, we examined the effect of Dox on lysosomal acidification by staining cells with the acidophilic fluorescent dye acridine orange to determine the pH of lysosomes. Acridine orange emits red fluorescence under acidic conditions, whereas it emits green fluorescence under neutral pH conditions. The green and red fluorescence intensities of acridine orange were measured by flow cytometry. Bafilomycin, an H+-pump inhibitor of lysosomes, significantly inhibited lysosomal acidification. In contrast, lysosomal acidification increased considerably after Dox treatment. We further explored the effect of Dox on the expression of transcription factor EB (TFEB), which is known to regulate lysosomal function. Unexpectedly, Dox did not affect the expression of TFEB. These results indicated that Dox does not affect lysosomal acidification or function.

Phospholipase C like protein PRIP1 PH-domain-containing liposomes enhance apoptotic cell death in cisplatin resistant breast cancer cells

PLC様タンパク質PRIP1のPH domain内包リポソームはシスプラチン耐性乳ガン細胞のアポトーシス細胞死を促進する

○浅野 智志[1]、吾郷 由希夫[1]、兼松 隆[1,2]

[1]広島大・院医系科学・細胞分子薬理、[2]九州大・院歯・口腔細胞工学

Cisplatin is one of the most frequently used chemotherapeutic agents for the treatment of several human malignancies, and induces caspase-9-mediated apoptosis. Here, we examined whether phospholipase C-related catalytically inactive protein (PRIP) enhances cisplatin-induced apoptosis of cisplatin resistant breast cancer cells. PRIP depletion increased expression of X-linked inhibitor of apoptosis protein (XIAP) by inhibiting protein degradation, which is downstream of PI3K/AKT pathway and inhibits apoptotic signaling by blocking caspase-9 activation. Conversely, XIAP was decreased by expression of PRIP1 or pleckstrin homology domain of PRIP1 (PRIP1-PH domain) that blocked $PI(4,5)P_2$ metabolism. The expression levels of cleaved caspase-9 and downstream cleaved caspase-7 and poly-ADP ribose polymerase were greater in PRIP1 or PRIP1-PH domain-expressing MCF-7 cells treated with cisplatin than in control cells. In an orthotopic transplantation model, combined administration of PRIP1-PH domain-containing liposomes and cisplatin reduced the size of MCF-7 tumors compared with cisplatin alone. Our findings demonstrate that PRIP promotes XIAP degradation by inhibiting $PI(3,4,5)P_3$/AKT signaling and enhances cisplatin-induced apoptotic cell death. Therefore, we propose that PRIP1-PH-liposomes are a novel agent to avoid cisplatin resistance.

Differentiation inducing factor-1 suppressed the epithelial-mesenchymal transition via degradation of Yes-associated protein

細胞性粘菌由来分化誘導因子DIF−1はYAPの分解促進によりEMTを阻害する

○高橋 富美、有岡 将基、岸上 赳大、石兼 真

産業医大・医・薬理

Differentiation-inducing factors (DIFs), produced by *Dictyostelium discoideum*, show anti-tumor activity via several different signaling molecules, including glycogen synthase kinase-3 (GSK-3) and signal transducer and activator of transcription 3 (STAT3). However, the exact mechanism of action of DIFs is still poorly understood. Therefore, to better understand the action of DIF, we performed DNA microarray analysis and found that the activity of the Hippo signaling pathway may be modified. Therefore, we analyzed mRNA expressions of Hippo signaling pathway target genes. Although the mRNA expression of the target genes including CTGF and Cyr61were elevated by DIF-1, surprisingly the protein expression themselves were reduced. In an attempt to elucidate the mechanism, we found that DIF promoted proteolysis of Yes-associated protein (YAP), a transcriptional co-activator of the Hippo signaling pathway, in the human cervical cell line HeLa and the human colon cancer cell line HCT-116. YAP has also been reported to promote epithelial-to-mesenchymal transition (EMT). Indeed, cell migration, cell invasion and expressions of EMT-related proteins (fibronectin, vimentin and N-cadherin) were reduced by DIF-1. These results suggest that DIF-1 suppresses EMT via degradation of YAP in human cancer cell lines.

Probenecid has diverse effects on 3D-cultured prostate cancer cells.

プロベネシドが3次元培養された前立腺がん細胞に与える多様な効果

○宇和田 淳介、中澤 瞳、益岡 尚由

金沢医科大・医・薬理学

Probenecid, an uricosuric drug, has diverse pharmacological targets, including multidrug resistance-associated protein (MRP). Since inhibition of MRPs contributes to suppression of efflux of anti-cancer drugs, probenecid has been investigated for cancer therapy as a chemosensitizer. In this study, we examined the effects of probenecid on 3D-cultured prostate cancer cells. In the 3D-cultured spheroids of 22Rv1 cells that were less sensitive to cisplatin and doxorubicin than the 2D-cultured monolayer cells, probenecid treatment (100 and 300 μM) increased sensitivity to those anti-cancer drugs. On the other hand, a higher concentration (500 μM) of probenecid showed no chemosensitizing effect, which are consistent with increase in ABCG2, a drug-efflux transporter, at the dose. Furthermore, we found that probenecid has various anti-cancer effects other than alteration of chemosensitivity in 3D culture. Probenecid itself inhibited the viability of 22Rv1 spheroids and suppressed spheroid compaction rather than growth inhibition in the spheroids of another prostate cancer cell line PC-3. In addition, probenecid inhibited colony formation of 22Rv1 and PC-3 cells in soft agar and decreased the protein levels of focal adhesion kinase (FAK), which is important for anchorage-independent growth. In this presentation, we will also discuss about the latest findings including the target of probenecid in these anti-cancer effects.

Search for chromone derivatives that show high tumor-specificity against human oral squamous cell carcinoma, and evaluation of their adverse effects on normal cells

ヒト口腔扁平上皮がん細胞に対して高い腫瘍選択性を示すクロモン誘導体の探索と副作用の検討

○坂上 宏[1]、田沼 靖一[1]、天野 滋[1]、魚田 慎[1]、植沢 芳広[2]、黒崎 宏太[2]、高尾 浩一[3]、杉田 義昭[3]

[1]明海大、[2]明治薬科大・医療分子解析学、[3]城西大・薬

Introduction: Many anticancer drugs have been reported to cause serious side effects such as oral mucositis, neurotoxicity, and extravascular leakage. We have reported that among 291 chromone derivatives, 7-methoxy-3-[(1*E*)-2-phenylethenyl]-4*H*-1-benzopyran-4-one (compound A) showed the highest tumor-specificity against human oral squamous cell carcinoma (OSCC) cell lines, exceeding that of doxorubicin and 5-FU. In this study, newly synthesized 65 chromone derivatives were investigated for their TS and side effects. Method: The 50% cytotoxic concentration (CC_{50}) for four OSCC (Ca9-22, HSC-2, HSC-3, HSC-4), normal oral cells (gingival fibroblast, periodontal ligament fibroblast, pulp cell), oral epithelial cells (HOK, HGEP) and differentiated PC12 neuronal cells was determined from the dose-response curve. TS was calculated as the ratio of the mean CC_{50} for normal cells to that for OSCC. Apoptosis was assayed by cell cycle analysis. Results and Discussion: Newly synthesized indolychromones, indole-aurone hybrids, capsaicin derivatives, 6,7-styrylchromones, 3-benzylidenechromanones showed much lower TS value than compound A. Compound A should much lower keratinocyte toxicity than doxorubicin and 5-FU. 20 h treatment of Ca9-22 with compound A induced plateau level of cytotoxicity and accumulation of subG_1 and G_2/M population. *In silico* study suggests the inhibition of compound A against the estrogen related receptor-alpha signaling pathway, that is identified as an adverse marker for breast cancer progression and poor prognosis.

Canagliflozin, a sodium-glucose transporter 2 (SGLT2) inhibitor, suppresses the growth of glioblastoma through the AMPK-mTOR signaling

SGLT2阻害剤CanagliflozinはAMPK−mTORシグナルを介してglioblastomaの増殖を制御する

○江田 岳誉[1]、棗田 学[2]、大石 誠[2]、藤井 幸彦[2]、武井 延之[3]

[1]新潟大・病院薬剤部、[2]新潟大・脳研・脳神経外科学、[3]新潟大・脳研・腫瘍病態学

Glucose is a major metabolic source required for cancer cell survival and growth. The up-take of glucose by its transporters is enhanced in cancer cells. Cellular glucose levels are sensed by 5′ -AMP-activated protein kinase (AMPK). Upon glucose insufficiency, AMPK is activated and suppresses anabolic processes such as mammalian target of rapamycin (mTOR) system. mTOR is a key molecule for cellular growth and dysregulated activation is observed in cancer cells.

Therefore, we focused on the sodium-glucose transporter 2 (SGLT2) in glioblastoma survival and growth, because its expression was observed in these cells. We examined the effects of pharmacological inhibition of SGLT2 by canagliflozin. Canagliflozin reduced the growth of glioblastoma cell lines of human origin in a dose-dependent manner. Canagliflozin enhanced the phosphorylation of AMPK and suppressed S6 protein and p70S6 kinase phosphorylation. Canagliflozin inhibited the protein synthesis evaluated by the SUnSET assay. Canagliflozin inhibited the growth of glioblastoma also in xenograft model.

Canagliflozin activates AMPK and inhibits mTOR pathway thus inhibits the glioblastoma growth both in vitro and in vivo.

LAT1 plays a critical role in cell proliferation via CDK1 and CDK2 in cabazitaxel-resistant prostate cancer cells

アミノ酸トランスポーターLAT1はカバジタキセル耐性前立腺癌細胞においてCDK1とCDK2を介して細胞増殖に寄与する

○梨井 隼菱[1]、坂本 信一[1]、斎藤 心平[1,2]、新井 隆之[1]、溝上 敦[3]、安西 尚彦[2]、金井 好克[4]、市川 智彦[1]

[1]千葉大・院医・泌尿器科学、[2]千葉大・院医・薬理学、[3]金沢大・院医・泌尿器科学、[4]大阪大・院医・生体システム薬理学

Background: L-type amino acid transporter 1 (LAT1) is known to be highly expressed in various cancer types. We explored the role of LAT1 in cabazitaxel-resistant prostate cancer cells using phosphoproteome analysis.
Materials and Methods: We used PC-3, and a cabazitaxel-resistant strain generated based on PC-3 (PC-3-TxR/CxR). JPH203, a specific inhibitor of LAT1, was used to inhibit LAT1 function. Phosphoproteome analysis was used to quantitatively investigate the proteins and sites of phosphorylation that are altered by JPH203 administration.
Results: Compared to PC-3, LAT1 expression was significantly upregulated in PC-3-TxR/CxR. JPH203 significantly inhibited the migration and invasion of PC-3-TxR/CxR cell. Phosphoproteome analysis showed that JPH203 treatment reduced the activity of CDK1 and CDK2 as kinases more than previously known mTOR in PC-3-TxR/CxR cell. The decrease of phosphorylation in Cdc6 and Rb, substrates of CDK1 and CDK2, respectively, by treatment with JPH203 was confirmed by Western blotting.
Conclusions: Current date may indicate that LAT1 has a crucial role to progression of cabazitaxel-resistant prostate cancer via CDK1 and CDK2.

Potential of lysophospholipids in the prevention and treatment of Alzheimer's disease

アルツハイマー型認知症の予防及び治療におけるリゾリン脂質の可能性

○上芝 洸貴[1]、上田 勝也[2]、馬 闖[2]、泉谷 惇[2]、塚原 完[3]、松田 佳和[4]、齋藤 直人[5]、羽二生 久夫[1,2,5]

[1]信州大・院総合理工、[2]信州大・院医理工、[3]長崎大・院医歯薬、[4]日本薬科大・薬、[5]信州大・バイオメディカル研究所

Alzheimer's disease (AD) is a disorder in which cognitive dysfunction appears due to neuronal damage caused by abnormal aggregation of amyloid-beta protein (Aβ) in the brain. The number of patients is increasing year by year, not only in Japan but also around the world. However, all drugs currently approved in Japan for the treatment of AD are symptomatic treatments and cannot fundamentally cure the condition. Therefore, establishing methods of prevention and therapeutic of AD is an important issue. The authors have shown that porcine liver decomposition product (PLDP) exhibit a variety of biological activities in living organisms. In this study, we evaluated the effect of PEL, lipids extracted from PLDP, on the aggregation process of Aβ, a key protein in AD. PEL was extracted from PLDP by the Bligh & Dyer method. The thioflavin T assay was used as the evaluation method to monitor changes over time in fluorescence caused by Aβ aggregation. The results revealed that PEL dissociates as Aβ aggregates. In addition, the thioflavin T assay was also performed on various lysophospholipids, which are abundant in PEL. The results confirmed the phenomenon of inhibition of Aβ aggregation in certain molecular species. These results suggest that PEL could be a first step in the development of AD drug discovery.

Effects of 3,3‘-diindolylmethane, a phytochemical in Brassica vegetables, on the activation of cardiac fibroblasts.

心臓線維芽細胞活性化に対するアブラナ属野菜由来 3,3 ‘−ジインドリルメタンの効果

〇斉藤 麻希

医療創生大・薬・医療薬学

Phytochemicals are bioactive substances produced by plants so as to protect them from infection by fungi, bacteria, and viruses. Nowadays, many scientists have focused on and reported their beneficial effects against the activation of inflammatory cells and/or various types of cancer cells. In the present study, I investigated the effects of 3,3‘-diindolylmethane (DIM), an indole derivative contained in Brassica vegetables, on the activation of cardiac fibroblasts, which is deeply involved in cardiac fibrosis and furthermore it would cause heart failure. Human cardiac fibroblasts (hCF) were purchased and grew on collagen type I-coated culture dishes in the medium for hCF. The hCF were stimulated with 0.03~3 micro M of angiotensin II for 24 hrs present or absent of various concentrations of DIM. After the stimulation periods, the total RNA of the cells was conventionally extracted and then used for reverse transcription followed by real-time PCR.

The stimulation with angiotensin II tended to increase the expression of connective tissue growth factor (CTGF), one of the indicators for fibroblast activation, and concomitant application with DIM seemed to suppress these increases. The expression levels of other molecules which seemed to be involved in the activation of the fibroblast, we investigated, had a similar tendency as CTGF. These results suggest that DIM has the potential to be a candidate compound for the alleviation of the progression of cardiac fibrosis.

Discovery of hypnotic effect of two Japanese traditional herbal medicines on Drosophila insomnia models by using a newly developed automated sleep and rhythm analysis system (AutoCircaS)

新規開発した睡眠リズム自動解析システム（AutoCircaS）を用いた不眠症モデルショウジョウバエに対する2種類の和漢薬の鎮静効果

○井上 栄二[1,2]、鈴木 孝洋[2,3]、清水 康晴[1]、岩城 良和[4]、川崎 陽久[2]、石田 直理雄[2]

[1]救心製薬東京研、[2]国際科学振興財団　時間生物学研究所、[3]シグレイ、[4]タイセー

【Purpose】 Sleep in *Drosophila* was defined in 2000 by using an equipment named Drosophila Activity Monitor (DAM) system. However, this DAM system has too narrow space for *Drosophila* (fly) to analyze their social behavior. To overcome such demerits of DAM system, we developed a novel automated sleep and rhythm analysis system (AutoCircaS) which can monitor and record any behaviors like social mating, sleep, and circadian rhythm in flies in free space using the time-lapse (one frame per 10 sec) imaging. This study aimed to investigate the hypnotic effect of Japanese traditional herbal medicines on fly insomnia models by using the AutoCircaS.

【Method】 The caffeine-induced insomnia wild-type flies and the short-sleep mutant [*fumin* (*fmn*)] flies were used to assess the hypnotic effects of Japanese traditional herbal medicines *in vivo*. Flies were placed individually into 24-well microplates with drug-containing medium and acclimated for 3 days. The sleep duration was measured for 3 days by using the AutoCircaS.

【Results and Discussion】 Shortening of the sleeping duration was recorded in the caffeine-induced wild-type and the *fmn*-mutant flies using AutoCircaS. In this new system, we found Japanese traditional herbal medicines, KKG, NK and a Kampo hypnotic prescription Sansoninto, significantly improved the shortening of the sleep duration in the caffeine-induced insomnia flies. Moreover, KKG also significantly improved the decrement of the sleep duration in the *fmn*-mutant insomnia flies. The data provide new insights into the use of KKG and NK for human insomnia.

Elucidation of improving effects of ninjinyoeito, a traditional Japanese herbal medicine, on $A\beta_{25-35}$-induced neurite damage

人参養栄湯の$A\beta_{25-35}$誘導神経突起障害に対する改善効果の解明

○窪田 香織、永松 拓海、岡村 尚幸、坂本 真由、石田 恵理奈、渡辺 拓也、桂林 秀太郎、岩崎 克典

福岡大・薬

Ninjinyoeito (NYT), a Japanese herbal medicine, is widely used to treat patients with insomnia, anemia, amnesia, and neurosis. Recently, NYT was reported to be clinically effective in Alzheimer's disease (AD). To address the mechanism underlying effect of NYT on AD, we examined the effects of NYT in β-amyloid ($A\beta_{25-35}$)-exposed primary cocultured astrocytes and neurons. The effects of NYT on neurotoxicity induced by $A\beta_{25-35}$ were assessed by immunocytochemical assays and Sholl analysis of microtubule-associated protein 2 (MAP2)-positive and tau-positive neurites. $A\beta_{25-35}$ treatment attenuated the arborization of axons and dendrites of single autaptic hippocampal neurons in a concentration-dependent manner. NYT treatment ameliorated the $A\beta_{25-35}$-induced impairment of tau-positive axon outgrowth. However, NYT did not ameliorate the $A\beta_{25-35}$-induced suppression of MAP2-positive dendrite arborization. RT-PCR analysis demonstrated that NYT increases the expression of nerve growth factor (Ngf) but not brain derived neurotrophic factor (Bdnf) in $A\beta_{25-35}$-exposed primary cocultured astrocytes and neurons. Our results indicate that NYT protects against $A\beta_{25-35}$-induced neuronal injury through induction of nerve growth factor expression. These findings provide a mechanistic basis for treating AD with NYT.

Action of Rikkunshi-To on the rivastigmine-induced nausea in mice

認知症治療薬リバスチグミンが誘発する悪心に対する六君子湯の作用

○山本 浩一[1]、佐藤 雄己[2]

[1]森ノ宮医療大・医療技術・診療放射線、[2]福山大・薬・臨床薬効解析

Rivastigmine, which is a cholinesterase inhibitor used for the treatment of mild to moderate Alzheimer's disease, is known to be associated with significant gastrointestinal (GI) adverse reactions including nausea, vomiting, anorexia. If severe GI adverse reactions are observed during the course of treatment, the patient should be instructed to stop treatment. Rikkunshi-To (RKT), a traditional herbal Japanese medicine, has been prescribed for patients with various GI symptoms, and we have reported that RKT on therapy-induced nausea in mice. We also reported that pica, kaolin ingestion behavior, could be used to evaluate nausea in mice; thus, in this study, we investigated the effects of rivastigmine on pica in mice and the effects of RKT on inhibition of rivastigmine-induced nausea.

Male mice were consecutively administered rivastigmine (i.p.) for 5 days, and their kaolin intakes were measured. Additionally, we examined the effects of a serotonin 5-HT_3 (granisetron: i.p.), dopamine D_2 (domperidone: i.p.) and muscarinic (butylscopolamine: i.p.) receptor antagonists rivastigmine-induced pica. Furthermore, we investigated whether RKT has the therapeutic effects on the rivastigmine-induced pica.

We found mice showed pica during the course of donepezil administration. Among the tested anti-emetic drugs, none of the anti-emetic drugs led to any change in the rivastigmine-induced pica. On the other hand, mice fed the diet supplemented with RKT significantly inhibited rivastigmine-induced pica. These findings suggest that RKT is useful to prevent rivastigmine-induced nausea.

Effect of lactic acid bacteria-fermented milk whey on melanin production

乳酸菌発酵物ホエイによるメラニン産生抑制作用とそのメカニズムの解析

○五十嵐 信智[1]、西中 ゆい[1]、篠﨑 優衣[1]、吉田 涼太郎[1]、田端 慶斗[1]、今 理紗子[1]、酒井 寛泰[1]、畑中 美咲[2]、細江 智夫[1]

[1]星薬科大、[2]アサヒグループ食品

Melanin is generated in the melanosomes of melanocytes and is an important factor determining the skin color. Melanogenesis is catalyzed by tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and dopachrome tautomerase (DCT). Therefore, these enzymes are suitable targets for the development of cosmetics aimed at skin whitening. In this study, we investigated the effect of milk whey on melanin production. Whey obtained from five strains of lactic acid bacteria (L. delbrueckii bulgaricus, L. helveticus, L. acidphillus, L. casei, and L. helveticus CM4 strains) was used. The melanin contents in B16 melanoma cells treated with α-MSH was significantly increased by about 2-fold compared to control cells. In contrast, melanin production by α-MSH was significantly suppressed in whey obtained from any lactic acid bacteria. These wheys inhibited α-MSH-induced increased expression of TYR, TYRP1, and DCT. When the whey obtained from L. helveticus was examined in detail, it was found that the fraction with a molecular weight of 3 kDa or more had a strong melanin production inhibitory effect. These results demonstrated that whey decreases the expression of TYR, TYRP1, and DCT and suppresses melanin production. It was also suggested that substances with a molecular weight of 3 kDa or more may be involved in this action of whey.

Alteration of stress granule formation and clearance by *S*-Nitrosylation modification of G3BP1

G3BP1 の *S*-ニトロシル化修飾によるストレス顆粒形成・クリアランスの変化

○伊藤 和、藤河 香奈、上原 孝

岡山大・院医歯薬・薬効解析学

Nitric oxide (NO) is a signaling molecule that exerts a variety of regulatory functions in physiological states and stress responses. NO has been proposed to modulate protein function through *S*-nitrosylation of cysteine thiol residues in proteins. Appropriate amounts of NO result in neuroprotective effects via increasing moderate *S*-nitrosylated proteins and the cGMP pathway. On the other hand, excess amounts of NO promote neurodegenerative signaling pathways by increasing aberrant *S*-nitrosylated proteins.

We found that Ras GTPase-activating protein-binding protein 1 (G3BP1), which forms stress granules (SGs) implicated in neurodegenerative diseases, was a target of *S*-nitrosylation. Treatment with NO stimulated this modification in a concentration-dependent manner. It has been known that SGs are rapidly formed by oxidative and heat shock stresses. In addition, SGs formation induced by proteasome inhibitor (MG132) was gradually degraded, whereas NO delayed this degradation. These findings suggest that *S*-nitrosylated G3BP1 not only promotes SGs formation but also delays SGs clearance.

Ghrelin restores the dopamine responses in the PFC to external stimuli via dopamine D1 receptor signaling and attenuates cognitive deficit in *MECP2* KO mice, a model mouse for Rett syndrome

グレリンはレット症候群モデルマウスの前頭前野D1受容体シグナルを介してドパミン応答を回復させ認知機能障害を改善する

○河原 幸江[1]、大西 克典[1]、高橋 知之[2,3]、岸川 由紀[1,4]、弓削 康太朗[2,3]、河原 博[5]、山下 裕史郎[2,3]、松石 豊次郎[2,6]、西 昭徳[1]

[1]久留米大・医・薬理、[2]久留米大・高次脳疾患研、[3]久留米大・医・小児科、[4]西九州大・リハビリテーション、[5]鶴見大・歯・歯科麻酔、[6]聖マリア病院・小児総合研究セ・レット症候群研究セ

Ghrelin improves cognition in various animal models with cognitive deficits. This study investigated the effects of ghrelin on cognitive deficits and modulation of D1 receptor signaling in the PFC, which plays a critical role in cognitive performance, in *Mecp2* knockout (KO) mice using *in vivo* microdialysis.

In the modified novel object recognition (NOR) test, KO mice showed the impairment of cognition. Ghrelin injection (8.6 microgram/mouse, s.c.) improved the cognition of objects and investigatory behaviors in KO mice. In microdialysis studies, saline injection and novelty induced increases in DA in WT mice, and the increase was not observed in KO mice. Thus, KO mice exhibit the low response of DA to external stimuli in the PFC. The results of the infusions of D1 receptor ligands into the PFC indicate that D1 receptor signaling in WT mice is involved in bidirectional regulatory mechanisms of DA release. In KO mice, the ability of D1 receptor signaling to inhibit DA release would be upregulated under tonic and D1 receptor-stimulated conditions. Ghrelin injection, but not saline injection, increased DA levels in the PFC, and the DA levels significantly increased in response to the novelty after ghrelin injection in KO mice. The DA responses to the ghrelin injection and to the novelty after ghrelin injection in KO mice were completely abolished by the infusion of SCH23390.

Ghrelin restores DA responses to external stimuli by adjusting the altered function of D1 receptor signaling, and the action of ghrelin may underlie the mechanism for ameliorating cognitive deficit in *Mecp2* KO mice.

Role of the microsomal prostaglandin E synthase-1 in imiquimod-induced psoriasis-like skin inflammation.

イミキモド誘発乾癬様皮膚炎における膜型プロスタグランジンE合成酵素−1の役割

○日置 優花[1,2]、榎本 大樹[1]、三浦 早貴[1]、小野寺 優[1]、板橋 輝[1]、飯塚 佳子[3,4]、前花 祥太郎[3,5]、久保 誠[3,5]、北里 英朗[6]、市川 尊文[2,3]、小島 史章[1,2,3]

[1]北里大・医療衛生・薬理学、[2]北里大・院医療・生体制御生化学、[3]北里大・医療衛生学部附属・再生医療・細胞デザイン研究施設、[4]北里大・院医療・食予防医科学、[5]北里大・院医療・環境微生物学、[6]北里柴三郎記念館

Background: Psoriasis is a chronic inflammatory disease that is accompanied by abnormalities in the immune system. It was recently reported that microsomal prostaglandin E synthase-1 (mPGES-1), a terminal enzyme for PGE_2 biosynthesis, highly expresses in the skin of psoriasis patients. However, the detailed role of mPGES-1 in psoriasis remains unclear. In this study, we investigated the role of mPGES-1 in psoriasis-like skin inflammation induced by imiquimod (IMQ), one of the well-established models of psoriasis.

Methods: Psoriasis-like skin inflammation was induced in mice lacking mPGES-1 (mPGES-$1^{-/-}$ mice) and wild-type (WT) mice by administrating IMQ under specific pathogen free condition. The expressions of mPGES-1 mRNA and protein in the skin were determined by real-time PCR and western blotting, respectively. The skin inflammation was evaluated based on scores with macroscopic symptoms and histological features. In addition, the expression levels of intereukin-17A (IL-17A) in inflamed skin was determined by real-time PCR.

Results: The expression of mPGES-1 was highly induced on both mRNA and protein levels in the skin of WT mice after IMQ administration. Interestingly, the mPGES-$1^{-/-}$ mice exhibited more severe symptoms of psoriasis-like skin inflammation compared to those of WT mice during administration of IMQ. Histological analysis further showed significant increase of epidermal thickness in mPGES-$1^{-/-}$ mice. The skin expression of IL-17A, a prominent target for the treatment of psoriasis, was highly up-regulated in mPGES-$1^{-/-}$ mice in response to IMQ administration.

Conclusion: mPGES-1/PGE_2 system plays a protective role in psoriasis, partly by reducing the expression of IL-17A.

Thromboxane A_2 receptor signaling inhibits angiogenesis and lymphangiogenesis in the endometriotic lesions in mice

トロンボキサンA_2受容体シグナルは子宮内膜症の血管およびリンパ管新生を抑制する

○古江 明子[1,2]、伊藤 義也[1]、本田 雅子[2]、服部 響子[2]、関口 和企[2]、山下 敦[1]、長田 真由子[1]、田邊 美奈[1]、細野 加奈子[1]、畑中 公[1]、馬嶋 正隆[3]、加藤 一喜[2]、天野 英樹[1]

[1]北里大・院医療・分子薬理、[2]北里大・医・産婦人科、[3]神奈川工科大学・健康医療科学部・病態治療

Angiogenesis and lymphangiogenesis contribute to the development of endometriosis. We recently reported that thromboxane A_2 (TXA_2) receptor signaling involves in angiogenesis in critical limb ischemia and lymphangiogenesis in inflamed diaphragm. In the present study, using wild-type mice (WT) and thromboxane prostanoid receptor (TP) knockout mice (TPKO), we examined whether TP signaling plays a role in the growth of endometriosis by angiogenic responses. Ectopic endometriosis model was created by transplantation of endometrial tissue fragments from donor mice (WT or TPKO) into the peritoneal wall of host mice (WT or TPKO). The implant sizes and density of blood and lymphatic vessels in the TPKO implants from host TPKO (TPKO→TPKO) were increased as compared with the WT→WT. The mRNA levels of markers for blood (CD31) and lymphatic vessels (LYVE-1) and of growth factors for angiogenesis (VEGF-A) and lymphangiogenesis (VEGF-C/D) in the TPKO→TPKO were higher than those in the WT→WT. Immunostaining showed that TP was expressed in F4/80-positive macrophages, but not in blood and lymphatic vessels in endometriosis lesions. The levels of M2 macrophage-related genes were higher in the TPKO→TPKO than in the WT→WT, while no statistically significant difference in M1 macrophage-related genes was observed These results suggest that TP signaling inhibits the growth of endometriosis by reducing angiogenesis and lymphangiogenesis.

Anorexigenic effects of central administered xenin was possibly induced via central nesfatin-1 cells in rats

ラットにおいて中枢投与したXeninの食欲減退効果は中枢のネスファチン−1細胞を介して誘導される可能性

○齊藤 将太、橋本 弘史、濱口 紀江、裵 祥存、齋藤 心平、霊園 良恵、平山 友里、安西 尚彦

千葉大・院医・薬理学教室

Xenin, which was identified in human gastric mucosa, is a 25-amino acid peptide. Xenin is widely expressed in peripheral and central tissues. Xenin has various physiological functions, such as stimulating intestinal motility. Central and peripherally administered xenin decreased food intake in rodents. Nesfatin-1/NUCB2 (nesfatin-1), which is an anorexic neuropeptide consisting of 82 amino acids, is widely expressed in peripheral and central tissues. This study examined the effect of intraventricular (icv) administration of xenin on central nesfatin-1 cells in rats. Fos immunohistochemistry was used to investigate the effect of icv administration of xenin on nesfain-1-like immunoreactive (LI) cells in rat brain. Fos-LI cells were observed in the supraoptic nucleus, paraventricular nucleus, arcuate nucleus, and nucleus of the solitary tract after icv administration of xenin. Double immunohistochemistry for Fos and nesfatin-1 showed that nesfatin-1-LI cells expressing Fos were significantly increased compared with a control group in these nuclei after icv administration of xenin. Anorexigenic effects of xenin were attenuated by nesfain-1 antisense pretreatment. These results suggested that the anorexigenic effect of xenin is partially mediated by nesfatin-1 cells in rats.

BQ788, a selective ET_B receptor antagonist alleviates inflammatory reactions after traumatic brain injury in mice

エンドセリンET_B受容体拮抗薬BQ788による頭部外傷マウスの炎症反応に対する抑制効果

○道永 昌太郎[1]、水口 博之[2]、小川 泰弘[1]、菱沼 滋[1]、小山 豊[3]

[1]明治薬大・薬・薬効、[2]大阪大谷大・薬・薬理、[3]神戸薬大・薬・薬理

Traumatic brain injury (TBI) is a fetal damage to the brain resulted from an external force to head by accidents and falls. One of the TBI-induced severe pathogenesis is an inflammatory damage. We previously suggested that BQ788, an endothelin ET_B receptor antagonist alleviated blood-brain barrier disruption and brain edema in TBI mice. In this study, we investigated the effects of BQ788 on inflammatory reactions in TBI mice. As a model of TBI, a fluid percussion injury (FPI) was performed by a hydraulic impact on the mouse dura mater. BQ788 (15 nmol/day) was repeatedly administrated into lateral cerebroventricle from 2 to 5 days after FPI. As a maker of neutrophil, myeloperoxidase (MPO) and Ly6G were examined by fluorescent immunostaining. Expressions of inflammatory cytokines (tumor necrosis factor-α: TNF-α and interleukin-1β: IL-1β) and chemokines (monocyte chemoattractant protein－1: MCP－1 and mouse macrophage inflammatory protein-2: MIP-2) were measured by Real-time PCR. After FPI, MPO- and Ly6G-posotive cells were increased in the mouse cerebrum. Administration of BQ788 decreased these positive cells. Additionally, BQ788 decreased FPI-induced increases in expressions of TNF-α, IL-1β, MCP-1 and MIP-2 in the mouse cerebrum. These results suggest that ET_B receptor antagonist alleviates TBI-induced inflammatory reactions.

cGAMP-induced metabolic alterations in astrocytes and their impacts on tumor immune responses in the CNS

cGAMPが惹起するアストロサイトの代謝変容と中枢免疫応答に与える影響

○佐藤 洋美[1]、菊池 望恵[1]、後藤 杏子[1]、大川 柊弥[1]、松本 千佳[2]、田中 浩揮[2]、秋田 英万[3]、樋坂 章博[1]
[1]千葉大・院薬、[2]千葉大・院薬・薬物学、[3]東北大・院薬・薬物送達学分野

It has been shown that cGAMP (Cyclic 2'3'-GMP-AMP) is delivered via gap junction from cancer cells to adjacent astrocytes in metastatic brain tumors (BrM) and contributes to tumor progression. We aimed to reproduce the peritumoral astrocytic conditions by introducing cGAMP directly into astrocytes and to quantify the metabolic changes around the glutamine (Gln)-glutamate (Glu) cycle, as major CNS metabolism. In cGAMP-treated cells, the synthetic flux of Gln from $^{13}C5,^{15}N$-Glu was reduced to about half that of control cells, while Glu secretory flux was increased two-fold. Furthermore, when Glu was evaluated as a chemoattractant for migration using HL60-derived neutrophils, which have been implicated in immunosuppression of BrM, exposure to 10-50 μM Glu tended to increase the migration. Downstream of the STING, which accepts cGAMP, a marked upregulation was observed for IL-6, CCL5, CCL2, CCL7, and CXCL1 by several tens of fold. They are target genes of the NF-κB pathway that may be involved in interferon activation as well as IRF3, and were completely suppressed by NF-κB blockage. Glu-induced oxidative stress has been reported to mediate activation of NF-κB. These results suggest that induction of NF-κB by cGAMP may be involved in Glu metabolic alterations, as well as in tumor microenvironment formation, including immune responses.

Possible involvement of monomeric forms of visfatin around the cerebral blood vessels in the pathogenesis of cerebral infarction with diabetes

脳血管周囲における単量体 visfatin は糖尿病合併脳梗塞の病態進展に関与する

○岩谷 結衣、山本 春菜、市川 美月、増田 紋、森山 慶之、林 秀樹、高木 教夫

東京薬科大・薬・応用生化学

Changes in visfatin, as an adipocytokine, after cerebral infarction with diabetes and its pathophysiological significance are not clear. We attempted to elucidate the relationship between changes in visfatin after cerebral infarction with diabetes and pathogenesis. Protein levels of visfatin in brain tissue after middle cerebral artery occlusion/reperfusion (MCAO/R) in db/db mice did not change compared with those of the non-diabetic and control groups, but they were significantly increased in the cerebrovascular fraction. This increase would be derived from astrocytes as a monomer, which is involved in inflammation, around the cerebral blood vessels. The increase in MMP-9 mRNA of the cerebrovascular fraction might be due to the increased monomeric form of visfatin. Interestingly, there were no changes in visfatin mRNA in the cerebrovascular fraction, while protein levels of dimeric form of visfatin, which contributes to neuroprotection, were increased in serum after MCAO/R with diabetes. Therefore, visfatin could be secreted into the blood from peripheral tissues as a dimer after MCAO/R with diabetes, and a part of it changes into a monomer in the process of infiltration into the brain parenchyma. Monomeric forms of visfatin may be involved in an inflammatory reaction and the pathogenesis of cerebral infarction with diabetes.

Upregulation of nicotinic acetylcholine receptors in pulmonary arterial hypertension

肺動脈性肺高血圧症におけるニコチン性アセチルコリン受容体の発現増加

○山村 彩[1]、中浜 光哉[2]、Alamgir Hossain[1]、北村 文也[3]、高橋 理恵[1]、山村 寿男[2]、佐藤 元彦[1]

[1]愛知医科大・医、[2]名古屋市大・院薬・細胞分子薬効解析学、[3]愛知医科大・医・腎臓リウマチ膠原病内科

Smoking causes hypoxic vasospasm, thickening, and inflammation. Such responses are similar to the pathological mechanism of pulmonary arterial hypertension (PAH). PAH is a progressive and fatal disease that is characterized by the irreversible remodeling of the pulmonary artery. Pulmonary vasospasm, thickening, and inflammation are triggered by a chronic increase in cytosolic Ca^{2+} concentration. Here, we focused on the expression of nicotinic acetylcholine receptors (nAChRs), which are associated with cytosolic Ca^{2+} signaling, in PAH and hypoxic stress. The expression of the α subunits of nAChRs in pulmonary arterial smooth muscle cells (PASMCs) from normal subjects and idiopathic pulmonary arterial hypertension (IPAH) patients was analyzed by RT-PCR. Normal-PASMCs expressed nAChR α5 and α9 subunits. On the other hand, IPAH-PASMCs expressed nAChR α1, α5, and α7 subunits. As a result of Western blotting, the expression of nAChR α1and α7 proteins was upregulated in IPAH-PASMCs. In addition, the expression of nAChR α1 subunits was also increased in PASMCs from monocrotaline-induced pulmonary hypertensive rats. Furthermore, hypoxic exposure (1% O_2) increased nAChR α7 expression in normal-PASMCs. In conclusion, the expression of nAChR α1 and α7 subunits is upregulated in chronic respiratory diseases including PAH and hypoxic stress.

Involvement of mitochondrial Na^+/Ca^{2+} exchanger in hypoxia-induced pulmonary arterial hypertension

低酸素誘発性肺高血圧症におけるミトコンドリアNa^+/Ca^{2+}交換輸送体の関与

○喜多 紗斗美[1]、田頭 秀章[2]、披田 真里[1]、谷 和佳奈[1]、根本 隆行[2]、喜多 知[2]、岩本 隆宏[2]

[1]徳島文理大・薬・薬理、[2]福岡大・医・薬理

Pulmonary arterial hypertension (PAH) is a severe and progressive disease that leads to right heart failure. The pathogenesis of PAH is generally characterized by vasoconstriction, upregulated proliferation, migration, and pulmonary vascular remodeling in lung tissue. Recent studies using genetic analyses and experimental models have suggested that the hypercontraction of pulmonary arteries induced by Ca^{2+} signaling abnormality may be involved in the pathogenesis of PAH. The Na^+/Ca^{2+} exchanger type-1 (NCX1) is a bidirectional transporter that is controlled by membrane potential and transmembrane gradients of Na^+ and Ca^{2+}. We recently showed that the upregulation of vascular smooth muscle NCX1 contributes to the development of hypoxia-induced PAH, using NCX1 knockout mice and specific NCX1 inhibitor SEA0400. In the present study, we investigated the pathological role of mitochondrial Na^+/Ca^{2+} exchanger (NCLX). NCLX knockout mice exhibited significant reduction in right ventricular systolic pressure compared with wild-type mice. Furthermore, specific NCLX inhibitor CGP-37157 significantly suppressed hypoxia-induced PAH in wild-type mice. These findings indicate that NCLX contributes to the development of hypoxia-induced PAH, suggesting that NCLX inhibition might be a novel approach for the treatment of PAH.

Involvement of xenobiotic efflux transporter MRP5/ABCC5 in neurite outgrowth

薬物排出輸送体MRP5/ABCC5の神経突起伸長への関与

○石本 尚大、八木 寛史、増尾 友佑、加藤 将夫

金沢大・薬学系

Multidrug resistance associated protein 5 (Mrp5/Abcc5) is highly expressed in mouse primary cultured neurons (PCN) compared to other Abcc family members. However, the physiological role of Mrp5 in neurons is still unknown. In the present study, we examined the involvement of Mrp5 in neurite outgrowth because its physiological substrates cGMP and cAMP are important modulators of the neurite outgrowth. Immunocytochemical analysis showed that Mrp5 was expressed in the PCN and neuronal cell line Neuro2a. Efflux activity of Mrp5 in Neuro2a was then examined using CMFDA, which is metabolized to a fluorescent substrate for Mrps after diffusion into the cells. Knockdown of Mrp5 by transfection with siRNA for Mrp5 (siMrp5) showed a higher fluorescence intensity during incubation with CMFDA compared with control siRNA. In addition, exposure to an Mrp5 inhibitor zaprinast increased the fluorescent intensity. These results show the functional expression of Mrp5 in Neuro2a. Furthermore, both siMrp5 and zaprinast significantly increased number of cells having longer neurites. The knockdown with siMrp5 tended to increase protein expression of cGMP-dependent protein kinase 1 (PKG1), while a PKG inhibitor significantly suppressed the neurite outgrowth induced by Mrp5 knockdown with a concomitant decrease in expression of PKG1, suggesting that inhibition of MRP5 would promote neurite outgrowth at least partially through activation of PKG1. Further studies are needed to elucidate the possible involvement of clinically used Mrp5 inhibitor drugs in the neurite outgrowth.

Intragingival application of *Porphyromonas gingivalis*-derived lipopolysaccharide induces an increase in plasma TNF-α levels in anaesthetised rats

*Porphyromonas gingivalis*由来のリポ多糖のラットの歯肉内への投与が血漿中のTNF-α量に及ぼす影響

○青野 悠里、斉藤 幸治、三枝 禎

日本大・松戸歯・薬理

Periodontal disease is a chronic inflammation of the gingiva resulting in the destruction of periodontal tissue. *Porphyromonas gingivalis* (*Pg*), a gram-negative bacterium, appears to play a role in the development of periodontal disease. Lipopolysaccharide (LPS), a component of the gram-negative bacterial cell wall, plays a role in periodontal tissue destruction. We have shown that intragingival application of *Pg*-derived LPS (*Pg*-LPS) in rats increases gingival TNF-α without affecting IL-6 (Taguchi et al., Int. J. Oral Sci., 2015). Since periodontal infection is known to be a potential threat to general health, we examined the influence of intragingival application of *Pg*-LPS on plasma TNF-α and IL-6 levels in urethane-anaesthetized rats. For comparison, effects of LPS derived from *Escherichia coli* (*Ec*-LPS) were also studied. Male SD rats were used. The external jugular vein was cannulated and connected via Teflon tubing to a blood sampling system. Either *Pg*-LPS (1 μg), *Ec*-LPS (6 μg) or vehicle was injected into gingiva of upper incisors via a microsyringe, with 500 μl of blood sampled every 1 hr. Plasma TNF-α and IL-6 levels were determined by enzyme-linked assays. Unlike IL-6, basal plasma TNF-α was lower than the detection limit (5 pg/ml). *Pg*-LPS failed to affect IL-6 levels but increased TNF-α. Neither IL-6 nor TNF-α were altered by *Ec*-LPS. These results suggest that increased *Pg*-LPS in gingiva may have systemic effects through induction of plasma TNF-α.

ATP release from astrocyte induced by mechanical stimulation and its visualization

機械刺激によるアストロサイトからのATP放出の誘導と可視化

○加藤 奥穂、井上 貴文

早稲田大・先進研・生医 井上研

ATP, a gliotransmitter released from astrocytes, works in glia-glia and glia-neuron communications. Still, the functional significance of astrocyte-derived ATP remains to be elucidated, since there has been no efficient and selective way to inhibit the ATP release. While two types of ATP release from astrocytes are known: vesicular and channel-mediated ATP release, the mechanisms of the channel-mediated ATP release are still unknown. To investigate those, we established a method that can directly detect single ATP release events from single astrocytes: ATP release was evoked by pressing cultured astrocytes with a glass pipette (mechanical stimulation), and released extracellular ATP was detected using GRAB-$ATP_{1.0}$, a protein ATP sensor together with the changes in intracellular Ca^{2+} with a Ca^{2+} indicator, Fura-2. Extracellular ATP and intracellular Ca^{2+} were observed to increase in synchrony. Through pharmacological screening with inhibitors, maxi-anion channels, connexin hemichannels, and volume-regulated anion channels revealed as candidates involved in astrocytic ATP release. Further analysis will provide detailed mechanisms and functions of ATP release from astrocytes.

Bitter taste receptor14 regulates expression of histamine receptors, E-Cadherin and N-Cadherin

苦味受容体14はヒスタミン受容体、E−カドヘリン、N−カドヘリンの発現を制御する

○小笠原 正人[1]、福田 尚代[2]、石河 太知[3]、徳弘 圭造[2]、加茂 政晴[4]、山田 浩之[5]、石崎 明[4]

[1]岩手医科大・歯・薬理、[2]関西医科大・生命科学・ゲノム編集、[3]岩手医科大・歯・微生物、[4]岩手医科大・歯・生化学、[5]岩手医科大・歯・口腔外科

Background

Bitter taste receptor 14 belongs (TAS2R14) to GPCR family, stimulated by epigallocatechin gallate (EGCG). We reported the EGCG administration induced downregulation of histamine receptors in oral epithelial cancer cell lines. It is reported that usage of histamine receptor antagonists inhibited cancer cell metastasis through suppressing epithelial to mesenchymal transition (EMT). The current study investigates the function of TAS2R14 for histamine receptors and EMT.

Method

We performed knock out of TAS2R14 in HSC4 oral epithelial cancer cell line by genome editing method. The cultured cells extractions were prepared for the western blot analysis. Histamine H1 and H2 receptor antibodies were used for detection of protein expression. For the biochemical analysis of EMT, E-cadherin and N-cadherin antibodies were used.

Results

Genome editing of TAS2R14 in three HSC4 cells resulted in integration of pretermination codon in the gene. TAS2R14 protein in edited HSC4 cells was not detected by western blot analysis. E-cadherin was upregulated and N-cadherin was down regulated. Furthermore, histamine H1 and H2 receptors were down regulated.

Conclusions and discussion

TAS2R14 is involved in regulation of EMT marker proteins and histamine H1 and H2 receptors expression. EGCG administration to HSC4 cells means that there might be other molecular targets of EGCG or EGCG might function as inverse agonist considering the reports that bitter taste receptors work as constitutive active receptors.

Involvement of Kir2.1 K^+ channel in osteoblast differentiation mediated by ATP-conductive hemichannels

骨芽細胞分化制御における ATP 透過性ヘミチャネルを介した内向き整流性K^+チャネル Kir2.1 の役割

○鬼頭 宏彰、劉 澤成、雑賀 紀明、遠藤 京子、梶栗 潤子、大矢 進

名古屋市立大・院医

Osteoblast growth and differentiation, which are controlled by Ca^{2+} signaling, are important for bone formation and homeostasis. It has been reported that the inward rectifier Kir2.1K^+ channel is responsible for osteoblast differentiation by maintaining the resting membrane potential to regulate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). ATP released from hemichannels, such as pannexins and connexins located within the cell membrane, also contributes to osteoblast differentiation. The purpose of this study is to elucidate the functional relationship between Kir2.1 and ATP signaling in osteoblast differentiation of the osteoblastic cell line MC3T3-E1, established from mouse calvaria. We found that the alkaline phosphatase ALP mRNA expression in MC3T3-E1 cells and the endochondral ossification of metatarsal bones were suppressed by the treatment with ML133, a Kir2.1 inhibitor or CBX, a hemichannel blocker. The pharmacological blockade of hemichannels or Kir2.1 also decreased the amount of released ATP from MC3T3-E1 cells during osteoblast differentiation. ATP stimulation induced a transient Ca^{2+} influx through ATP-sensitive Ca^{2+} permeable channels, and this ATP-stimulated $[Ca^{2+}]_i$ rises were attenuated by the treatment with ML133 and 5-BDBD, a P2X4 inhibitor. These results suggest that both Kir2.1 and hemichannels may be involved in ATP signaling during osteoblast differentiation. The functional coupling between Kir2.1 and P2X4 channels plays an essential role in maintaining the bone homeostasis via modulating osteoblast differentiation.

Involvement of FAT/CD36 expression in mouse parotid ducts on salivary secretion

マウス耳下腺導管の脂肪酸輸送体FAT/CD36発現の唾液分泌への関与

○佐藤 慶太郎[1]、大野 雄太[2]、長瀬 春奈[2]、柏俣 正典[2]、安達 一典[1]

[1]明海大・歯・薬理、[2]朝日大・歯・薬理

Xerostomia is commonly observed in middle-aged and elderly patients. Since the adipose tissue infiltration is frequently observed in the parotid gland (PG) of alder animals. We hypothesized that the alteration in expression of fatty acid translocase (FAT/CD36), which facilitates fatty acids transportation, induces xerostomia through the hyposecretion in the PG. We firstly examined the *CD36* expression in the three major salivary glands of male BALB/c mice. The PG expressed significantly substantial *CD36* among them. In addition, the immunohistochemical analysis revealed that the CD36 protein localized in duct cells, but not acinar cells, in the PG of BALB/c mice. Then, the effect of CD36 inhibitor, sulfosuccinimidyl oleate, treatment on the salivary secretion in 48-weeks BALB/c mice was assessed. The inhibitor reduced pilocarpine (Pilo)-induced salivation. Moreover, the involvement of the PG CD36 in the salivary secretion of male 48-weeks senescence-accelerated mouse (SAM) was investigated. Compared with SAM resistant 1 (SAMR1), the Pilo-induced salivation in age-matched SAM prone 1 (SAMP1) was decreased. In addition, the protein expression of PG CD36 in SAMP1 was significantly lower than that of SAMR1. These results suggest that the CD36 in ducts of PG plays an important role in the aging-induced hyposecretion.

Humanin promotes exocytosis in PC12 cells

ヒューマニンはPC12細胞からの開口分泌を促進させる

○小塚 彩里、新倉 貴子

上智大・理工

PC12 cells, rat pheochromocytoma cells, are neuroendocrine cells and widely used for the study of exocytosis. PC12 cells release catecholamine (CA) from vesicles in response to the neurotransmitter acetylcholine (ACh) by the fusion of the vesicle membrane and the cell membrane. In the models of neurodegenerative disorders, such as Alzheimer's disease (AD), alteration of exocytosis mechanisms has been reported. Humanin (HN) is a 24-residue polypeptide and initially identified as a neuroprotective factor against AD-related insults. HN suppresses neuronal death caused by amyloid beta, an AD-associated cytotoxic insult, in vitro and ameliorates memory deficit of AD mouse models. We have recently found that HN increases the levels of neurotransmitters including catecholamines in the hippocampal region of normal mice. This finding suggests that HN may have the direct effect on promoting exocytosis in neurons. In this study, we examined the effect of S14G-HN (HNG), a highly potent HN derivative, on exocytosis. In PC12 cells, HNG increased ACh-induced CA secretion by the Bath application experiment. On the other hand, neuroprotection-defective HN derivatives, S7A-HN and C8A-HN, did not affect CA secretion. Furthermore, no secretagogue activity of HNG was observed by inhibiting JAK, a signal molecule of gp130 receptor. These results suggest that the receptor-mediated JAK-STAT pathway is involved in the HN's activity for promoting exocytosis.

MicroRNA targeting *Skp1* regulates inflammatory response through NF-κB signaling pathway

*Skp1*を標的とするmicroRNAによるNF−κB経路抑制を介した抗炎症効果

○佐野 朋美[1]、李 栄智[2]、溝上 顕子[3]、西村 英紀[2]、兼松 隆[1]

[1]九州大・院歯・口腔機能分子科学、[2]九州大・院歯・歯周、[3]九州大・院歯・OBTセ

Nuclear factor-kappa B (NF-κB) is one of the transcription factors that play a central role in the immune response and is involved in many physiological phenomena such as acute and chronic inflammatory reactions, cell proliferation and apoptosis. It has been reported that microRNA (miRNA) binds mainly to 3'UTR of the target mRNA, suppresses its gene expression, and plays an important role in various diseases such as cancer. Therefore, the purpose of this study was to identify miRNA that regulates the activation of NF-κB signaling. We identified miR-582-5p as a significantly downregulated miRNA in inflamed mouse adipose tissue. We found miR-582-5p was suppressed in LPS-stimulated RAW264.7 cells, a macrophage-like cell line. Subsequently, S-phase kinase-associated protein 1 (SKP1), a component of the E3 ubiquitin ligase that regulates the NF-κB pathway, was detected as a potential target for miR-582-5p predicted by Targetscan. The dual luciferase reporter assay confirmed that miR-582-5p binds to the 3'UTR site of *Skp1*, and the transfection of miR-582-5p mimic suppresses SKP1 expression in RAW264.7 cells. In addition, miR-582-5p suppressed the production of the inflammatory cytokine TNF-α by inhibiting the degradation of IκBα, which subsequently suppressed the phosphorylation of p65 resulting in its nuclear translocation. Our findings demonstrated that exogenously applied miR-582-5p can attenuate the activation of NF-κB signaling pathway by targeting *Skp1* gene expression, which provides a prospective therapeutic strategy for treating inflammatory diseases.

The role of primary cilia in zebrafish fin regeneration.

ゼブラフィッシュヒレ再生における一次線毛の役割

○白水 崇[1]、山川 大史[2]、稲垣 昌樹[1]、西村 有平[1]

[1]三重大・院医・統合薬理、[2]三重大・院医・分子生理学

Primary cilia are immobile structures that extend from the surface of various cells and have been suggested to be involved in signal transduction from extracellular stimuli. Signal transduction through primary cilia has been reported to work in tissue regeneration, but the detailed mechanism remains unclear. Zebrafish has a higher regenerative capacity than mammals and is a suitable animal model for observing the tissue regeneration process. In this study, we investigated the role of primary cilia in tissue regeneration using knockout zebrafish of genes involved in the regulation of primary cilia formation. Trichoplein is localized in the basal bodies of primary cilia and works as a suppressor of primary cilia formation through activation of Aurora A kinase. KCTD17 is also involved in primary cilia formation through the degradation of trichoplein. In the zebrafish fin injury-repairing model, the trichoplein knockout fish showed higher regenerative capacity than the wild type. In this presentation, we demonstrate the role of trichoplein and KCTD17 in the process of zebrafish fin-regeneration.

Evaluating drug efficacy by visualizing the spatiotemporal dynamics of intracellular states using a novel Covariation Network analysis

「細胞状態」の時空間的変化を活写する共変動ネットワーク解析法による薬効評価

○國重 莉奈[1]、野口 誉之[2]、米谷 信彦[3]、中津 大貴[1]、村田 昌之[1,2,4]、加納 ふみ[1,4]

[1]東京工業大・科創研・細胞制御工学、[2]東京大・国際高等研・IRCN、[3]（株）ニコン、[4]東京工業大学・マルチモーダル細胞解析協働研究拠点

To capture the spatiotemporal dynamics of biomolecules for examining the pharmacological action of drugs, we developed a novel protein network analysis, Protein Localization and Modification-based Covariation Network (PLOM-CON) analysis method. This method quantifies the temporal changes in quantity, quality (post-translational modifications such as phosphorylation), and (co)localization of proteins that are activated or inactivated in response to specific signals input from the immunofluorescence images, and visualizes them as a "covariation network" using the "strength of temporal correlation" of the feature quantities.

In this study, we performed PLOM-CON analysis method to obtain covariation networks for ~50 proteins in insulin-stimulated rat hepatoma H4IIEC3 cells. The results showed that both Akt, a central molecule in insulin signaling, and its phosphorylated form p-Akt (Ser473) are the hub of the network. Furthermore, we revealed that the actin domain, a specific structure temporally created upon insulin stimulus, is the site of accumulation of various molecules for the initiation of glycogen synthesis via phosphorylated GSK3β. Thus, the PLOM-CON analysis method can visualize how signals are widely propagated into cells, and can be used as a technology that can capture the main and side effects of drugs.

取り下げ

NMDA-induced activation of the CaMKII-RhoA-Rho-kinase pathway regulates aversive learning

NMDAによるCaMKII–RhoA–Rho–kinase経路の活性化が忌避学習を制御する

○船橋 靖広[1,2]、Ahammad Rijwan Uddin[1,2]、張 心健[3]、Emran Hossen[1,2]、Faruk Md. Omar[1,2]、王 緩緩[4]、呉 敏華[5]、許 伊凡[1,2]、坪井 大輔[1,2]、西岡 朋生[1,2]、黒田 啓介[4]、天野 睦紀[4]、崎村 建司[6]、内野 茂夫[7]、山田 清文[5]、永井 拓[3]、貝淵 弘三[1,2,4]
[1]藤田医科大・医科学研究センター・神経・腫瘍のシグナル解析プロジェクト研究部門、[2]藤田医科大・精神・神経病態解明センター・細胞生物学部門、[3]藤田医科大・精神・神経病態解明センター・神経行動薬理学研究部門、[4]名古屋大・院医・神経情報薬理学、[5]名古屋大・院医・医療薬学、[6]新潟大・脳研究所・モデル動物開発分野、[7]帝京大・理工・バイオサイエンス学科

Glutamate induces Ca^{2+} influx in neurons through NMDA receptors (NMDARs) and activates Ca^{2+}-dependent protein kinases, including CaMKII, which play critical roles in synaptic plasticity and learning. However, how these kinases regulate synaptic plasticity and learning remains largely unknown. Here, we performed phosphoproteomics and identified 160 proteins including ArhGEF2 whose phosphorylation were promoted by NMDA. CaMKII phosphorylated ArhGEF2 and stimulated its RhoGEF activity. Aversive stimuli induced CaMKII-mediated ArhGEF2 phosphorylation and Rho-kinase/ROCK activation in the nucleus accumbens (NAc). Inhibition of Rho-kinase in the NAc attenuated aversive learning. We also screened Rho-kinase substrates and identified 221 proteins including Shank3 which links actin filaments with NMDARs and AMPA receptors via Dlgap3. The Rho-kinase-mediated phosphorylation of Shank3 increased its interaction with Dlgap3. Manipulation of Shank3 in the NAc regulated dendritic spine formation and aversive learning in a phosphorylation-dependent manner. These results demonstrate that NMDA activates the CaMKII-ArhGEF2-Rho-kinase pathway to induce Shank3 phosphorylation for aversive learning.

Effects of *S*-allyl-L-cysteine on phosphorylation of insulin-like growth factor type-I receptor tyrosine kinase in primary cultures of adult rat hepatocytes.

成熟ラット初代培養肝実質細胞における*S*-allyl-L-cysteineのIGF-I受容体チロシンキナーゼリン酸化活性促進作用に関する検討

○茂木 肇、荻原 政彦、木村 光利

城西大・薬

We previously reported that *S*-allyl-L-cysteine (SAC)-induced cell proliferation was involved in intracellular IGF-I secretion via Janus kinase 2 (JAK2) / phospholipase C (PLC) pathway in primary cultures of adult rat hepatocytes. In this study, we investigated the involvement of IGF-I receptor tyrosine kinase (RTK) in the SAC-induced hepatocyte proliferation by using Western blot analysis. IGF-I RTK (p95 kDa) phosphorylation in cultured hepatocytes peaked 20 min after SAC stimulation. SAC-induced IGF-I RTK phosphorylation was suppressed not only by the IGF-I RTK inhibitor AG538 but also by the JAK2 inhibitor TG101209 and the PLC inhibitor U-73122. Furthermore, the SAC-induced cell proliferation was significantly suppressed by PI3 kinase inhibitor LY294002, MEK inhibitor PD98059, and mTOR inhibitor rapamycin. These results suggested that the SAC-induced IGF-I RTK phosphorylation is mediated by JAK2/PLC phosphorylation and subsequently released IGF-I phosphorylates IGF-I RTK. Then hepatocyte proliferation may be induced via IGF-I RTK / PI3 kinase / MEK / mTOR pathway.

Cytoprotective effect of Rab proteins against PRAF protein-induced cytotoxicity

PRAFタンパク質誘発細胞毒性に対するRabタンパク質の細胞保護効果

○渡部 正彦

帝京大・医療共通教育研究セ

The prenylated Rab acceptor 1 (PRA1) superfamily member PRAF3 plays crucial roles in membrane traffic as a GDI displacement factor *via* protein interaction with a variety of Rab proteins, as well as in the modulation of antioxidant glutathione through its interaction with the amino acid transporter EAAC1. It is known that the overexpression of PRAF3 induces the toxicity of the host cell, however, the factors capable of cancelling the cytotoxicity remained unknown. Our findings demonstrate that Rab1a can protect from the cytotoxicity of PRAF3-overexpressed human cells. Cytoprotective effects of Rab1a protein could further suggest that PRAF3 and Rab1a are closely related to each other physiologically and genetically.

Roles of MFN2 and MFN2 associated protein in chemotaxis of neutrophil-like differentiated HL-60 cells

好中球様細胞に分化させたHL−60細胞のケモタキシスにおけるMFN2及びMFN2結合タンパク質の役割

○真崎 雄一[1]、東 恒仁[1]、小林 純子[2]、小野寺 康仁[3]

[1]北海道大・院医・細胞薬理、[2]北海道大・院医・組織細胞、[3]北海道大・医・医理工

Neutrophils are important in innate immunity and in the initiation of an acute response to infection. Under normal conditions, the mitochondrial membrane potential of neutrophils is low, and neutrophils energy depends fundamentally on glycolysis. In contrast, neutrophils require energy supplied from mitochondrial oxidative phosphorylation (OXPHOS) during infection. The inhibition of mitochondrial OXPHOS blocks the chemotaxis of neutrophils. Here, we examined the mitochondrial morphology of neutrophil-like differentiated HL-60 cells after chemoattractant *N*-formyl-Met-Leu-Phe (*f*MLP) stimulation. We found that mitochondrial morphology changes to a tubular form after *f*MLP stimulation. Mitochondrial OXPHOS activity and mitochondrial complex II significantly increased after *f*MLP stimulation. On the other hand, the silencing of mitochondrial fusion protein *mitofusin 2* (*MFN2*) suppresses mitochondrial morphological changes. *MFN2* silencing suppressed OXPHOS activation and chemotaxis after *f*MLP stimulation. Furthermore, the silencing of MFN2 associated protein suppresses also mitochondrial morphological changes and chemotaxis upon *f*MLP stimulation. These results suggest that MFN2 and MFN2 associated protein are involved in chemotaxis of differentiated HL-60 cells.

Cigarette smoke gas phase induces ferroptosis via PKC in tracheal epithelial cells

タバコ煙ガス相は気管上皮細胞に対してPKC依存的にフェロトーシスを誘導する

○東 恒仁[1]、眞井 洋輔[2]、真崎 雄一[1]

[1]北海道大・院医・細胞薬理、[2]北海道大・院医・皮膚科

Cigarette smoking is one of the risk factors for respiratory diseases such as chronic obstructive pulmonary disease and emphysema. Cigarette smoke can be divided into two phases; tar (particle) phase including nicotine and gas phase. Although both phases of cigarette smoke have cytotoxic activity and affect respiratory system, the molecular mechanism for cytotoxicity has remained to be clarified. In this study, we have examined the effects of cigarette smoke extracts on tracheal epithelial cells and lung cells. Both tar and gas phases induced cell death. Ferrostatin-1 suppressed gas phase-induced cell death, whereas it had no effects on tar phase-induced cell death. Several unsaturated carbonyl compounds such as acrolein (ACR) and methyl vinyl ketone (MVK) are major cytotoxic compounds in the gas phase. Ferrostatin-1 also suppressed ACR- and MVK-induced cell death in tracheal epithelial cells. These results indicate that ACR and MVK in gas phase are critical factors for ferroptosis induction by cigarette smoke in the respiratory system. Since we have previously reported that ACR- and MVK-induced cell death is PKC-dependent in aorta smooth muscle cells, we have examined PKC inhibitors. The results suggest that novel and/or atypical PKCs involve in cigarette smoke-induced ferroptosis induction in tracheal epithelial cells.

Involvement of glycerophosphodiesterase 7 in the intracellular production of cyclic phosphatidic acid

グリセロホスホジエステラーゼ7による環状リン脂質メディエーターの細胞内産生

○北風 圭介[1]、Ali Hanif[2]、木本 来希[1,3]、竹之内 康広[1]、石丸 浩靖[1]、山下 純[4]、上田 夏生[5]、田中 保[2]、岡本 安雄[1]、坪井 一人[1]

[1]川崎医大・医、[2]徳島大・院生物資源、[3]奈良医大・医、[4]帝京大・薬・生物化学、[5]香川大・医・生化学

Cyclic phosphatidic acid (cPA) is a lipid mediator present in tissues and plasma, which regulates physiological and pathological processes via the suppression of peroxisome proliferator-activated receptor γ (PPARγ). Glycerophosphodiesterase 7 (GDE7) is an endoplasmic reticulum-localized lysophospholipase D-type enzyme, and mouse GDE7 was reported to catalyze the production of cPA in cell-free systems. However, it remains unknown whether this reaction occurs in living cells. In this study, we found that overexpression of human GDE7 in COS-7 cells increased not only cPA-producing activity in the membrane fraction but also cPA levels in living cells. Furthermore, we demonstrated that active site of human GDE7 is directed toward the luminal side of the endoplasmic reticulum membrane. Mutagenesis revealed that amino acid residues F227 and Y238 of human GDE7 play an important role in cPA production. Finally, GDE7-knockout decreased the cPA levels and derepressed mRNA expression of PPARγ target genes in human mammary MCF-7 cells, suggesting that cPA acts as an intracellular lipid mediator. These findings lead to a better understanding of the biological role of GDE7 and cPA in cells.

Establishment of the novel method to culture primary neurons from aged rodent brain

加齢マウスを用いた新たな神経細胞初代培養手法の確立

○笠井 悠哉[1]、野崎 千尋[2]、柴田 重信[1]

[1]早稲田大・先進理工学部・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

Recent study showed that cannabinoid CB1 receptor-mediated signaling may take a huge role in age-related modification of brain function. CB1 deficient animals show significant cognitive deficit in old age, however they show better learning ability in young age. Even though the finding is surprising, the molecular mechanisms of such difference, especially for better learning in young age remains unknown mainly due to lack of suitable in-vivo/ex-vivo models. Here we will purpose to use the primary cultured neurons as the ex-vivo model of aging, which can be enabled with gentleMACS technology. This method is said to enable the primary cell culture from adult rodents but up to age of P60. In the present study we aimed to establish a method for primary culture of brain neurons using aged mice, which can be also used for knockout animals of CB1 receptors.
We first tried to prepare the primary culture of brain neurons from neonatal (P7), young (P50) and mature (P105) mice. After 3-days of culture we could find that all of them survived well and start to extend their axons. Notably, P105 is twice an older age than previously reported suggesting that this method could be usable to evaluate the neuronal growth and cellular activity after the certain aging. Further, they could even survive for next 10-days with continuous axon growth. Using this method, we will further conduct the primary cell culture in knockout animals of CB1 as well as endocannabinoid producing enzyme DAGL-a to see the effect of endocannabinoid deficiency to the neuronal growth and cellular activity.

Differences in expression of fat metabolism-related genes in liver and adipose tissue after ingestion of high-fat diet in obese and obesity-resistant mouse strains

易肥満および肥満抵抗性マウス系統における高脂肪餌摂取後の肝および脂肪組織での脂肪代謝関連遺伝子の発現の違いについて

○山口 瑞希[1]、神原 遥[1]、渕 美樹[1]、吉田 真尋[1]、金子 啓一郎[1]、藤田 融[1]、前田 利男[2]、田邉 由幸[1]

[1]横浜薬科大・薬・薬理学研究室、[2]静岡県立大・薬・臨床薬剤学講座

We have isolated inbred mouse strains that stably express either obesity-prone (ddY-H) or obesity-resistant (ddY-L) phenotypes. The ddY-H mice spontaneously develop hyperglycemia and hepatic steatosis along with a significant increase in body weight and fat mass even by fed with normal diet, whereas the ddY-L mice hardly develop these metabolic syndrome-like phenotypes. To investigate metabolic differences between ddY-H and ddY-L mice during high-fat diet (HFD) feeding, we analyzed the expression of transcripts related to glucose- and fat-metabolisms in the liver and adipose tissues. At 6 weeks of age with normal diet, no significant difference was observed in the expression of CD36, a major fatty acid transporter, in the liver of both strains, although the expression of PPARγ, a key transcription factor for fat synthesis, tended to be higher in the liver of ddY-H mice than that of ddY-L mice. Afterward, ingestion of HFD from 6 to 9 and 12 weeks of age dramatically induced the expression of PPARγ and CD36 in the liver of ddY-H mice. Neither PPARγ nor CD36 was induced in the liver of HFD-fed ddY-L mice, however, both transcripts were induced significantly in epididymal fat tissue. These results suggest that the low efficiency of hepatic expression of PPARγ and CD36 may be involved in the obesity-resistant phenotype of ddY-L mice.

Involvement of glucose on TGF-β_1-induced epithelial-mesenchymal transition in epithelial keratinocytes

上皮ケラチノサイトにおけるTGF−β_1誘導性上皮間葉転換に対するグルコースの関与

○武石 幸容[1]、長岡 良礼[1]、髙橋 千代[1,2]、武田 佳奈[1,2]、岡村 和彦[3]、大徳 浩照[4]、八田 光世[1]

[1]福岡歯科大・歯・細胞分子生物学講座　分子機能制御学分野、[2]福岡歯科大・矯正歯科学分野、[3]福岡歯科大・歯・病態構造学分野、[4]筑波大・生存ダイナミクス研究センター

Epithelial-to-mesenchymal transition (EMT) is a unique program in which epithelial cells become more like mesenchymal cells. EMT is involved in several processes, including development, wound healing, fibrosis, and cancer progression, but the molecular mechanism remains to be elucidated.

We analyzed the involvement of glucose on transforming growth factor (TGF)-β_1-induced EMT in human keratinocyte HaCaT cells. Using fluorescent staining, TGF-β_1-treated HaCaT showed the formation of actin stress fiber, regardless of glucose concentration. EMT-related markers were partially suppressed in TGF-β_1-treated cells under low-glucose conditions. Furthermore, both intracellular glucose and lactate, a glucose metabolite, were decreased under low-glucose conditions. We focused on the relationship between autophagy and the partial suppression of EMT under low-glucose conditions because we found in a previous study that LC-3 and GABARAPL1, autophagy markers, may be associated with TGF-β_1-induced EMT. The expression of those markers were regulated in a glucose- and TGF-β_1-dependent manner.

Therefore, it is likely that TGF-β_1-induced EMT is regulated by glucose-induced autophagy in epithelial keratinocytes.

Investigation of dephosphorylation of synaptic phosphoproteins engaged in mammalian sleep-wake regulation

哺乳類の睡眠覚醒制御に関与するシナプスリン酸化タンパク質の脱リン酸化の研究

○曹 思鈺[1]、戸根 大輔[1]、山田 陸裕[2]、隅山 健太[3]、上田 泰己[1,2]

[1]東京大・院医、[2]理研・生命システム研究センター・合成生物学チーム、[3]理研・生命システム研究センター・高速ゲノム変異マウス作製研究チーム

Sleep-wake cycle is an organism-level phenomenon that is precisely controlled by multi-layered systems such as circuits, cellular and molecular levels in a brain. Recent studies have provided a deeper understanding of the molecular basis of mammalian sleep-wake regulation and some studies suggested dynamic changes in neuronal protein phosphorylation under the control of the sleep-wake cycle. However, the core molecular mechanism of the dynamic changes in phospho-proteins and whether it could drive the transition between sleep and wake is still unclear. In this study, we identified a gene known to be involved in the dephosphorylation process in various signaling pathways in mammals as a novel sleep-regulating factor. Exogenous expression of the active form of GeneX in excitatory postsynapses resulted in a significant increase in sleep duration. Besides, knockout of one of geneX regulators which works as the scaffold protein of the proteinX in excitatory postsynapses resulted in a significant decrease in sleep duration. These results suggest that the sleep-wake cycle is modulated by dephosphorylation processes involving proteinX in the excitatory postsynapses.

Neuronal nitric oxide synthase is regulated by supersulfides

神経型NO合成酵素は超硫黄分子により制御される

○土屋 幸弘、荒木 笙馬、渡邊 泰男

昭和薬科大・薬・薬理

〈Introduction〉 Neuronal nitric oxide synthase (nNOS) is a Ca^{2+}/calmodulin (CaM)-dependent enzyme that catalyzes the metabolism of L-arginine (Arg) to nitric oxide (NO) in the presence of NADPH and O_2. nNOS is only active as a dimer, however, a reduction of cofactor tetrahydrobiopterin (BH_4) levels drastically destabilizes the dimer and thereby uncoupling from NADPH and production of superoxide rather than NO occur. Protein polysulfidation at specific Cys residues plays pivotal roles in the protein function. In this study, we investigated the molecular mechanism of supersulfides-induced inactivation of nNOS.

〈Results and discussion〉 Incubation of recombinant nNOS with Na_2S_4 in the presence of Arg and BH_4 resulted in a dose-dependent inhibition of NO production which was cancelled by reducing agent, dithiothreitol. Likewise, NADPH oxidation was inhibited with Na_2S_2 in the presence of BH_4 either with or without Arg but was not inhibited when both Arg and BH_4 were omitted. This indicates that inhibition targets NADPH oxidation with dimer formation of nNOS and without sign of uncoupling. The dimer/ monomer ratio of nNOS was not affected by Na_2S_4. Treatment of Na_2S_4 resulted in decreased ADP- but not CaM-agarose binding ability to nNOS. We successfully did generate the Na_2S_4-insensitive nNOS mutant in that its specific Cys reside within the NADPH-binding domain were mutated with serine. Thus, NADPH oxidation activity of dimerized nNOS is regulated by supersulfides via its regulatory cysteine residue modification.

Search for tyrosine kinases overexpressed in the hypertrophied right ventricular wall of monochromotaline-induced pulmonary hypertensive rats

モノクロタリン誘発肺高血圧ラットの肥大化右心室壁で過剰発現するチロシンキナーゼの探索

○田邉 由幸、金子 啓一郎、吉田 真尋、藤田 融

横浜薬科大・薬

Pulmonary arterial hypertension (PAH) results in right ventricular failure due to markedly elevated pulmonary arterial pressure and compensatory right ventricular hypertrophy. Although the involvement of tyrosine kinase (PTK) in the smooth muscle pathogenicities of PAH has been suggested, attempts to treat with a “multi-target” PTK inhibitor (PTKI) have not made sufficient progress due to harmful effects such as cardiotoxicity. In this study, we searched for PTK, whose expression is selectively increased in the hypertrophied right ventricular wall of PAH rats. RT-PCR targeting PTK domain consensus sequence was carried out for right ventricular wall (RV) and left ventricular wall with septum (LV+S) from monocrotaline-induced (MCT-) PAH rats and control rats. Southern hybridization was performed using various PTK probes derived from MCT-PAH rat RV. Selective increased expression of at least three types of PTK in MCT-PAH rat RV was observed, including PDGF-Rβ, which we previously reported its overexpression in pulmonary arteries of MCT-PAH rats. There was no difference in the expression of c-abl, which is thought to be involved in PTKI cardiotoxicity, in the LV+S and RV in both PAH and control rats. Our results suggest that a more specific inhibition of PTK isoform would be suitable for safely treating PAH using PTKI.

The production of hydrogen sulfide in glioblastoma cell lines

ヒトがん細胞における生理活性物質硫化水素の産生経路

○澁谷 典広、佐藤 彩湖、河津 咲穂、伊藤 凌大、木村 英雄

山口東京理科大・薬

Hydrogen sulfide (H_2S) functions as a signaling molecule and a cytoprotectant. The pathway to produce H_2S includes cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST). 3MST produces H_2S from 3-mercaptopyruvate that is provided from l-cysteine and α-ketoglutarate (α-KG) by cysteine aminotransferase (CAT) and from d-cysteine by d-amino acid oxidase (DAO). Recently, a new concept emerges in H_2S biology, showing tumor cells upregulate the production of H_2S and utilize it to promote tumor growth and chemotherapy resistance. In this study, we investigated the production of H_2S in cell lines derived from glioblastoma, the most common- and aggressive-type of brain tumors among adults. Lysates of glioblastoma cells produced H_2S from l-cysteine and α-KG. In the absence of α-KG, the production of H_2S was significantly decreased, suggesting that H_2S production is dependent on the activity of CAT. The production of H_2S from l-cysteine and α-KG was correlated with the amount of bound sulfane sulfur, a storage form of H_2S. Unlike the l-cysteine pathway, d-cysteine pathway does not operate in the glioblastoma cells. Further investigation will be needed to clarify whether regulation of the l-cysteine pathway changes proliferation and anti-cancer drug resistance of the glioblastoma cells.

Behavioral analysis of mice overexpressing PP5 specifically in the mice CA3 region of hippocampus.

マウス脳海馬CA3領域特異的PP5過剰発現マウスの行動解析

○宇野 恭介、河原井 康介、金城 俊彦、倉本 展行

摂南大・薬

[Purpose] We have explored the possibility that protein phosphatase 5 (PP5), together with PP1 and PP2A, promotes desensitization of GABAB receptors and attenuates receptor action by dephosphorylating the 892nd serine residue of mouse GABAB receptors. In this study, we investigated the possibility that dephosphorylation of GABAB receptors in the hippocampal CA3 region affects the behavior of mice.

[Methods]The PP5 gene was cloned in a pAAV-CMV-GFP vector to create an adeno-associated viral vector (AAV). Mice in which the PP5 gene was overexpressed in the hippocampal CA3 region (PP5 mice) using the created AAV or mice in which AAV containing only the GFP gene was introduced as a control group (GFP mice) were created, and the effect on mouse behavior was analyzed.

[Results] In the Open field test, PP5 mice tended to stay in the center area longer than GFP mice, and a significant difference was observed especially for last 10 minutes of the session. In an elevated cross-maze test, PP5 mice tended to stay longer in open zones than GFP mice. In the Y-maze test, PP5 mice showed a decreasing trend in alternation behavior compared to GFP mice. In the novel object search test, a significant increase in the time of interest in the novel object was observed in GFP mice, but in PP5 mice there was no significant difference in the time of interest in the two objects.

[Conclusion] The behavioral experiments show that overexpression of PP5 in the CA3 region may affect mice to reduce anxiety-like behavior and cognitive function.

A novel endothelin A receptor antagonist eliminates the analgesic tolerance and recovers the attenuated analgesic effects for long-term use of opioids

新規エンドセリンA受容体拮抗薬はオピオイド製剤の長期使用で起こる鎮痛耐性および鎮痛減弱作用を解除する

○大島 佳織[1,2]、野中 美希[2]、黒田 唯[2,3]、宮野 加奈子[2]、高柳 広[1]、上園 保仁[2]

[1]東京大・院医・病因・病理学、[2]東京慈恵会医科大・医・疼痛制御、[3]順天堂大・医・麻酔科学・ペインクリニック

Endothelin-1 (ET-1) is known as a vasoconstrictor. Several studies have reported that ET-1 induced pain signals through the ETA receptor (ETAR). Although ETAR antagonists are reported to enhance analgesic effects of opioid agonists and relieve its tolerance, the mechanisms remain unclear. We previously revealed the novel and highly selective ETAR antagonist (provided by Eisai Co., Ltd.) enhanced morphine-induced opioid receptor (OR) activities with HEK293 cells stably expressing either ETAR (ETAR cells), μOR (μOR cells), or both ETAR and μOR (ETAR/μOR cells). Further, we found that μOR and ETAR form a dimer as a possible target of this antagonist, and ET-1 internalized the ETAR/μOR heterodimer into the cytosol, resulting in decreased numbers of μOR on the cell membrane, followed by reduced responses by morphine. There are opioid agonists prone to internalize μOR or not; morphine is hardly to internalize, whreas fentanyl preferentially internalizes μOR among opioid agonists. Such difference in μOR internalization is believed to involve in the analgesic tolerance. In the present study with cells expressing ETAR/μOR, we evaluated the effects of the ETAR antagonist on the enhancement of μOR activities by several opioid agonists in addition to morphine, and compared the selectivity of the ETAR antagonist as an analgesic adjuvant.

Role of arachidonic acid-containing phospholipids in neuropathic pain

アラキドン酸含有リン脂質が担う神経障害性疼痛における役割

○山本 将大[1]、清水 孝雄[1,2]、進藤 英雄[1,3]

[1](国研)国立国際医療研究センター研究所・脂質生命科学研究部、[2]公益財団法人微生物化学研究会　微生物化学研究所、[3]東京大・院医・脂質医科学連携講座

Glycerophospholipids (PLs) play pivotal roles in our body as main structural components of biological membranes and precursors of lipid mediators. Recently, we revealed that peripheral nerve injury (PNI) increased arachidonic acid-containing PLs (ARA-PLs) in the dorsal root ganglion (DRG), spinal microglia, and spinal astrocytes. Therefore, we focused on LPCAT3 (also called LPLAT12), a biosynthetic enzyme of ARA-PLs, and established cell type-specific *Lpcat3*-knockout mice: DRG neuron (*Advillin*^{Cre}), macrophage/microglia (*Cx3cr1*^{CreERT2}), and satellite glia/astrocyte (*Gfap*-Cre). We found that PNI-induced mechanical allodynia were attenuated in DRG neuron-specific and satellite glia/astrocyte-specific *Lpcat3*-knockout mice. These results suggest that alteration of quality of lipid in these cells may be a cause of neuropathic pain. Now, we are analyzing the mechanisms by which ARA-PLs are involved in neuropathic pain.

Synergistic effect of topical application of TRP channel antagonists to gingiva on orthodontic force-induced pain in rats

矯正力負荷に伴う疼痛に対するTRPチャネル拮抗薬の歯肉への併用塗布の効果

○湯川 未郷[1]、佐藤 慶太郎[2]、須田 直人[1]、安達 一典[2]

[1]明海大・歯・形態機能成育学講座　歯科矯正学分野、[2]明海大・歯・病態診断治療学講座　薬理学分野

The electrical stimulation to gingiva induced the jaw-opening reflex (JOR). We have previously reported that the threshold for inducing jaw-opening reflex (JOR-TH) is significantly reduced by application of orthodontic force to teeth, and the intraperitoneal administration of TRPV1 antagonist recovers the JOR-TH. However, serious adverse side effects (e.g., hyperthermia) are reported by general administration of TRPV1 antagonists, and effective and safety application has to be established. In this study, TRPV1 and or TRPA1 antagonists were topically applied to gingiva, and the orthodontic force-induced JOR excitation was investigated with other features (e.g., trigeminal excitation, inflammatory cytokine alteration, rectal and gingival temperatures, and temperature sensitivity of the plantar and gingiva). Topical application of TRP antagonists immediately (D0) or one-day (D1)after orthodontic force application significantly increased JOR-TH the next day in dose-dependent and synergistic manner. On the other hand, there were no alterations in rectal and gingival temperature. In D0, the analgesic effect was associated with a significant reduction of CINC2 (cytokine-induced neutrophil chemoattractant-2) in the periodontium. In addition, chemical application-induced increase in temperature sensitivity was only observed in plantar. Taken together, topical application of TRP antagonists cocktail may reduce clinical orthodontic pain via CINC-2 reduction without adverse side effects.

PAC1 receptor antagonist PA-81004 provides an excellent preventive effect against oxaliplatin-induced cold allodynia

PAC1受容体拮抗薬PA-81004はオキサリプラチン誘発性冷的アロディニアに対し優れた予防効果を示す

○足原 佑弥[1]、斎藤 弘樹[2]、宮田 篤郎[2]、栗原 崇[2]、髙﨑 一朗[1]

[1]富山大・院医薬理工・生体情報薬理、[2]鹿児島大・院医歯・生体情報薬理

Oxaliplatin (OXA), a third-generation platinum-based anticancer agent, is widely used for colorectal cancer. However, OXA causes acute peripheral neuropathy in approximately 80-90% patients. The most common symptom is cold allodynia in the hands and feet. At present, there are few drugs that can prevent OXA-induced peripheral neuropathy. Previously, we demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1 are important role for the chronic pain, and developed a small-molecule antagonist of PAC1 receptor (PA-81004, Takasakiet al., JPET, 2018; Eur J Med Chem, 2022). In this study, we investigated whether PA-81004 has a preventive effect on oxaliplatin-induced cold allodynia.

Cold allodynia developed 1 day after single injection of OXA (5 mg/kg, i.p.), peaked at 4 days, and lasted for 6 days. The administration of OXA also caused transient weight loss. Repetitive administration of PA-81004 (0.1-30 mg/kg) showed a dose-dependent inhibitory effect, but weight loss was observed, possibly due to repetitive administration. A single intraperitoneal dose (0.1-3 mg/kg) administered 30 min prior to OXA administration showed no weight loss and an anti-allodynic effect. Intravenous administration of PA-81004 was more potent than intraperitoneal administration in preventing the onset of cold allodynia and also in inhibiting weight loss.

The present results suggest that PAC1 receptors are involved in OXA-induced cold allodynia and that PA-81004 may become a preventive agent against OXA-induced peripheral neuropathy.

Effects of meloxicam and morphine on nociceptive behaviour in rats with bilateral intraplantar injections of carrageenan into hind paws

両後肢足底へcarrageenanを投与したラットの疼痛関連行動へmeloxicamおよびmorphineが及ぼす効果

○三枝 禎、川島 央暉、青野 悠里

日本大・松戸歯・薬理

Intraplantar injection of carrageenan into the hind paw of rodents is used to induce experimental inflammatory symptoms, including nociceptive behaviour. The unilateral injection of carrageenan into a hind paw to provoke inflammation was reported to enhance nociceptive sensitivity in the contralateral paw in rats. In order to provide balanced stimulation to the both sides, carrageenan should be injected bilaterally into the hind paws. In the present study we analysed the effects of systemic administration of meloxicam, a non-steroidal anti-inflammatory drug, or morphine on paw withdrawal and threshold forces in response to tactile stimuli in rats with bilateral intraplantar injections of carrageenan into hind paws. For comparison, effects of these analgesic drugs were also analysed in rats with a bilateral sciatic nerve ligation to produce neuropathic pain. In both carrageenan-treated and sciatic nerve-ligated rats, the paw withdrawal responses and threshold forces in response to tactile stimuli were increased and decreased, respectively. These nociceptive behaviours of carrageenan-treated rats were each inhibited by meloxicam and morphine. Morphine, but not meloxicam, inhibited the behavioural changes in sciatic nerve-ligated rats. The inhibitory effect of morphine in carrageenan-treated rats was much larger than that in nerve-ligated rats. These results suggest that rats with bilateral intraplantar injection of carrageenan into hind paws can be considered an animal model of inflammatory pain.

Pain-induced potentiation at synapses between pain-activated neurons in the parabrachial nucleus and central amygdala of FosTRAP mice

マウス外側腕傍核ー扁桃体中心核痛み活性化ニューロン間シナプス伝達の痛み誘発増強

○内山 瑳和子[1,2]、奥田 崇雄[2]、高橋 由香里[2]、津田 誠[1]、加藤 総夫[2]

[1]九州大・院薬・薬理学分野、[2]東京慈恵会医科大・医・神経科学研究部

The lateral parabrachial nucleus (LPB) collects nociceptive information from the spinal cord and trigeminal sensory system and sends it to the central nucleus of the amygdala (CeA). This LPB-CeA pathway undergoes extensive synaptic plasticity in various rodent models of persistent pain and plays an essential role in "nociplastic" expression of widespread sensitization (Miyazawa et al, 2018; Sugimoto et al, 2021). However, as this pathway also transmits signals other than nociception, it remains elusive whether the plastic changes at LPB-CeA synapses are specific to pain-associated neuron pairs. We selectively activated synaptic transmission between pain-associated pairs of LPB and CeA neurons using the FosTRAP technique, in which channelrhodopsin-2 (ChR2) and td-Tomato were expressed in LPB neurons and CeA neurons, respectively, following persistent inflammatory pain to activate c-Fos expression in these regions. In brain slices from the FosTRAP mice, we recorded light-evoked excitatory postsynaptic currents (leEPSCs) from "FosTRAPed" CeA neurons and found 1) leEPSC amplitude was larger specifically in these pairs and 2) a second-shot stimulation given 4-8 weeks after the first one resulted in larger leEPSC amplitude in these pairs, which was, unexpectedly, observed in animals also with non-inflammatory brief stimulation. These results suggest that the LPB-CeA synaptic potentiation occurs specifically between pain-associated pairs, which would be primed for plastic changes in response to future aversive events of various types.

Role of spinal cord-projecting cortical neurons in Aβ fiber-derived neuropathic allodynia in mice

神経障害性アロディニアにおける大脳皮質－脊髄後角神経路の役割

○藤森 一樹、津田 誠

九州大・院薬・薬理学分野

Mechanical allodynia, pain caused by innocuous mechanical stimulation, is a characteristic symptom of neuropathic pain that develops after peripheral nerve injury (PNI). In this study, we used Thy1-ChR2 mice, a transgenic mouse line that expresses channelrhodopsin-2 in touch-sensing myelinated primary afferent fibers but not in nociceptors. Light illumination to the plantar skin of Thy1-ChR2 mice with PNI produced pain-like withdrawal behavior and increased c-FOS expression in superficial spinal dorsal horn (SDH) neurons. This light-induced pain-like behavior disappeared by silencing Aβ fibers, but was not suppressed by morphine administration. Furthermore, using pathway-selective gene expression by adeno-associated viral vectors, we demonstrated that chemogenetic silencing of primary sensory cortex (S1) neurons projecting to the SDH attenuated pain-like behavior and reduced the number of c-FOS-expressing SDH neurons evoked by photostimulation of Aβ fibers. These findings indicate that spinally projecting S1 neurons contribute to Aβ fiber-derived neuropathic allodynia.

Augmented mechanical response of spinal dorsal horn neurons in a rat model of fibromyalgia.

線維筋痛症モデルラットにおける脊髄後角ニューロンの機械的刺激に対する反応の増強。

○歌 大介[1]、坪島 功幸[2]、西条 寿[2]、水村 和枝[3]、田口 徹[4]

[1]富山大・院医薬・応用薬理、[2]富山大・院医薬・システム情動、[3]日本大・歯・生理学、[4]新潟医療福祉大・リハビリテーション学部

Purpose: Fibromyalgia (FM) is characterized by chronic widespread pain with mechanical allodynia and hyperalgesia. However, the neural mechanisms of nociception/pain are poorly understood. The aim of this study was to examine the responsiveness of superficial dorsal horn (SDH) neurons using a rat model of FM.
Methods: Reserpine, a depleter of biogenic amines in the nervous system, was subcutaneously injected to make the mode. Extracellular recording of the SDH neurons *in vivo* was performed at the spinal segments L4/L5 under urethane anesthesia. Mechanical stimulation with a series of calibrated von Frey filaments was applied for 10 seconds to an identified receptive field of the SDH neurons.
Results: The SDH neurons showed mechanical stimulus intensity-dependent increases in the discharge rate both in the control and the reserpine-injected group. However, the magnitude of the mechanical response was significantly greater in the reserpine-injected group. Some SDH neurons in the reserpinized rats exhibited background discharges of low frequencies, although those in the control rats did not.
Conclusion: These results suggest that facilitated mechanical sensitivity of the SDH neurons is involved in mechanical allodynia and hyperalgesia in a rat model of reserpine-induced pain. Similar spinal mechanisms may underlie in FM patients.

Effects of calcium channel blockers on stiffness of the aortic and femoral arterial segments in anesthetized rabbits: comparison of the actions of nifedipine and cilnidipine

カルシウム拮抗薬が大動脈と大腿動脈の血管弾性に与える影響－ニフェジピンとシルニジピンの作用比較－

○佐藤 啓[1,2]、鈴木 保菜実[1]、佐久間 清[1,2]、千葉 達夫[1,3]、相本 恵美[1]、永澤 悦伸[1]、高原 章[1]

[1]東邦大・薬・薬物治療、[2]東邦大医セ佐倉病院・薬剤部、[3]東邦大医セ大橋病院・薬剤部

Ca^{2+} channel blocker is one of the vasodilators with excellent efficacy in preserving organ blood flow, which may be partly affected by functional stiffness of the conduit arteries. In this study, we assessed effects of L-type Ca^{2+} channel blocker nifedipine and L/N-type Ca^{2+} channel blocker cilnidipine on stiffness of the aortic and femoral arterial segments (aortic β and femoral β, respectively) in anesthetized rabbits. The methodology to obtain aortic β and femoral β was essentially same as that for cardio-ankle vascular index with the VaSera devise for human. Antihypertensive dose of nifedipine (300 μg/kg, i.v.) increased the aortic β but hardly affected the femoral β. However, nifedipine decreased the femoral β in the presence of an α-adrenoceptor blocker doxazosin (1 mg/kg, i.v.). Antihypertensive dose of cilnidipine (30 μg/kg, i.v.) increased the aortic β but decreased the femoral β. These results suggest that the increment of the aortic β reflecting the stiffening of the aortic segment and the decrement of the femoral β reflecting the softening of the femoral arterial segment are common effects of Ca^{2+} channel blockers on the conduit arteries. In addition, the stiffness of femoral arterial segment is considered to be modified by vasoconstriction associated with sympathetic reflex.

Possible pathophysiological importance of C-terminal tyrosine phosphorylation of $Ca_V1.2$ in vessel remodeling

動脈硬化における$Ca_V1.2$のC末端チロシンリン酸化の重要性の検討

○冨田 拓郎[1]、川岸 裕幸[1]、中田 勉[2]、山田 充彦[1]

[1]信州大、[2]信州大・基盤研究支援センター・機器分析部門

Voltage-dependent $Ca_V1.2$ L-type calcium channels play a critical role in the regulation blood pressure. Besides, $Ca_V1.2$ is implicated to play a pivotal role in chronic vessel remodeling such as atherosclerosis because several clinical trials indicate that $Ca_V1.2$ inhibitors delay the progression of atherosclerosis. However, how $Ca_V1.2$ participates in atherogenesis still remains obscure. Vascular smooth muscle cells (VSMC) are critically involved in atherogenesis. Medial VSMCs migrate and proliferate into the intima and form a part of plaque upon endothelial injury. Recently, we have identified that two tyrosine residues in the C-terminus (Tyr1709 and Tyr1758) of VSMC $Ca_V1.2$ are phosphorylated by Src-family kinases in response to PDGF. *In vitro* studies demonstrated that PDGF enhances $Ca_V1.2$ channel activity through this phosphorylation, thereby induceing VSMC migration. Thus, $Ca_V1.2$ may contribute to vessel remodeling in atherosclerosis. In order to verify this hypothesis, we established a knock-in mice line harboring a mutation at one of the tyrosine residues (Y1709F by using the CRISPR/Cas9 system. Although these mice exhibited no obvious developmental defects, we are now analyzing the effect of carotid artery ligation, a model of intimal thickening in these mice.

Differential effects of Na^+ channel blockers on the conduction velocity and the effective refractory period in the guinea pig left atrium and pulmonary vein myocardium

モルモット摘出左心房－肺静脈連結標本における伝導速度・有効不応期に対するNa^+チャネル遮断薬の影響

○濵口 正悟、土屋 真優、冨山 陽、行方 衣由紀、田中 光

東邦大・薬・薬物

We compared the effects of Na^+ channel blockers on the electrophysiological properties in the isolated pulmonary vein-left atrium connected preparation from guinea pig. Four concentric electrodes were attached to the pulmonary vein and left atrium to measure the intra-pulmonary vein and intra-atrial conduction velocity and the effective refractory period. Pilsicainide (10 μM), a Na^+ channel blocker, decreased the conduction velocity and prolonged the effective refractory period in both regions. GS-458967 (1 μM) and eleclazine (10 μM), known as Na^+ channel blockers at open and inactivated states, prolonged the effective refractory period in the pulmonary vein but not in the left atrium without affecting the conduction velocity in both regions. The wavelength, calculated by the conduction velocity × the effective refractory period, in the pulmonary vein was prolonged by GS-458967 and eleclazine but shortened by pilsicainide, while these drugs did not prolong that in the left atrium. These results suggest that certain types of Na^+ channel blockers, such as GS-458967 and eleclazine, appear to be effective therapeutic agents for atrial fibrillation in which the reentrant excitation in the pulmonary vein is important.

Roles of vascular smooth muscle NCX1/2 in the development of pulmonary arterial hypertension

肺高血圧発症における血管平滑筋NCX1/2の役割

○根本 隆行[1]、小松 知広[1,2]、喜多 知[1]、田頭 秀章[1]、上原 吉就[2]、喜多 紗斗美[3]、岩本 隆宏[1]

[1]福岡大・医・薬理学、[2]福岡大・スポーツ科学、[3]徳島文理大・薬・薬理学

Pulmonary arterial hypertension (PAH) is a severe and progressive disease that causes right heart failure. Recent studies suggested that the hypercontraction and excessive proliferation of the pulmonary artery induced by Ca^{2+} signaling abnormality may be involved in the pathogenesis of PAH, though their molecular mechanisms remain unclear. The Na^{+}/Ca^{2+} exchanger (NCX) is a bidirectional transporter that is controlled by membrane potential and transmembrane gradients of Na^{+} and Ca^{2+}. Vascular smooth muscle (VSM) NCX plays an important role in intracellular Ca^{2+} homeostasis and Ca^{2+} signaling. In this study, we investigated the pathophysiological roles of NCX1/2 in hypoxia-induced PAH, using VSM-specific NCX1/2 knockout mice and NCX1/2 inhibitors. VSM-specific NCX1-knockout mice exhibited attenuation of hypoxia-induced PAH and right ventricular hypertrophy compared with wild-type mice. In addition, administration of NCX1 inhibitors suppressed hypoxia-induced PAH and pulmonary vessel muscularization. These findings indicate that genetic knockout and pharmacologic inhibition of NCX1 attenuate the development of hypoxia-induced PAH. Furthermore, our next studies with VSM-specific NCX2-knockout mice will provide new evidence that NCX2 differentially contributes to the development of hypoxia-induced PAH.

The alteration of contractile reactivity in isolated mesenteric arteries from Zucker fatty diabetes mellitus rats

Zucker fatty diabetes mellitusラット摘出腸間膜動脈の収縮反応の変化

船田 拓志、○大谷 紘資、兒玉 朋子、岡田 宗善、山脇 英之

北里大・獣医・獣医薬理

Obesity and type 2 diabetes (T2D) are major risk factors for cardiovascular diseases. Zucker fatty diabetes mellitus (ZFDM)-*Lepr*$^{fa/fa}$ (Homo) rats develop obesity and T2D at a young age, while ZFDM-*Lepr*$^{fa/+}$ (Hetero) rats are normal. We recently reported that blood pressure in Homo is normal until 35 weeks of age, while blood adrenaline level and sympathetic nervous activity are lower than Hetero. In the present study, we tested the hypothesis that contractile reactivity to adrenaline in peripheral blood vessels from Homo increases. After the isolated mesenteric arteries were cumulatively administrated with adrenaline (1 nM-30 μM) in the presence or absence of propranolol (1 μM), isometric tension was measured by a Magnus method. Body weight and blood glucose levels in Homo were significantly higher than Hetero, while heart rate was significantly lower. The adrenaline-induced contraction in the isolated mesenteric artery from Homo was significantly higher than Hetero. In Hetero, pretreatment with propranolol enhanced the adrenaline-induced contraction, while it had no effect in Homo. These results suggest that the adrenaline-induced contraction in the isolated mesenteric artery from Homo may be increased due to the altered expression of adrenaline receptors. In addition, the increased adrenaline responsiveness of peripheral arteries in Homo would maintain the blood pressure normal despite the decrease of sympathetic nerve activity.

A study on a pathophysiological role of a macromolecular complex of the cardiac KCNQ1 channel

心筋KCNQ1チャネル分子複合体の病態生理学的役割に関する研究

○黒川 洵子[1]、服部 希海[1]、野間口 財[1]、杉本 早穂[1]、岩鶴 果奈[1]、児玉 昌美[2]、渡邉 泰秀[1]、清水 聡史[1,3]、永森 收志[3]、坂本 多穂[1]

[1]静岡県立大・薬・生体情報分子解析学、[2]順天堂大・医・薬理学教室、[3]東京慈恵会医科大・医・臨床検査医学

The I_{Ks} channels, which contribute to the repolarization phase of the cardiac action potential, are composed of the alpha subunit KCNQ1 and beta subunit KCNE1. Mutations in these genes are associated with the development of lethal arrhythmias due to congenital QT prolongation syndrome and are influenced by sympathetic nerve stimulation and sex hormones. As a molecular mechanism, we have demonstrated the involvement of a molecular complex of the KCNQ1 channel in the I_{Ks} regulation by intracellular Ca^{2+}, cAMP, and NO. Recently, membrane proteomics has shown an association between the KCNQ1 molecular complex and Ca^{2+} signaling, but the pathophysiological role of this association has not been elucidated. Therefore, we aimed to test whether I_{Ks} channels activated by pathological Ca^{2+} overload may compensate for arrhythmias using genetically engineered (I_{Ks}-Tg) mice expressing cardiac human I_{Ks} channels. We employed a sepsis model for pathological Ca^{2+} overload condition. We found that the sepsis score I_{Ks}-Tg mice was significantly lower than that in wild-type mice, suggesting a protective role of the I_{Ks} channel on cardiac pathological modification by sepsis.

SARAF and ALG-2 are degraded under the ER stress condition in heart failure

心不全病態のERストレス環境下で、SARAFとALG-2は分解されている

○横江 俊一、朝日 通雄

大阪医科薬科大・医・薬理

SARAF is an ER membrane protein, that inactivates store operated calcium entry (SOCE). ALG-2 regulates SARAF expression by modulating E3 ligases that are known to target SARAF. Although SARAF and ALG-2 are expressed in the heart, their pathophysiological role remains unknown. Here, we found that SARAF protein expression level was significantly decreased due to its polyubiquitination in cardiomyocytes excised from dilated cardiomyopathy (DCM) mice compared to control mice. The ALG-2 expression level was also significantly decreased in cardiomyocytes excised from DCM mice compared to control mice. We next observed that the expression level of both SARAF and ALG-2 are significantly decreased in ER stress-exposed HEK293 cells. Furthermore, we observed that knockdown of *alg-2* decreased SARAF expression level in HEK293 cells. These data indicate that SARAF is degraded via ALG-2-mediated polyubiquitination under the ER stress condition in failing hearts. Further work is necessary to determine whether SARAF and/or ALG-2 can regulate cardiac function in DCM especially through in ER stress conditions.

YAP promotes aerobic glycolysis by upregulating GLUT1 in cardiomyocytes in response to acute pressure overload

急性圧負荷刺激により活性化したYAPはGLUT1の発現増加を介して心筋細胞の解糖系代謝を亢進する

○柏原 俊英[1]、中原 努[1]、佐渡島 純一[2]

[1]北里大・薬・分子薬理、[2]Rutgers New Jersey Med. Sch., Cardiovasc. Res. Inst., Dept. of Cell Biol. and Mol. Med.

Yes-associated protein 1 (YAP), a major transcriptional cofactor in the Hippo signaling pathway, is known to regulate cell growth and homeostasis. We have shown previously that YAP is activated and mediates adaptive cardiac hypertrophy in response to acute pressure overload (PO). However, how YAP induces adaptive hypertrophy is unknown. Glycolysis is intimately involved in cell growth, including cardiac hypertrophy. Here we examined whether YAP regulates glycolysis during acute PO to promote adaptive cardiac hypertrophy. Evaluation of extracellular acidification rate using the Seahorse XF analyzer in freshly isolated adult ventricular myocytes (AVMs) from wild-type mice revealed that glycolytic flux was increased by acute PO. The PO-induced glycolysis was attenuated in isolated AVMs from cardiac-specific heterozygous YAP knockout mice. We found that YAP promoted glycolysis by upregulating glucose transporter 1 (GLUT1), which in turn caused accumulation of glucose metabolites, including L-serine, L-aspartate, and malate, during acute PO. YAP overexpression increased GLUT1 protein levels, glycolytic flux, and cardiomyocytes size in isolated AVMs. These results suggest that YAP induces adaptive cardiac hypertrophy through activation of aerobic glycolysis in response to acute PO.

TNF-α induces human aortic valve interstitial cell calcification by inhibiting CD34 gene expression

石灰化大動脈弁狭窄症患者より得た大動脈弁間質細胞においてTNF−αはCD34発現低下により異所性石灰化を誘発する

○于 在強[1]、大徳 和之[1]、皆川 正仁[1]、今泉 忠淳[2]、元村 成[3]、古川 賢一[4]、瀬谷 和彦[2]

[1]弘前大・院医・胸部心臓血管外科学、[2]弘前大・院医・脳血管病態学、[3]弘前大・院医・病態薬理学、[4]弘前大・院医・整形外科学

Aortic valve stenosis (AVS) occurs frequently in the elderly, which is exacerbated by valve ectopic calcification. Recently, we found that mesenchymal undifferentiated cells were rich in human aortic valve interstitial cells (HAVICs), and hematopoietic stem cell marker CD34-negative cells were higher sensitive on various calcification stimulations such as tumor necrosis factor (TNF)-α. These cells were also vascular endothelial growth factor receptor 2-positive. We aimed to investigate whether TNF-α affects the gene expression of CD34 and calcification-related proteins in HAVICs, isolated from the calcified aortic valves of AVS patients. After 8h culture of HAVICs with TNF-α (30 ng/mL), the gene expressions of bone morphogenetic protein (BMP) 2 and distal-less homeobox 5 were accelerated. In addition to CD34, gene expressions of extracellular matrix proteins tenascin X and matrix Gla protein were significantly decreased after 6 days culture of HAVICs with TNF-α, resulting in accelerated calcification. Transforming growth factor-β1, known another calcification inducing factor, did not change these gene expressions. These results suggest that TNF-α-BMP2 signaling mainly contributes to valve ectopic calcification by inhibiting CD34 expression.

Relevance of characterizing calcitonin gene-related peptide knockout mice in the Parkinson‘s disease model

パーキンソン病モデルにおけるカルシトニン遺伝子関連ペプチドノックアウトマウスの特徴づけの関連性

○米山 佳和[1]、孫 熙文[2]、荒 智大[2]、橋川 直也[1,2]、橋川 成美[1,2]

[1]岡山理科大・理学研究科、[2]岡山理科大・理

Depressive disorders occur in 40%–50% of patients with Parkinson‘s disease. Calcitonin gene-related peptide (CGRP) is a neuropeptide known as a pain transmitter. Our previous study suggested that intracerebroventricular administration of CGRP improves depressive-like behaviors. Here, we presented evidence that CGRP-deficient knockout (CGRP KO) mice exhibit Parkinson‘s disease symptoms with depression-like behaviors. We also investigated whether intranasal administration of CGRP affects the amelioration of Parkinson‘s disease-like symptoms in CGRP KO mice. Saline or CGRP (0.1 mM) was intranasally administered to 10-week-old CGRP KO mice for 2 weeks. We performed several behavioral paradigm tests to evaluate the mice‘s motor and cognitive functions: open field, catalepsy, pole, rotarod, hind-limb, and adhesive removal. Motor dysfunction was observed in the CGRP KO mice in all behavioral tests compared with the wild-type control C57BL6/J mice. Intranasal administration of CGRP ameliorated motor function in the rotarod test and tended to improve it in the catalepsy test. This administration also increased the level of tyrosine hydroxylase in the substantia nigra in the CGRP KO mice. This study‘s results suggest that CGRP replacement is a novel therapeutic approach for Parkinson‘s disease.

The impacts on fetal brain by maternal hypoxic stress

妊娠時低酸素曝露が胎仔脳に与える影響

○徳留 健太郎[1]、植木 正明[1,2]、中村 敦輝[1]、本間 拓二郎[1]、松永 慎司[1]、冨田 修平[1]

[1]大阪公立大・院医・分子病態薬理、[2]西脇病院・麻酔科

Maternal hypoxic stress such as threatened abortion at early pregnancy is thought to be a risk factor for onset of children's neurodevelopmental disorder. In fact, we previously demonstrated that maternal stress by rearing at hypoxic condition caused communication deficit and learning disability of rat pups. To clarify the onset mechanism of neurodevelopmental disorder-like phenotype, we conducted immunohistochemical studies by using rat fetal brain. Firstly, TUNEL stain revealed that maternal hypoxic stress didn't induce cell death. Then, immunofluorescent double staining for analyzing proliferation and differentiation of neural stem cells (NSCs) were conducted. NSCs proliferation ability analysis revealed that the rate of $Ki67^{+}SOX2^{+}$ cells were significantly increased in fetal rat brain received maternal hypoxic stress. In addition, analysis of asymmetric division which means initial step of neurogenesis, by using SOX2 and TBR2 (intermediate progenitor cell marker) double staining, showed that maternal hypoxia induced the increase of the number of NSCs. Furthermore, qPCR results showed that maternal hypoxia reduced astrogliogenesis-related genes and excitatory neurogenesis-related gene expression. This study indicated that maternal hypoxia influenced the function of NSCs and their destination.

Evaluation of Autosomal Dominant Nocturnal Frontal Lobe Epilepsy Symptoms in S284L Mutant Transgenic Rats

S284L変異トランスジェニックラットの常染色体優性夜間前頭葉てんかん様症状の評価

○パブラック 晶子[1]、佐伯 健輔[1]、笠井 重幸[2]、村上 惇[2]、小栗 均[2]、村澤 寛泰[1]、小林 洋之[1]、伊藤 貴博[1]、加藤 正巳[1]、平澤 康史[1]、長瀬 孝彦[1]

[1]日本バイオリサーチセンター、[2]オリエンタルバイオサービス

Drug-induced and electroshock-induced animal models have been used in the development of antiepileptic drugs, but these models are seizure models, not epilepsy models. For the development of antiepileptic drugs, it is important to develop epilepsy models that mimic the mechanism of seizure generation in humans. Mutations in the gene encoding the nicotinic acetyl receptor (nAChR) causes autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) in humans. In the present study, we evaluated the epileptic phenotype of CHRNA4 S284L mutant transgenic rats by measuring EEG and EMG, following the report of Zhu et al. We also evaluated the antiepileptic effects of carbamazepine and zonisamide. In addition, cognitive function, anxiety symptoms, and circadian rhythm were evaluated to assess the animals' psychiatric symptoms. As a result, we confirmed ADNFLE seizures with symptoms of "paroxysmal arousal," "paroxysmal dystonia," and "wandering" in TG rats. We also confirmed interictal discharges and confirmed that zonisamide was more effective than carbamazepine for these abnormal discharges. On the other hand, no cognitive dysfunction, anxiety symptoms, or circadian rhythm abnormalities were observed in TG rats. Although these TG rats did not show abnormal psychiatric symptoms in this study, they exhibited symptoms similar to those of human epilepsy and were responsive to drugs, suggesting that they may assist in the development of antiepileptic drugs.

Kaufman oculocerebrofacial syndrome gene, *Ube3b*, is crucial for the maintenance of synapse numbers in the young adult brain

カウフマン症候群原因遺伝子である*Ube3b*は若年成人期のシナプス数維持に重要である

○勝部 早紀[1]、小金澤 紀子[1,2]、花村 健次[1,2]、Cuthill Katherine J.[3]、Ambrozkiewicz Mateusz C.[3]、川辺 浩志[1,2]

[1]群馬大・医・薬理、[2]群馬大・院医・薬理、[3]ベルリン・シャリテ医科大学　細胞生物学神経科学研究所

A large number of patients are suffering from developmental disabilities, including Kaufman oculocerebrofacial syndrome (KOS). KOS is a severe autosomal recessive disorder characterized by general developmental delay with intellectual disability, hypocholesterolemia, and seizures. *Ube3b*, an E3 ligase gene, has been reported as the causative gene for KOS. Our previous report demonstrated a loss of murine *Ube3b* increases the number of dendritic spines at the age of three weeks, indicating that *Ube3b* is a negative regulator of synaptogenesis for the excitatory synapses in the early developmental stage. In this study, using brain-specific conditional *Ube3b* knockout (*Ube3b* bKO) mice, we investigated the further role of *Ube3b* in the young adult period. Mouse brain sections were prepared from 9-10 weeks old mice and immunostained with antibodies to Bassoon and Homer1, respective markers for presynapse and excitatory postsynapse. Images from hippocampal CA1 region were acquired with 3D Stimulated Emission Depletion (3D-STED) microscopy to estimate the excitatory synapse numbers. We found that the excitatory synapse density significantly decreased in *Ube3b* bKO as compared to the control. Together with our previous reports, our results indicate the novel role of *Ube3b* in the maintenance of synapse numbers in the young adult period.

Short-term intranasal rotenone-administrated mice exhibit multiple prodromal symptoms in Parkinson‘s disease

パーキンソン病の前駆症状を示す短期間ロテノン鼻腔内投与マウス

○佐藤 元[1]、豊田 博紀[2]、野崎 一徳[3]、佐藤 慶太郎[1]、片桐 綾乃[2]、安達 一典[1]、加藤 隆史[2]

[1]明海大・歯・薬理、[2]大阪大・院歯・口腔生理、[3]大阪大・歯・医療情報

Parkinson‘s disease (PD) causes multiple non-motor symptoms as well as distinctive motor deficits. It is well known that chemosensory and mild cognitive impairments frequently occur in the early stage of PD, but the mechanisms underlying these PD-related prodromal impairments remain unknown. We examined whether intranasal rotenone-administrated male C57BL/6J mouse was a suitable model for elucidation of PD-related prodromal/non-motor symptoms. The 1-week rotenone treatment induced chemosensory and conditioned taste aversion memory impairments without locomotor impairments. Subsequent catecholamine (CA) neuron and fiber loss was observed in the first central nervous system for chemosensory information relays (the olfactory bulb and nucleus of solitary tract) and the critical brain region of taste associative memory (the dysgranular insular cortex) in 1-week rotenone-treated mice, respectively. Despite no apparent changes in the number of CA neuron in the substantia nigra and the ventral tegmental area of 1-week rotenone-treated mice, those of 4-week rotenone-treated mice were significantly reduced (> 70%) at the fifth week. These results suggest that this model employing short-term intranasal rotenone administration will be useful for the elucidating the prodromal stage of PD pathophysiology.

Exposure to social defeat stress as juveniles leads to activated brain immune systems and impaired neuronal morphology

幼若期社会的敗北ストレス負荷が脳内免疫系および神経形態に与える影響

○吉田 樹生[1]、鈴木 千晴[2]、濱田 眞里亜[2]、片田 ひかり[2]、肥田 裕丈[2]、毛利 彰宏[3]、吉見 陽[2,4]、尾崎 紀夫[4]、野田 幸裕[1,2,4]

[1]名城大・院薬・病態解析学Ⅰ、[2]名城大・薬・病態解析学Ⅰ、[3]藤田医科大・院保健・レギュラトリーサイエンス分野、[4]名古屋大・院医・精神医学

Exposure to psychosocial stress (e.g., bullying) in juveniles is a risk factor of stress-related psychiatric disorders later in life. The exposure to stress activates microglia, which plays an important role in brain immunity, and induces neuroinflammation. It is unclear to what degree the exposure to psychosocial stress as juveniles is affected to brain immunity systems and neuronal morphology. The present study was examined expression of inflammatory cytokines or inflammatory signal-related molecules and neuronal morphology in the prefrontal cortex (PFC) by using the mice exposed to social defeat stress as juveniles. We found that inflammation or immune system-related genes of defeated mice were significantly changed, compared to those of non-defeated mice in transcriptome analysis. Especially, the high expressions of Ca2+ binding protein, S100A8, and S100A9 genes were observed in the PFC of defeated mice. The levels of TNF-α and the numbers of spines in defeated mice were significantly increased and decreased, respectively, compared to that in non-defeated mice. There were no significant changes of TNFR1, NF-κB, or I-κB levels in defeated mice. Administration of R-7050, a TNF-α receptor antagonist, didn't develop the impairment of social behaviors induced by social defeat stress exposure as juveniles. Our findings suggest that exposure to social defeat stress as juveniles induces TNF-α mediated neuroinflammation via the activated microglia.

Mass spectrometric analysis of Parkinson's disease brain by Py-Tag derivative reagent.

Py-Tag 誘導体化試薬によるパーキンソンモデルラット脳内の解析

○鹿野 仁美[1]、池田 明夏里[2]、寺内 勉[2]、横山 順[2]、平 修[1]

[1]福島大学・農学群・食農学類、[2]太陽日酸株式会社・SI事業部

To demonstrate the accurate analysis of catecholamines and amino acid using derivatization reagents, we investigated the reaction conditions for 2,4,6-triethyl-3,5-dimethyl pyrylium trifluoromethanesulfonate (Py-Tag), derivatization of the targets dopamine (DA) and γ-aminobutyric acid (GABA) on tissue sections, and constructed an optimized reaction compartment. Ten different Py-Tag reaction conditions with the targets were considered. The optimal condition for the Py-Tag reaction with the targets was identified as a 70% methanol with 5% trimethylamine solution at 60 ℃ under homogenous conditions. To reproduce this reaction on tissue sections, we constructed a reaction compartment to maintain humidity levels and facilitate the derivatization reaction. Moreover, visualization of DA and GABA was archived by derivatized-imaging mass spectrometry. Brain sections of unilateral Parkinson's disease (PD) model rats showed Py-Tag DA in the unilateral striatum and Py-Tag GABA in the cerebral cortex, striatum, hippocampus and hypothalamus. Using the PD model rat brain, images with left-right differences were obtained for the localization of DA and GABA. These findings indicate, it is important to consider the reaction conditions that allow high reaction efficiency between DA or GABA and Py-Tag as well as high quality imaging of sections.

Sympathetic reactivities by restraint stress change during peri-pubertal stages in rats

ラットにおける拘束ストレス負荷による交感神経反応は思春期前−思春期間で変化する

○上田 昌史、山口 奈緒子、岡田 尚志郎

愛知医科大・医・薬理学講座

There are differences in stress reactivity between pre-puberty and adult rats. Previous studies showed that significant shifts in the reactivity of hypothalamic-pituitary-adrenal (HPA) axis during the pubertal stages of development. Although sympathetic nervous system (SNS) is activated by stressor including restraint stress (RS) in adult rats, it is not well understood whether RS induces the SNS activations in peri-pubertal stages. The aim of this study is to investigate the age-dependent changes in sympathetic reactivities, as stress response by a single and repeated RS, in peri-pubertal male rats.

Male Wistar rats at 25-28 postnatal days of age (PND) (pre-puberty) and PND 39-42 (mid-puberty), and PND 84-87 (adult) were used in this study. Rats were exposed to a single RS (1RS) or repeated RS for 4 consecutive days (4RS). Blood samples were collected immediately after termination of RS. Plasma catecholamines were measured by HPLC with electrochemical detection to evaluate the SNS activations.

As results, the levels of plasma catecholamines were significantly increased by 1RS in pre-pubertal rats compared to mid-pubertal and adult rats. In contrast, 4RS did not induce the increasing of the levels of plasma catecholamines in pre-pubertal rats. These results indicate that the SNS in pre-puberty is more severely activated by a single RS than that in mid-puberty and adult.

The analysis of synapse numbers in an autism spectrum disorder (ASD) model mouse with 3D-STED microscopy

3D−STED顕微鏡による自閉症スペクトラム症モデルマウスの興奮性シナプス数の解析

○中村 友哉[1]、花村 健次[1,2]、堀 啓[3]、星野 幹雄[3]、川辺 浩史[1,2]

[1]群馬大・医・薬理、[2]群馬大・院医・薬理、[3]国立精神・神経医療研究セ・神経研・病態生化学研

Autism spectrum disorders (ASD) is a developmental disorder with an estimated prevalence of ˷1.6%. Growing financial and caregiving burdens for patients‘ families are getting social problems. Diagnostic criteria of ASD include deficits in social communications and interactions, restricted interests, and repetitive behaviors. Based on studies of monozygotic and dizygotic twins, it has been proposed that a part of ASD is a genetic disorder. Indeed, many causative genes of ASD, such as Autism susceptibility candidate 2 (AUTS2) have been identified. It has been reported that the murine orthologue of AUTS2, Auts2 activates a small GTPase Rac1, and thus regulates neuronal migration by forming lamellipodia at the growth cone. Given that lamellipodia play crucial roles in synapse formation as well, we estimated synapse numbers in Auts2 mutant mouse in this study. Excitatory synapses were immunolabeled by antibodies to Bassoon and Homer1, markers for pre-synapse and excitatory post-synapse respectively. Synapses at hippocampal CA1 region were imaged with super-resolved three-dimensional stimulated emission depletion (3D-STED) microscopy. As a result, we discovered the change of the number of excitatory synapses in Auts2 mutant mouse. Our finding sheds light on unidentified pathological changes in ASD at the synapse level.

Investigating the effects of ultrasound exposure on emotion using animal models

動物モデルを用いた超音波曝露の情動への影響の検討

○山内 つぐみ[1,2]、吉岡 寿倫[1]、山田 大輔[1]、濱野 匠[1]、公木 彩夏[1]、入山 聖史[3]、吉澤 一巳[4]、市川 寛子[2]、西野 彰一[5]、宮崎 智[6]、斎藤 顕宜[1]

[1]東京理科大・薬・薬学科、[2]東京理科大・教養教育研究院・心理学研究室、[3]東京理科大・理工・量子情報力学研究室、[4]東京理科大・薬・疾患薬理学研究室、[5]株式会社フジミック、[6]東京理科大・薬・生命情報科学研究室

Recently, ultrasound exposure has been shown to be a noninvasive method for modulating brain activity and has to be applied to treat mental disorders, such as depression. However, its underlying mechanism remains unclear. Therefore, there is a need for animal models that can investigate the mechanism of ultrasound exposure. In this study, we utilized olfactory bulbectomized (OB) rats as an animal model of depression and investigated their emotional state following ultrasound exposure. As a result, following exposure to high-frequency ultrasonic vocalizations (USV)s of rats, the hyperemotionality of OB rats was significantly suppressed. Also, USV exposure significantly reduced the latency to the first entry into the open arm in the elevated plus maze tests and the plasma corticosterone levels of OB rats. Furthermore, artificial ultrasounds (50 and 100 kHz) also significantly decreased the hyperemotionality score of OB rats. These results suggested that ultrasound exposure, regardless of vocal or artificial sound, ameliorated depressive-like behavior and stress level in OB rats. We proposed that OB rats might be an appropriate animal model to identify mechanisms underlying the effects of ultrasound exposure.

The effects of DOP agonists on the extinction and reconsolidation of fear memory in mice.

恐怖記憶の消去および再固定化に対するオピオイドδ受容体作動薬の作用

○河南 絢子[1]、山田 大輔[1]、柳澤 祥子[1]、白方 基揮[1]、畠山 梓摘[1]、飯尾 啓太[2]、長瀬 博[2]、斎藤 顕宜[1]

[1]東京理科大・薬・薬理学研究室、[2]筑波大・国際統合睡眠医科学研究機構

Facilitation of fear extinction and inhibition of reconsolidation of fear memory are useful for the treatment of fear-related disorders, such as post-traumatic stress disorder (PTSD). Here, we investigated the effects of two DOP agonists, KNT-127 and SNC80, with different chemical structures on the fear extinction and reconsolidation of fear memory in mice. On day 1, male C57BL/6J mice were contextually conditioned with 8 footshocks. On day 2, the mice were re-exposed to the conditioning chamber as a memory retrieval session (re-exposure 1). DOP agonists were administered subcutaneously 30 min before (for extinction) or immediately after (for reconsolidation) the re-exposure 1. On day 3, mice were re-exposed to the chamber as a memory testing session (re-exposure 2). The duration of re-exposure was 6 min for extinction and was 2 min for reconsolidation. As a result, KNT-127 (3–10 mg/kg), but not SNC80 (1–10 mg/kg), administered to the mice following both 6-min and 2-min re-exposure 1 significantly decreased the freezing rates of mice during re-exposure 2. These effects of KNT-127 were abolished by pretreatment with a selective DOP antagonist. In conclusion, we propose that KNT-127 facilitates fear extinction and inhibits reconsolidation of fear memory via DOP.

Prolonged, but not single, administration of nandrolone inhibits morphine-induced increases in accumbal dopamine efflux in rats

Morphineが誘発したラットの側坐核のdopamine放出の促進作用はnandroloneの単回ではなく反復投与により抑制される

○川島 央暉[1]、青野 悠里[1]、榛葉 繁紀[2]、三枝 禎[1]

[1]日本大・松戸歯学部・薬理学、[2]日本大・薬・健康衛生

Nandrolone (NDL) is an anabolic androgenic steroid known to be misused by elite athletes as well as some adolescents due to its muscle-building properties. We have shown that a 4-week period of repeated administration of NDL to 6-, but not 10-week-old rats enhances the developmental increase in grip strength without influencing developmental increases in body weight. Furthermore, systemic administration of morphine induced a smaller increase in dopamine (DA) efflux in the nucleus accumbens (NAc) of rats that had received NDL treatment for 4 weeks relative to those without NDL treatment. Recently, a single administration of NDL to mice was shown to enhance DAergic neural firing in the ventral tegmental area (VTA: Bontempi & Bonci, 2020). The DAergic neurons in the VTA are known to send projections to the NAc. Accordingly, we further analyzed the effects of a single administration of NDL on baseline as well as morphine-induced increases in accumbal DA efflux using *in vivo* microdialysis. Male Sprague-Dawley rats approximately 10 weeks old were used. A single administration of NDL (5.0 mg/kg s.c., 24 hr before morphine treatment) did not alter either basal levels of accumbal DA or the increase in accumbal DA efflux induced by systemic administration of morphine (1.0 mg/kg, s.c.). These results, together with our previous findings, show that prolonged but not a single administration of NDL in rats can inhibit morphine-induced increases in accumbal DA efflux.

Labeling method for memory-related synapses

恐怖記憶形成に関与するシナプスの検出法の開発

○大西 泰地[1]、坂本 寛和[1]、大久保 洋平[2]、並木 繁行[1]、廣瀬 謙造[1]

[1]東京大・院医・細胞分子薬理学、[2]順天堂大・医・薬理

In the auditory cued fear conditioning, memories are stored in synapses between engram neurons, which increased activity during the memory formation. Although molecular imaging of memory-related synapses contributes to deciphering the molecular basis of memory, there is no established method for visualizing them. In this study, we developed a method to selectively visualize synapses between engram neurons, using the c-fos promoter-driven Tet-On system. Tag-fused synaptophysin (tagSyp) and FingR.PSD95 were expressed in presynaptic auditory cortex neurons and postsynaptic lateral amygdala neurons, respectively, in an activity-dependent manner. We found that the number of synapses positive for both tagSyp and FingR.PSD95 in the lateral amygdala was 15-fold higher in mice with cued fear conditioning than in control mice. Thus, we concluded that synapses both positive for tagSyp and FingR.PSD95 correspond to memory-related synapses. Furthermore, combining this method with immunohistochemistry, we found greater amounts of several kinds of synaptic molecules accumulate in memory-related synapses compared to other synapses. It is expected that our method enables comprehensive analysis of molecular changes in synapses induced by fear memory formation.

Changes in spontaneous firing frequency of striatal cholinergic interneurons in aged mice.

老齢マウスにおける線条体コリン作動性介在ニューロンの自発発火頻度の変化

○鈴木 江津子、籾山 俊彦

東京慈恵会医科大・医

It has been reported that frequency of action potential firing of striatal cholinergic interneurons increases with postnatal development. On the other hand, changes in spontaneous firing frequency and firing properties during aging have not been investigated. In this study, cell-attached and whole-cell patch-clamp studies were carried out to investigate changes in firing properties of striatal cholinergic interneurons during aging. Brain slices were prepared from 2–3-month-old, 11–12-month-old and 24-month-old mice of either sex. Frequencies of spontaneous firing at 2–3-month-old, 11–12-month-old and 24-month-old were 4.55 ± 1.01 Hz (n = 18), 8.73 ± 2.28 Hz (n = 8) and 2.88 ± 0.99 Hz (n = 11), respectively. Firing frequency at 14 month of age was significantly decreased than that of 11–12-month-old (p = 0.028). Since spontaneous firing appeared to be irregular in 24-month-old mice, the coefficient of variation (CV) of the inter-event interval of spontaneous firing was analyzed. CV at 24 months of age (0.68 ± 0.1, n = 10) was significantly larger than that of 2-3-month of age (0.34 ± 0.05, n = 18, P = 0.015), indicating that the regular pattern of spontaneous firing is disrupted in 24-month-old mice. These findings suggest prominent changes in firing properties of striatal cholinergic interneurons during aging.

Increased neuronal activity of serotonin neurons in the median raphe nucleus attenuates reward-related consummatory responses and facial expression

正中縫線核セロトニン神経活動の増加は舌応答や表情変化などの報酬関連応答を減弱させる

○大村 優[1]、ブシェキワ ユセフ[1,2]、河合 洋幸[3,4]、永安 一樹[3]、金子 周司[3]

[1]北海道大・院医、[2]City University of New York・Queens College・Dept. Psychology、[3]京都大・院医薬・生体機能解析、[4]大阪公立大・院医・脳神経機能形態学

We have previously demonstrated that serotonin neurons in the median raphe nucleus (MRN) facilitate anxiety- and fear-related behavior evaluated by elevated plus maze and fear conditioning tests, respectively. However, we still cannot conclude that the neuronal activity of serotonin neurons in the MRN directly causes negative emotion because the time gap between the experimental manipulations and the evaluation of resulting behavioral changes is relatively long in these behavioral tests. In other words, learning or planning processes could affect performance in these tests. To address this issue, we optogenetically stimulated serotonin neurons in the MRN and evaluated quick responses to sucrose reward in mice. We delivered a sucrose reward from a blunt-tipped needle placed at a licking distance and measured mice licking behavior. The optogenetic activation of serotonin neurons in the MRN reduced the number of licks. Furthermore, to assess hedonic responses more directly, we intraorally infused a drop of sucrose solution and analyzed taste reactivity and facial expression. We found that activating serotonin neurons in the MRN significantly reduced the number of tongue reactions and sweet sucrose-driven facial expressions. These results suggest that increased activity of serotonin neurons in the MRN mediates aversion.

Histamine neurons promote the recall of associative memories

ヒスタミン神経による連合記憶の想起促進

○髙村 侑希[1]、西村 京華[1]、人羅（今村） 菜津子[1,2]、南 雅文[1]、野村 洋[1,3]

[1]北海道大・薬・薬理学研究室、[2]熊本大・院生命科学、[3]名古屋市立大・院医・認知機能病態学寄附講座

In the brain, histamine is produced mainly in the tuberomammillary nucleus (TMN) and involved in learning/memory, sleep/wakefulness, feeding, and neuroendocrine regulation. Previously, we demonstrated that activation of the histaminergic neurotransmission by histamine H_3 receptor inverse agonists promotes the recall of forgotten memories. However, it remains unclear whether histamine neurons are activated during memory recall and whether activation of histamine neurons is required for memory recall. In this study, first, we used fiber photometry to measure the activity of histamine neurons. We introduced GCaMP6s into histamine neurons of HDC-Cre mice and acquired the fluorescence via optic fibers. The mice were subjected to auditory-reward conditioning. The activity of histamine neurons increased when the mice recalled the reward memory by the conditioning tone. Next, we used optogenetics to test whether the activation of histamine neurons is involved in memory recall. We introduced NpHR into histamine neurons of HDC-Cre mice and inhibited their activity. Inhibition of histamine neurons reduced tone-induced licking behavior that is based on recall of reward memory associated with auditory stimulus. These results suggest that histamine neurons contribute to promoting the recall of associative memories.

Effects of systemic inflammation and histamine on the network oscillation in the anterior cingulate cortex

前帯状回神経回路オシレーションに対する全身性炎症とヒスタミンの作用

○村越 隆之[1]、平尾 鮎美[1]、村上 元[2]、伊藤 吏那[1]、魚住 尚紀[1]

[1]埼玉医科大・医・生化、[2]埼玉医科大・医・教養教育

Since the arousal and cognition are considered to be maintained by the network oscillation generated by neural circuits in the anterior cingulate cortex (ACC), and are deeply influenced by histaminergic system and disturbed under the systemic inflammation, it is expected that elevated inflammatory cytokines would alter ACC network oscillations along with the behavioral changes. We studied the effects of lipopolysaccharide (LPS) on ACC-related task and kainic acid (KA) -induced network oscillations in an ACC slice, to search the relationship among LPS-induced elevation of cytokine level, the profiles of behavior and network oscillations.
<Materials & Methods> Male C57BL/6J mice were intraperitoneally administered with LPS (30 μg/kg). Three hours later, novel object recognition test (NOR) was performed, followed by preparations of blood and cortical tissue samples, and brain slices containing ACC for electrophysiology.
<Results> LPS treatment significantly increased serum IL-6 level and tended to increase brain tissue IL-6 level. Network oscillation was evoked by KA (0.3-3 μM) perfusion alone or with histamine (10 μM) as field potential activities within the ACC *in vitro*. Power of KA-induced oscillation increased in the LPS group compared with the control. Histamine enhanced oscillation power in the control group, but this was not observed in the LPS group. In the NOR, exploration time for a novel object decreased in the LPS group but without a significant difference in the recognition index between the two groups.

Region-specific plastic changes in the prefrontal cortex drive compulsive task performance

課題学習による前頭皮質内亜領域選択的なシナプス可塑的変化は強迫的な課題遂行様式の形成に関与する

○浅岡 希美、林 康紀

京都大・院医・システム神経薬理

Compulsive actions, defined by maladaptive perseveration and excessive repeat, are shown in various mental diseases. Recent evidence suggests that habit-based decision-making causes such perseveration; however, neural mechanisms for developing habitual, and also excessive, action remain unclear. Here, we developed an original operant overtraining protocol that induces habitual and excessive action in mice. Such compulsive-like action is only observed in about half of overtrained mice, whereas other mice show habitual but not excessive action. During the course of training, training-induced plastic changes in excitatory synaptic inputs, as assessed by AMPA/NMDA ratio, was observed in several regions of the prefrontal cortex. As mice learned the task, an increase in AMPA/NMDA ratio was observed in layer 5 pyramidal neurons of the anterior cingulate cortex (ACC) and lateral orbitofrontal cortex (OFC). When mice were overtrained on a slightly modified task for additional 4 days, the increase in AMPA/NMDA ratio was no longer observed in the ACC of overtrained mice regardless of the excessiveness, whereas the increase was only detected in the lateral OFC of mice showing both habitual and excessive action. Consistently, chemogenetic activation of ACC neurons of overtrained mice restored goal-directed action and inhibition of lateral OFC neurons of compulsive-like mice suppressed excessive response. These results indicate that region-specific changes in excitatory inputs in the prefrontal cortex are critical for the development of compulsivity.

Evaluation of addiction-inducing drugs based on the electrical activity of human iPS cell-derived dopamine neurons

ヒトiPS細胞由来ドーパミンニューロンの電気活動に基づく依存症誘発薬の評価

○石橋 勇人、永福 菜美、鈴木 郁郎

東北工業大

Addiction is known to be caused by various compounds such as frequently used medicines, luxury goods, and illegal drugs, and there is a need to establish an evaluation system for side effects to prevent the formation of addiction. However, a method for evaluating addiction has not yet been established. In this study, we constructed an evaluation system to detect addiction-positive compounds by measuring the electrical activity of iPS cell-derived dopamine neurons using microelectrode arrays (MEAs). The dopamine neurons (iCell) and astrocytes (iCell) were co-cultured on MEA. After 35 days of culture, we conducted a chronic administration test of 10 addiction-related compounds and the results before and after the chronic administration were compared using principal component analysis (PCA). In the cumulative administration experiment using dopamine neurons, it was possible to detect different responses for addiction-positive and addiction-negative compounds acting on the same receptors. In the chronic administration test, four out of five compounds were detected for the addiction compound, and the non-addiction compound was not misclassified as an addictive compound, suggest that this evaluation method is capable of detecting the addiction compound. These results suggest that the evaluation system used in this study is useful as a screening system for addiction-positive compounds.

Our pre-clinical support for neurotransmitter evaluation in the brain of rodents using a microdialysis method

マイクロダイアリシス法による小動物の脳内神経伝達物質の評価に向けた当社の前臨床サポート

○荒木 康平、齋藤 慶太、鈴木 孝太郎、野々村 徹、山崎 則之

株式会社新薬リサーチセンター・非臨床研究部

【Objective】

In vivo microdialysis is a technology to measure the concentration of a target substance in extracellular fluid at a localized site by collecting the substance through a dialysis membrane. In the brain, the dialysate enables us to assess real-time changes in neurotransmitters. Therefore, the technique is a useful method for the pharmacological action of central nervous system compounds. In order to fully support researchers, we would like to present an example of the antidepressant evaluation systems using the dialysate from various brain regions in rodents.

【Methods & Results】

A microdialysis probe was inserted into each of the hippocampus or the medial prefrontal cortex (mPFC) in C57BL/6J mice. After 2hour of pre-perfusion, the dialysate was collected at 20-minute intervals at a flow rate of 1μL/min. Then, mice were treated with intraperitoneal injection of R-fluoxetine, a selective serotonin reuptake inhibitor for the treatment of depression, and the dialysate was recollected. The next day, dopamine and serotonin levels were measured by HPLC-ECD. As a result of the study, serotonin release was significantly increased in the hippocampus compared to pre-administration. On the other hand, dopamine release was significantly increased in mPFC compared to pre-administration.

【Conclusions】

In this study, we confirmed the effects of a single antidepressant administration on brain monoamines in wild-type mice. We will continue to introduce a wide range of technologies and evaluation systems to provide value-added *pre*-clinical testing services.

Roles of brain carbon monoxide in micturition of rats

脳内一酸化炭素がラット排尿反射へおよぼす影響の薬理学的解析

○山本 雅樹[1]、清水 孝洋[2]、Zou Suo[2]、清水 翔吾[2]、東 洋一郎[2]、藤枝 幹也[1]、齊藤 源顕[2]

[1]高知大・医・小児思春期医学、[2]高知大・医・薬理学講座

Endogenous carbon monoxide (CO) produced by heme oxygenase (HO) is reported as a relaxation factor in the urethral smooth muscle, while roles of CO in the brain in regulation of the micturition reflex remain unclear. In this study, to elucidate roles of brain endogenous CO in regulation of the micturition reflex, we investigated effects of centrally administered CORM3 (CO donor) and ZnPP (non-selective HO inhibitor) on the micturition reflex in urethane-anesthetized (0.8 g/kg, ip) male Wistar rats. A catheter was inserted into the bladder to perform cystometrograms (CMG), which was started 2 h after the surgery, and 1 h after the start, CORM3 or ZnPP was intracerebroventricularly (icv) administered. CORM3 (1 or 10 nmol/rat, icv) dose-dependently prolonged intercontraction intervals (ICI), while ZnPP (10 or 30 nmol/rat, icv) dose-dependently shortened ICI. The ZnPP (30 nmol/rat, icv)-induced ICI shortening was reversed in the presence of CORM3 (10 nmol/rat, icv). In addition, ZnPP (30 nmol/rat, icv) significantly reduced single-voided volume and bladder capacity without affecting post-voiding residual volume or voiding efficiency. These results suggest that brain endogenous CO can inhibit the micturition reflex in rats.

TRPC3/6 channels inhibitor L862 exhibits protective effect against PAN-induced cellular damage in mouse podocyte

新規TRPC3/6チャネル阻害剤に関する細胞薬理研究

○松田 由宗[1,2]、坂口 怜子[1]、岡田 亮[1,3]、木原 隆典[2]、永田 龍[4]、森 誠之[1]

[1]産業医科大・医・医学科生命科学分野生体物質化学、[2]北九州市立大学・国際環境工学研究科・環境システム専攻、[3]産業医科大・医療保健・人間情報科学、[4]大阪大・院薬

Transient Receptor Potential Canonical (TRPC) 6 is highly expressed in glomerular epithelial cells (podocytes) and is known to contribute to the maintenance of glomerular protein filtration. It has been reported that patients with nephrotic syndrome, which is a disease characterized by proteinuria due to renal glomerular damage, show elevated expression of TRPC6 that causes the disorganization of actin filaments in podocytes. It has also been reported that gain-of-function mutations of TRPC6 are associated with nephrotic syndrome. Therefore, specific inhibition of TRPC6 would be an attractive strategy for suppressing proteinuria.
In this study, we have identified a novel TRPC3/6 channels inhibitor, L862. To evaluate the effect of L862 on podocytes, MPC-5 (Mouse Podocyte Clone 5) were treated with L862. Comparison of the viability of L862-treated group to non-treated group showed no significant difference, indicating that L862 does not have toxicity at the cellular level. Next, we prepared MPC-5 cells treated with Puromycin Aminonucleoside (PAN), which is known to cause podocyte damage. Treatment of L862 to these damaged MPC-5 exhibited protective effect on cell morphology. These results suggest the potential of L862 as a drug for suppression of proteinuria.

Utilizing split-luciferase-based HTS platform and natural product extracts library yielded cyclosporin A as candidate drug for Alport syndrome

Split-Luciferase評価系により見出したAlport症候群に対する新規治療候補薬Cyclosporin A

○スイコ メリーアン、桑水流 淳、大町 紘平、小嶋 遥、加世田 翔大、首藤 剛、甲斐 広文

熊本大

Alport syndrome (AS) is a hereditary kidney disease caused by mutation in type IV collagen alpha (Col4A) 3, 4, 5 chains, which disrupts trimerization, leading to kidney dysfunction. Mutations in Col4A5 comprise more than 80% of AS-associated mutations. These mutations can hinder trimer formation and/or trimer secretion. Correcting the trimerization and secretion of Col4A3/4/5 is a feasible therapeutic approach, but is hampered by the absence of high-throughput screening (HTS) platforms for assessment. We previously created an HTS system based on split nanoluciferase in which Large BiT (LgBiT) or Small BiT (SmBiT) subunits were fused to Col4A monomers. Proper trimerization results in complementation of LgBiT and SmBiT to produce quantifiable luminescence. Here, we used this system to screen natural product extracts library for AS drug candidate. For screening, we established HEK293T cells stably expressing wild-type Col4a3-SmBiT, Col4a4 and Col4a5 G1244D mutant-LgBiT. The library we screened is composed of extracts from fungi, bacteria, marine sponge and plants (>15,000 extracts screened). The hit extracts were subjected to mass spec that revealed cyclosporin A (CsA) as the main component of the extracts. CsA increased the trimer secretion but not trimer formation of Col4a3/4/5. The cyclophilin binding domain of CsA is important for its secretion-inducing activity, with cyclophilin D/PPIF being involved in the effect of CsA. Overall, we found a compound that can enhance the secretion of mutant Col4A trimer as candidate therapeutic for AS.

Synthesis and evaluation of activities of new pantetheine derivatives

新規パンテテイン誘導体の合成および活性評価

○細畑 圭子[1]、米山 弘樹[2]、金 徳男[3]、宇佐美 吉英[2]、髙井 真司[3]

[1]大阪医科薬科大・薬・臨床薬学教育研究センター、[2]大阪医科薬科大・薬・有機薬化学研究室、[3]大阪医科薬科大学大学院・医学研究科・創薬医学研究室

Background: Acute kidney injury (AKI) is associated with incomplete recovery after the onset of the disease. However, there is no fundamental treatment for this disease. Previously, we identified vanin-1, which appears or the cell surface of proximal tubules early after oxidative stress and induces various inflammatory responses. In this study, we developed and evaluated vanin-1 inhibitors. We synthesized novel vanin-1 inhibitors (OMP compounds) by chemical modification of pantetheine analogues RR6 (IC50 = 0.54 μM), which was reported by Schalkwijk J et al. To evaluate the candidate compounds, vanin-1 activity in serum and renal tissues collected at 1 and 4 hours after subcutaneous administration of 10 mg/kg of RR6 or OMP7 to hamsters was compared with that in the normal group. Results/Conclusions: Serum vanin-1 activity was significantly decreased at 1 hour after subcutaneous administration of RR6, but there was no significant difference at 4 hours. On the other hand, vanin-1 activity was significantly suppressed at 1 and 4 hours after subcutaneous administration of the OMP7. Vanin-1 activity in renal tissue was also not inhibited at 1 hour after subcutaneous administration of RR6. In contrast, the OMP7 significantly inhibited vanin-1 activity at 1 hour after subcutaneous administration. These results suggest that compared to RR6, the OMP7 has a sustained inhibitory effect on vanin-1 activity in serum, and a certain inhibitory effect can be expected in renal tissues as well.

Eucommia leaf extract improves renal impairment and vascular endothelial dysfunction in a type 2 diabetes mellitus rat model.

杜仲葉エキス慢性摂取は糖尿病に併発する腎障害および血管内皮機能障害を改善する

○中川 恵輔[1]、中川 愛海[1]、田中 雅美[1]、石川 梨乃[1]、犬塚 理奈[1]、平田 哲也[2]、松村 靖夫[1]、大喜多 守[1]

[1]大阪医科薬科大・薬・病態分子薬理、[2]小林製薬・中央研

Renal impairment and macrovascular endothelial dysfunction complicated with diabetes mellitus (DM) play a major role in the life expectancy of DM patients. *Eucommia* leaf extract (ELE) has anti-diabetic effects, but its effect on those complications with DM are unclear. Therefore, this study used the Zucker diabetic fatty (ZDF, type 2 DM) rat to examine the effects of ELE-containing diet (3% or 5%) ingestion before or after the onset of DM. Chronic ingestion of ELE prior to the onset of DM significantly suppressed the elevated blood glucose (BG) levels observed in ZDF rats and also remarkably improved glucose tolerance based on the results of the oral glucose tolerance test. Urinary protein excretion and albuminuria as important indicators of diabetic nephropathy (DN) were markedly suppressed by taking ELE 5% containing diet. A significant decrease in the acetylcholine-induced vasorelaxation response associated with DM was considerably improved by ELE ingestion. ELE ingestion after the onset of DM showed a reduction in BG levels, but no obvious effect on DN and endothelial dysfunction. These results indicate that chronic ingestion of ELE initiated before the onset of DM not only suppresses BG levels associated with DM progression but also has beneficial effects on DN and vascular endothelial dysfunction.

Evaluation of urination in a rat cystitis model of induced by hydrogen peroxide

過酸化水素誘発膀胱炎モデルにおける排尿評価

○清水 広夢、森田 枝美、吉原 佐江子、真壁 大地、水町 凉治、田代 貴士、片山 誠一、廣中 直行、西 勝英
㈱LSIM安全科学研究所・熊本研究所・薬理研究部

Few interstitial cystitis models can be used for evaluation over a long term although various animal models and evaluation methods exist for the development of novel clinical drugs for treatment of dysuria medicine. Currently, we produced the acetic acid, hydrochloric acid or cyclophosphamide–induced cystitis models, but it is difficult to evaluate the efficacy in the interstitial cystitis model since the symptoms of pollakiuria for all the models recover in a few days. Therefore, we tried to establish a cystitis model in which frequent urination symptoms continue for a long period of time using hydrogen peroxide (HP).

Female SD rats were used in the present study. First, we tested both the induction time of HP and the maintenance period of the model , comparing with those in the test groups of a sham group and the groups in which HP was stored in the bladder for 5, 15, or 30 minutes. The model rats were prepared by injection of 3% HP into the bladder under isoflurane inhalation anesthesia, and uroflowmetry was measured at 1, 2, 3, and 4 weeks after establishment of the model.

Results showed that since HP stored in the bladder for 5 minutes did not shorten the micturition interval and some rats died when HP was stored in the bladder for 30 minutes. Therefore, the model was prepared by storage of HP in the bladder for 15 minutes when a micturition interval was shortened. The micturition interval was found to be shorten up to 4 weeks in the uroflowmetry measurement. This model is considered useful for a long period of time for cystitis evaluation.

An estimation of urine flow rate using urinary creatinine excretion rate in rats

ラットにおける尿クレアチニン排泄速度に基づく尿流量推定の試み

小口 茜、○園田 紘子、川口 珠実、池田 正浩

宮崎大・農

Background:

In humans, urine collection is accessible because of their altruistic behaviors. On the other hand, since animals urinate spontaneously, urine collection is difficult. In this study, because it has been known that the urinary creatinine excretion rate is almost constant in normal animals of similar age of the same species, we try to estimate the urine flow rate using the excretion rate in normal age-matched rats.

Methods and Results:

First, using urine collected from normal male SD rats, the urinary creatinine excretion rate was calculated by dividing the total urinary creatinine amount by the collection time, and the resulting average value was 8.95 micro g/min. Next, we calculated an estimated urinary flow rate (eUF) using the value mentioned above and urinary creatinine concentration in each individual, and compared the resulting eUF with an actual urinary flow rate (aUF) obtained by considering collected urine volume and time. When the body fluid balance of rat was altered by changing an amount of drinking water or a diuretic-treatment, a high positive correlation was observed between the aUF and eUF for either a change in water intake ($r = 0.90$) or a diuretic treatment group ($r = 0.77$). A similar result was obtained, when we calculated free water clearance using aUF and eUF, respectively ($r = 0.89$).

Conclusion:

These results suggest that the urine flow rate can be estimated from the average urinary creatinine excretion rate in normal rats. In the future, animal species differences and individuals with altered urinary creatinine excretion rate should be examined.

Identification of efficient signal peptide in extracellular secretions for mRNA vaccine development

mRNAワクチンへの応用へ向けた分泌効率の良いシグナルペプチドの同定

○皆川 直樹、平田 悠朗、金子 雅幸、岡元 拓海

長崎大・院医歯薬・創薬薬理学

In mRNA vaccines, mRNA encoding antigens enclosed in lipid nanoparticles (LNPs) are injected into the muscle to induce intracellular antigens, which are then secreted extracellularly and recognized by antigen-presenting cells, thereby conferring immunity. Therefore, if the extracellular secretion of antigen is increased, immunity can be efficiently acquired with a smaller amount of mRNA; thus contributing to the improvement of vaccine supply.

The secretory efficiency ranking of artificial and natural signal peptides has already been reported. In this study, we transfected HEK293 cells with a vector in which the N-terminal IL-6 signal peptide of NanoLuc® luciferase (Nluc) was replaced by the signal peptides with high secretory signal strength. The amount of Nluc secreted extracellularly at each signal peptide was determined by luciferase assays and Western blotting. As per the results, Nluc with the signal peptide of Cystatin S, a natural signal peptide, had the highest extracellular secretion. The Western blotting result was consistent with that of the luciferase assay. Our future studies will test those peptides on C2C12 skeletal muscle cells and HepG2 liver cells. We also plan *in vivo* validation of those peptides using mRNA-LNP.

Elimination of Volatile Organic Compounds from indoor air by chemical filter significantly delays the development of atopic dermatitis in the mice model.

ケミカルフィルターによる室内空気からの揮発性有機化合物除去はアトピー性皮膚炎モデルマウスのアレルギー症状発症を有意に遅延させる

○大平 智春[1]、冨田 賢吾[2]、金木 真央[1]、早川 千春[1]、栗原 隆[2]、高木 哲[3]、福山 朋季[1]

[1]麻布大・獣医・薬理、[2]清水建設・技研・医療環境、[3]麻布大・獣医・小動物外科学

Allergic diseases including atopic dermatitis (AD) and allergic asthma are multifactor diseases, and the pathogenic mechanism is not fully understood yet. The environmental factor is one of the major contributors for allergy development, and several reports demonstrated that Volatile Organic Compounds (VOCs) are an exacerbating factors for allergy. In this study, we aim to examine the efficacy of elimination of VOCs by chemical filter in development of atopic dermatitis using a mice model.

A mouse model of hapten-induced AD were exposed to normal air and chemical filtered air (CF) with VOCs removed. There was a significant decrease in trans epidermal water loss (TEWL) 8 days after induction compared to the control group, but thereafter there was no change in the control group. Significant reductions in T and B cell counts by flow cytometry and pathology were observed in the CF group. The pathology of the control and CF groups became comparable in TEWL, skin thickness, and immune-related factors as the days passed. In study, the fact that significant effects were observed after the onset of the symptoms suggests that chemicals may be involved where they cause symptoms, furthermore, the impact of VOCs removal in the early stages of disease onset is currently under investigation.

Volatile organic compounds below guideline values in hospital facilities affect the development of pathology in mouse models of lung disease

病院施設における指針値濃度以下の揮発性有機化合物は肺疾患モデルマウスの病態形成に影響を与える

○冨田 賢吾[1]、大平 智春[2]、金木 真央[2]、早川 千春[2]、矢野 慧一[1]、栗原 隆[2]、高木 哲[3]、福山 朋季[2]

[1]清水建設・技研・医療環境、[2]麻布大・獣医・薬理、[3]麻布大・獣医・小動物外科

Indoor air quality (IAQ) has been suggested to affect respiratory diseases. Large amounts of volatile organic compounds (VOCs), emitted from indoor building materials, are known to cause healthy people to induce sick building syndrome., thereby being regulated as an IAQ guideline. However, the effects of VOCs below the IAQ guideline values on disease pathogenesis have not been adequately studied. If indoor VOCs affect disease development, they may also influence the treatment of respiratory diseases. In this study, we aimed to clarify the effects of indoor VOCs on the diseases of allergic asthma and acute lung injury (ALI) in model mice. We compared the pathological conditions of mice exposed to indoor air containing VOCs below the guideline values and those exposed to clean air whose VOCs were removed by a chemical filter. In addition, we compared the animal experimental environment with the clinical environment by collecting the IAQ data from the mouse breeding environment and the medical facility.

Our results indicated that clean air exposure significantly improved SpO_2, histological abnormalities, and gene expression of pro-inflammatory cytokines in asthma and ALI models. Our findings suggest that indoor VOCs even below the guideline values can influence the lung and immune function in asthma and ALI.

Generation of a DNA-aptamer targeting human galectin-7 as a lesion indicator for cholesteatoma

ガレクチン7を標的とした中耳真珠腫診断薬の開発

○劉 爽[1]、竹政 絵理香[1]、鈴木 康之[2]、羽藤 直人[3]、茂木 正樹[1]

[1]愛媛大・院医、[2]済生会松山病院・麻酔、[3]愛媛大・院医・耳鼻咽喉・頭頸部外

Aiming at complete excision of cholesteatoma during trympanomastoidectomy and therefore reducing the risk of recurrence, the current study was undertaken to develop a seed DNA-aptamer-based fluorophore-probe, which targets human galectin-7, as an intraoperative lesion-identifying indicator for the surgical treatment of cholesteatoma. A galectin-7-targeted DNA-aptamer library was generated for labeling the cholesteatoma matrix using cell-based systematic evolution of ligands by an exponential enrichment technique. The binding characteristics of the identified aptamers were analyzed, and structure optimization of the identified aptamers was carried out both in silico and in vitro. Using galectin-7-aptamer guided molecular imaging, the excision margins of cholesteatoma matrix and surrounding normal tissue were successfully observed in a xenografted cholesteatoma model. It is highly expected that specific galectin-7-aptamers could progress to future clinical trials for both imaging and therapeutic applications and therefore benefit cholesteatoma patients.

Topical treatment with high concentration of ozone water ameliorates inflammatory responses and breakdown of the cutaneous barrier in a mouse model of atopic dermatitis through antibacterial effect toward staphylococci

高濃度オゾン水の経皮塗布はブドウ球菌を殺菌することによりアトピー性皮膚炎モデルマウスにおける炎症反応及び皮膚バリア破綻を改善する

○金木 真央[1]、大平 智春[1]、高橋 美優[1]、内山 淳平[2]、阿野 哲也[3]、福山 朋季[1]

[1]麻布大・獣医・薬理、[2]岡山大・院医歯薬・病原細菌、[3]伯東

We focus on the ozone water to develop a novel treatment approach for staphylococci related inflammatory skin diseases. In the 95th Annual Meeting of the Japanese Pharmacological Society, we reported the anti-allergic and anti-microbial effect of 3 mg/L ozone water in a mouse model of atopic dermatitis (AD) and staphylococci, however, its effect was far from clinical application. The aim of this study is to evaluate the anti-microbial and anti-inflammatory property of high concentration (11 mg/L) of ozone water to advance our project. Ozone water showed a significant bactericidal effect against *Staphylococcus aureus*, *S. pseudintermedius* and *S. lentus*. Furthermore, *S. pseudintermedius* induced IL-1β and IL-6 secretion in human epidermal keratinocytes was significantly inhibited by pre-treatment of ozone water. *In vivo* experiment with a mouse model of AD, significant improvement of AD symptoms and trans epidermal water loss were also found in an ozone water treatment group. Surprisingly, local immune reactions such as type II conventional dendritic cells, effector helper T cells, and IgE-produced B cells are also regulated by ozone water application. Our findings strongly suggest that topical treatment with high concentration of ozone water significantly ameliorated AD symptoms through antibacterial effect toward staphylococci.

Verification of phenotype of novel atopic dermatitis model IL-33Tg mice

新規アトピー性皮膚炎モデルIL-33Tgマウスの表現型の検証

○野々村 徹、石田 裕紀、西川 英俊、大津 麻未、佐々木 麻衣、山崎 則之

株式会社　新薬リサーチセンター・非臨床研究部

In the development of drug discovery for atopic dermatitis（AD）, chemical-induced, special diet-induced or Tg mice have been used in experiments using rodents.

In order to overcome AD, it is expected that an animal model that appropriately reproduces the pathological condition of human AD can be created and utilized for pathological analysis and drug discovery research.

IL-33 is known to induce inflammatory cytokines or increase eosinophils by signaling from the nuclear transcription factor NFkB.

In the clinical picture of AD, it has been reported that IL-33 is highly expressed in human skin.

The model overproduces IL-33 in mice skin, induces inflammatory mediators via signaling from nuclear transcription factors, and results in Th2-dominant predominance, leading to AD.

In this presentation, introduce phenotypes such as itching behavior and histological examination of IL-33Tg mice.

Oral administration of *Lactobacillus* AZABU isolated from the gut microbiome of healthy dogs significantly prevents the development of atopic dermatitis and allergic asthma in mice models.

健常犬の腸内細菌叢から分離した*Lactobacillus*の経口投与はアトピー性皮膚炎モデルマウス及び喘息モデルマウスにおけるアレルギーの発症を抑える

○早川 千春[1]、大平 智春[1]、金木 真央[1]、安田 伊武希[1]、市川 茉南[1]、竹田 志郎[2]、内山 淳平[3]、福山 朋季[1]

[1]麻布大・獣医・薬理、[2]麻布大・獣医・食品科学、[3]岡山大・院医歯薬・病原細菌

Regulation of the gut microbiome using probiotics such as *Lactobacillus* spp. is recently focused on as one of the preventive measures for allergy. We originally isolated *Lactobacillus* sp. (*L.* AZABU) from the healthy dog guts. We here evaluated the anti-allergic effects of *L.* AZABU using hapten-induced atopic dermatitis (AD) and asthma mouse models. First, daily oral administration of live *L.* AZABU (2×10^8 CFU/ml) during the experimental period significantly prevented the development of AD symptoms, skin thickness, and trans-epidermal water loss in the AD mouse model. Significant decreased number of IgE-positive B cells in auricular lymph node was also observed in *L.* AZABU treatment group, indicating allergen-specific immunoreaction was regulated by *L.* AZABU. Group 2 innate lymphoid cells, which play a pivotal role in non-allergen-specific immunoreaction, also significantly suppressed by oral administration of live *L.* AZABU. Next, the anti-allergic effects of live *L.* AZABU was simultaneously demonstrated in the asthma mouse model, including significant decrease of inflammatory pathological change of lung, and both allergen-specific and non-specific immunoreaction in hilar lymph node and lung tissue. Our findings suggested that live *L.* AZABU has anti-allergic properties although the mechanism of action is still being investigated.

Examining anti-allergic properties of live and killed *Lactbacillus reuteri* isolated from healthy dogs in a mouse model of atopic dermatitis.

健常犬由来*Lactobacillus reuteri*の生菌および死菌のアトピー性皮膚炎モデルマウスにおける抗アレルギー効果の検討

○市川 茉南[1]、早川 千春[1]、大平 智春[1]、金木 真央[1]、安田 伊武希[1]、竹田 志郎[2]、内山 淳平[3]、福山 朋季[1]

[1]麻布大・獣医・薬理、[2]麻布大・獣医・食品科学、[3]岡山大・院医歯薬・病原細菌

We are currently developing *Lactobacillus reuteri* isolated from healthy dogs as a probiotic supplement for canine atopic dermatitis (AD). In this study, we examined the anti-allergic effects of live *L. reuteri* and killed *L. reuteri* strain mixture (i.e., strains M01, M11, M40, and M41) using a hapten-induced-AD mouse model. First, when UV-killed *L. reuteri* (overgrowth) was orally administered to AD mice, cutaneous barrier function evaluated by the trans epidermal water loss was maintained as normal in the *L. reuteri* treatment group, compared with the control group. Immune reactions including lymphocyte proliferation in auricular lymph nodes and total IgE levels in serum are significantly regulated by treatment of killed *L. reuteri*. In contrast, *L. reuteri* treatment improve neither AD score nor skin thickness. Second, the anti-AD effects were examined using 4 individual strains of live *L. reuteri* (i.e., M01, M11, M40, or M41). The symptoms of AD and immune responses such as lymphocyte proliferation and total IgE levels are significantly reduced by *L. reuteri* M11 administration compared with the vehicle control and other strains. Currently, the interaction with macrophages and regulatory T cells is being studied.

Effects of glycyrrhizic acid and its metabolite on the GIRK channel activity

甘草の主成分グリチルリチン酸とその代謝産物によるGIRKチャネル活性への影響

○陳 以珊、西谷（中村） 友重

和歌山県立医科大・医・薬理学講座

G-protein-gated inwardly rectifying K^+ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. GIRK channels are potential therapeutic targets for several diseases, such as atrial fibrillation and addiction. In the present study, we aimed to identify the effect of glycyrrhizic acid (GA), a main ingredient of licorice, and its metabolite glycyrrhetic acid (GRA) on GIRK channel activities. By electrophysiological recordings using *Xenopus* oocytes expressing different GIRK subunits, we observed that GA inhibits the current of heteromeric GIRK1/2 and GIRK1/4 but slightly activates the current of homomeric GIRK2 and GIRK4. This suggests that the inhibitory effect of GA is GIRK1-dependent. Mutation of a GIRK1-specific amino acid residue in the pore helix, Phe137, to Ser abolishes the inhibition of GIRK current by GA, suggesting that the Phe137 plays important roles in the sensitivity of channels to GA. Unlike GA, GRA activates all GIRK2, GIRK4, GIRK1/2 and GIRK1/4 channels. Taken together, these data indicate that GA and GRA have distinct actions on GIRK currents, and would provide clues to elucidate the diverse mechanisms of GIRK channel regulation by analyzing the difference of these compounds.

RAGE, which is the receptor for advanced glycation end-products, is involved in the regulation of KCC2 expression by the *Porphyromonas gingivalis* LPS treatment in PC-12 cells.

PC-12細胞において歯周病菌由来LPSによるKCC2発現制御に最終糖化反応生成物受容体であるRAGEが関与する

○富田 和男[1,2]、古川 紗圭[1,3]、五十嵐 健人[1,2]、田中 康一[1,2,4]、北中 純一[2]、北中 順惠[4]、西山 信好[2]、野口 和行[3]、佐藤 友昭[1]

[1]鹿児島大・院医歯・歯科薬理、[2]兵庫医科大・薬・薬理、[3]鹿児島大・院医歯・歯周病、[4]兵庫医科大・医・薬理

KCC2 has been shown to be important for neural maturation. We have shown that *Porphyromonas gingivalis*-derived lipopolysaccharide (*P. g*LPS) decreases KCC2 expression. We have also shown that oxytocin, Kamishoyosan, and Kamikihito restore the KCC2 decrease by *P. g*LPS treatment. However, the detailed molecular mechanism is still unclear. On the other hand, RAGE (receptor for advanced glycation end-products; AGE) was discovered as a receptor of AGE at first, it has been also reported as a receptor for LPS and oxytocin now. Therefore, we analyzed the relationship between *P. g*LPS and RAGE using PC-12 cells. SiRAGE Treatment reduced the expression of *Tlr4*, the receptor for *P. g*LPS, and canceled the KCC2 decrease by *P. g* LPS. In addition, *P. g*LPS treatment results in the nucleus localization of REST and MECP2 that bind to the transcriptional regulatory region of *Kcc2*, whereas siRAGE treatment inhibits the nuclear localization of REST and MECP2. Furthermore, Kamishoyosan treatment directly reduced the expression of *Rage*, and Kamikihito treatment increased the expression of *Oxytocin*, which is one of the ligands of RAGE. These results suggest that targeting RAGE can control the expression of KCC2, which is important for neurological maturation, and that Kamishoyosan and Kamikihito are potential therapeutic agents for neurological disorders.

Antiviral activity of curcumin and its analogs selected by artificial intelligence-supported activity prediction system in SARS-CoV-2-infected VeroE6 cells

人工知能支援活性予測システムによって選択されたクルクミンとその類縁体のSARS-CoV-2感染VeroE6細胞を用いた抗ウイルス活性

小松 弘嗣[1]、田中 剛史[1]、叶 正成[1]、池田 健[1]、松崎 尹雄[1]、城間 保[2]、細田 雅人[1]、安木 真世[3,4,5]、○手島 浩慈[2]

[1]インタープロテイン、[2]レキオファーマ・研究開発本部、[3]大阪公立大学・院獣医、[4]大阪公立大学・アジア健康科学研究所、[5]大阪公立大学・大阪国際感染症研究センター

Curcumin has been reported to exert its anti-SARS-CoV-2 activity through multiple mechanisms including inhibition of spike receptor-binding domain (RBD) to angiotensin-converting enzyme-2 (ACE2) binding. To identify more potent compounds, we tested curcumin and its analogs for spike RBD-ACE2 binding inhibitory activity and antiviral activity in SARS-CoV-2-infected cells. An artificial intelligence (AI) -supported activity prediction system was used to select the compounds, and 116 compounds with a docking score range of -8.7 to -4.3 kcal/mol were selected from 334 curcumin analogs. These compounds were narrowed down to 10 compounds, including curcumin, for confirmatory studies. These 10 compounds showed a significant correlation (r_s=0.685, P=0.029) between the IC_{20} values of spike-RBD-ACE2 binding inhibitory activity and EC_{50} values of antiviral activity, indicating that the antiviral activity was mediated by spike RBD-ACE2 binding inhibition. Based on the assumption that the binding site of curcumin and its analogs is different from that of anti-spike RBD antibody drugs, it is expected that these compounds through pharmaceutical or pharmacokinetic modification, or the development of more potent derivatives would contribute to supplementing the antiviral activity of antibodies against SARS-CoV-2.

Antimicrobial activities of ginseng saponins isolated from Red Ginseng to non-tuberculous mycobacteria

非結核性抗酸菌に対する紅参由来サポニンの抗菌作用の機序解明

○寒川 訓明[1]、山口 雄大[2]、徳留 健太郎[1]、本間 拓二郎[1]、松永 慎司[1]、冨田 修平[1]

[1]大阪公立大学・医・分子病態薬理学教室、[2](国研)国立感染症研究所・細菌第一部

Non-tuberculous mycobacterial lung disease (NTM lung disease) is the infection which is caused by mycobacteria, such as *Mycobacterium avium* (*M. avium*), *M. intracellulare*, *M. kansasii*. The prognosis of NTM lung disease is relative benign than that of TB, but in some cases NTM lung disease is highly resistant to chemotherapy. The incident rate of NTM lung disease is increased and it is estimated that the number of NTM patients exceeds the number of tuberculosis (TB) patients. Here, we investigated the antimicrobial activity of ginseng saponins from red ginseng (Red Ginseng Extract; RGE) against NTM.

We examined whether RGE had antimicrobial activity against *M. avium* MAH104 strain. RGE inhibited the growth of *M. avium* at more than 2.0 mg/mL. To elucidate antimycobacterial mechanism of RGE, we established a resistant strain to RGE. RGE-resistant strain grew slower than wild strain. In acid-fast staining, cell wall staining tended to be attenuated in RGE-resistant strain than that in wild type. Protein expression in cell wall fraction from RGE-resistant strain showed some differences from that from wild type.

These results suggest that RGE has the antimicrobial activity against *M. avium* in vitro. The mechanism of the antimicrobial activity is assumed to be through its effect on the cell wall synthesis.

Effectiveness of Lecture and Practical on Kampo Medicine for Nursing Undergraduates: The Relationship between Self-Reported Health Status and Interest in Kampo Medicine

看護大学生に対する漢方講義・演習の有効性：健康と漢方に対する関心との関連について

○金岡 麻希、野末 明希、児玉 みゆき、内田 倫子、竹山 ゆみ子、木下 由美子、柳田 俊彦

宮崎大・医・看

A survey was conducted using a self-administered questionnaire to clarify the relationship between nursing undergraduates' perceptions of their own health status and their interest in Kampo medicine, and to clarify their changes after a lecture and practical. About 85% of the nursing students considered themselves “very healthy” or “healthy”. Before the lecture, the higher health levels corrected with, lower interest in Kampo medicine. Conversely, after the lecture, interest in Kampo medicine in the healthy group increased. This suggests that undergraduate lecture can increase interest in Kampo medicine, especially among healthy nursing students. Regardless of their health status, the nursing students tended to recognize the need for knowledge of Kampo medicine before the lecture. This had a particular impact on the students in the healthy group, who accounted for the largest proportion of students surveyed. Before the lecture, students were aware of the need for Kampo medicine in nursing education. However, following the lecture, the healthy and poor health groups were more aware of the need for Kampo medicine.

Suppressive effects of ergothioneine on Aβ-induced hyperphosphorylation of tau protein in SH-SY5Y cells

SH−SY5Y細胞におけるAβ誘発タウの過リン酸化に対するエルゴチオネインの抑制効果

○柴垣 郁弥[1]、小菅 葉利[1]、板花 将輝[1]、加藤 優希[1]、松本 聡[2]、中道 範隆[1]

[1]高崎健康福祉大・薬・分子薬物治療、[2]エル・エス　コーポレーション

Ergothioneine (ERGO) is a hydrophilic antioxidant contained in the food and is distributed to the brain after oral intake, exhibiting a neuroprotective effect. ERGO protected PC12 cells against cellular toxicity induced by amyloid beta (Aβ) and improved impairment of learning and memory ability in mice administered with Aβ. However, the effects of ERGO on hyperphosphorylation of tau protein is unclear. In the present study, we investigated whether ERGO suppresses Aβ-induced hyperphosphorylation of tau protein in human neuroblastoma SH-SY5Y cells. SH-SY5Y cells were differentiated using culture medium containing 1% or 10% fetal bovine serum (FBS) with 10 μM retinoic acid. Exposure to $A\beta_{25-35}$ clearly decreased cellular viability in SH-SY5Y cells differentiated in the 10% FBS medium, but not 1% FBS medium. Pretreatment with ERGO protected SH-SY5Y cells against cellular toxicity induced by $A\beta_{25-35}$ in a dose-dependent manner. Exposure of SH-SY5Y cells to $A\beta_{25-35}$ increased expression of phosphorylated tau protein, and the increase was suppressed by pretreatment with ERGO in a dose-dependent manner. These results suggest that ERGO may suppress hyperphosphorylation of tau protein and alleviate neurotoxicity induced by Aβ.

Apolipoprotein E-containing lipoproteins protect retinal ganglion cells from NMDA-induced excitotoxicity with reducing α2-macroglobulin via an LRP1 in retinal glia

アポE含有リポタンパク質によるLRP1を介した網膜グリア細胞のα2−マクログロブリン発現抑制とNMDA誘発興奮毒性に対する網膜神経節細胞保護効果

○林 秀樹、森 みすず、南 厚徳、見世 加南子、高木 教夫

東京薬科大・薬・応用生化

Low-density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional receptor that is abundantly expressed in the central nervous system. In our previous study, glia-derived apolipoprotein E-containing lipoproteins (ELPs) protected retinal ganglion cells (RGCs) from glutamate-induced excitotoxicity via an LRP1 in vitro. We have also shown that α2-macroglobulin (a2M) interferes with the protective effect of ELPs. It is reported that a2M is increased in vitreous humor of glaucoma patients. However, the details of its function are unknown. Here we observed that *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity in retinae of rats showed RGC degeneration and increased amount of a2M in vitreous humor three days after NMDA injection into vitreous humor. Then, intravitreal injection of ELPs suppressed RGC degeneration and the increased amount of a2M. In addition, ELPs decreased a2M mRNA and protein levels in primary cultured retinal glia. LRP1 siRNA blocked the inhibitory effect of ELPs on a2M expression, and the addition of ELPs enhanced the phosphorylation of STAT3 in retinal glia. Thus, ELPs demonstrate optic nerve protection from excitotoxicity and also reveal an indirect protective effect by suppressing the a2M expression, which is a neuroprotective disturbing factor. We hope that a reduction of a2M mediated by the ELP-LRP1-STAT3 pathway might be an additional protective mechanism against excitotoxicity in the retina.

Regulations of cysteine uptake and intracellular glutathione levels by purine derivatives

プリン誘導体によるシステインの取り込みおよび細胞内グルタチオン量の調節

○松村 暢子、角田 ワッタナポン、青山 晃治

帝京大・医・薬理

Purine derivatives such as caffeine and uric acid have neuroprotective activities and reduce the risk of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. We have reported that caffeine, uric acid and paraxanthine, a major metabolite of caffeine, promote cysteine uptake in hippocampal slices. We have also reported that paraxanthine promotes the cysteine uptake and the synthesis of intracellular antioxidant molecule, glutathione (GSH), in HEK293 cells.

In this study, we examined the effect of uric acid, an end-product of purine metabolism, on cysteine uptake and GSH synthesis in HEK293 cells. Uric acid was treated to HEK293 cells for 30min at concentrations of 0, 10 and 100 μM in cystine-deprived condition, and then cysteine was added for 30 min at concentrations of 100 and 400 μM. High performance liquid chromatography analysis showed that uric acid decreased the extracellular cysteine levels. Fluorescent GSH detection by CMFDA indicated that uric acid increased intracellular GSH levels. These results suggest that uric acid promotes cysteine uptake leading to GSH synthesis in HEK293 cells.

Ghrelin enhances excitability of cerebellar molecular layer interneurons and facilitates GABAergic transmission onto cerebellar Purkinje cells

小脳抑制性介在ニューロンに対するグレリンの興奮作用

○廣野 守俊、中田 正範

和歌山県立医科大・医・生理学第2講座

Ghrelin is an endogenous orexigenic peptide for growth hormone secretagogue receptor type 1a (GHS-R1a). Ghrelin is produced not only in the stomach but also in the brain. A recent study has reported that rodent cerebellar Purkinje cells (PCs) express GHS-R1a and its activation facilitates spontaneous firing of PCs. However, little is known about whether ghrelin alters synaptic transmission onto PCs and modulates firing of PCs. We examined effects of ghrelin on inhibitory GABAergic transmission using patch clamp recordings applied to mouse cerebellar slices. We found that bath-application of ghrelin increased the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in PCs. The peptide did not affect miniature or stimulation-evoked IPSCs in PCs. Ghrelin significantly increased the spontaneous firing rate of molecular layer interneurons (MLIs). The ghrelin-mediated effect on MLIs was blocked by a GHS-R1a antagonist JMV3002 and attenuated by blockers of transient receptor potential canonical type 1 (TRPC1) or KCNQ channels, suggesting that ghrelin signaling in MLIs regulates both TRPC1 and KCNQ channels. These results indicate that the ghrelin-induced potentiation of spontaneous IPSCs is attributed to the firing facilitation of MLIs by the activation of GHS-R1a expressed most likely in somatodendritic sites of MLIs. Thus, ghrelin controls firing of PCs directly and indirectly, and could contribute to the regulation of motor coordination.

取り下げ

Effect of 5-aminolevulinic acid on the object recognition test in mice

マウス新規物体認識試験における5−アミノレブリン酸の影響

○小宮 素良、高橋 純平、古賀 愛理、山田 大輔、斎藤 顕宜
東京理科大・薬・薬理学研究室

An endogenous amino acid 5-aminolevulinic acid (5-ALA) is a precursor of heme in the porphyrin synthesis pathway. It has been reported that 5-ALA synthase is decreased in the brains of patients with dementia, suggesting an association between cognitive function and 5-ALA. However, little is known about the role of 5-ALA in cognitive function. To address this issue, we evaluated learning and memory performance in the novel object recognition (NOR) test by using ddY mice (4-6-week-old). 5-ALA hydrochloride or vehicle was intracerebroventricularly administered 30 min before the training session. On the test day, 48 hours after the training session, 5-ALA (3 mM) -treated mice showed significantly higher ratio of contact time to novel object compared to the vehicle-treated mice. This result suggested that 5-ALA produced the enhancement of the object recognition memory in mice. Next, we examined a memory-related synaptic plasticity, long-term potentiation (LTP), of field excitatory post-synaptic potential (fEPSP) in area CA1 of the hippocampus. As the results, 5-ALA (300 mM) perfusion in the hippocampal slices significantly increased the slope of the fEPSP, suggesting the enhancement of the LTP. Based on these results, we propose that 5-ALA could have the enhancing effects on cognitive function in rodents.

Neurotensinergic neurons in the lateral hypothalamus are important for physiological sleep-wake cycle.

視床下部外側ニューロテンシン神経は睡眠覚醒サイクルに重要である

○長沼 史登[1]、中村 正帆[1]、ベトリベラン ラマリンガム[2]、吉川 雄朗[3]、岡村 信行[1]

[1]東北医科薬科大・医、[2]ハーバード大学・医・神経学分野、[3]東北大・院医・薬理学

Neurotensin works as a neuropeptide in the brain. We have reported that experimental stimulation of neurotensinergic neurons in the lateral hypothalamus (Nts-LH) caused hyperarousal in mice. However, it remains unclear whether Nts-LH is involved in the physiological sleep-wake cycle. Thus, in the present study, we investigated the effect of Nts-LH selective lesion on physiological sleep-wake cycle. We also measured neuronal activities of Nts-LH during sleep and wakefulness. First, we performed Nts-LH lesion induced by diphtheria toxin-A. The Nts-LH lesion caused a fragmentation of wakefulness and decreased total amount of wakefulness, implying that Nts-LH was critical in the transition and maintenance of wakefulness. Next, we measured the neuronal activities of Nts-LH during sleep and wakefulness by using fiber photometory with a Ca^{2+} sensor, GCaMP7f. The calcium imaging clearly showed that Nts-LH was simultaneously activated at the transition from sleep to wakefulness, followed by continuous excitation during wakefulness. These data emphasize that Nts-LH mediate sleep-to-wake transition then their activation promote the following wakefulness. Taken together, the neuronal activity of Nts-LH maintains wakefulness, which is important for physiological sleep-wake cycles

Albumin suppresses hydrogen peroxide- and nimustine-induced cell death via increasing intracellular GSH levels in human astrocytoma U-251 MG cells.

ヒトアストロサイト―マU−251 MG細胞においてアルブミンは細胞内グルタチオン量を増加させることにより過酸化水素及びニムスチン誘発細胞死を抑制する

○吉岡 靖啓、砂川 和也、田野井 一、山室 晶子、石丸 侑希

摂南大・薬

We previously demonstrated that albumin increases intracellular glutathione (GSH) levels in human astrocytoma U-251 MG cells. GSH has been known to play an important in oxidative stress tolerance and anticancer drug resistance in tumor cells. In this study, we investigated the effect of albumin on hydrogen peroxide- and nimustine (ACNU)-induced cell death in U-251 MG cells using bovine serum albumin (BSA). Intracellular GSH levels were determined by the DTNB recycling assay. Cell death of U-251 MG cells was examined by Hoechst 33342/Propidium iodide staining. Treatment of U-251 MG cells with BSA (0.1-10 mg/mL) increased the levels of intracellular GSH and catalytic subunit of glutamate-cysteine ligase (GCLc), a rate-limiting enzyme in GSH synthesis, protein in a concentration-dependent manner. In addition, pretreatment with BSA for 24 h attenuated hydrogen peroxide (0.75 mM)- and ACNU (1 mM)-induced cell death in a concentration-dependent manner. This cytoprotective effect of BSA was not observed in the presence of DL-Buthionine-(S,R)-sulfoximine (1 mM), an inhibitor of glutathione synthesis. These results suggest that albumin increases intracellular GSH levels in U-251 MG cells by inducing GCLc protein and that albumin suppresses hydrogen peroxide- and ACNU-induced cell death via increasing intracellular GSH levels.

Activation of mechanosensitive Piezo1 channel suppresses brown adipocyte differentiation

機械刺激感受性チャネルPiezo1の活性化は褐色脂肪細胞の分化を抑制する

○内田 邦敏[1]、剣持 麻奈斗[1]、川原崎 聡子[2]、瀧澤 咲月[1]、岡村 和彦[3]、後藤 剛[2]

[1]静岡県立大・食品栄養科学・生体機能学、[2]京都大・院農・食品生物科学、[3]福岡歯科大・生体構造

Brown adipocytes cause an energy consumption by heat production and are thought to be a target for the prevention of obesity and related metabolic disorders. Piezo1 is a Ca^{2+}-permeable non-selective cation channel and activated by mechanical stimuli. While, Piezo1 has been reported to be involved in mechano-sensation in non-sensory tissues, the expression and role of Piezo1 in brown adipocytes have not been well clarified. In this study, we evaluated a brown adipocytes line from UCP1-mRFP1 transgenic mice and analyzed this cell. Application of Yoda-1, a Piezo1 agonistsuppressed brown adipocytes differentiation in a dose-dependent manner. This suppression was significantly recovered by co-application with a Piezo1 antagonist and a calcineurin inhibitor. In addition, knock-down of Piezo1 impaired Yoda-1-induced suppression of brown adipocyte differentiation and application of Yoda-1 enhanced the calcineurin activity. These results suggest that activation of Piezo1 might suppress the differentiation through calcineurin pathway in brown pre-adipocytes.

KPR-5714, a novel TRPM8 antagonist, improves frequent urination in a model with enhanced bladder afferent nerve activity

TRPM8拮抗薬 KPR−5714は膀胱求心性神経活動が亢進したモデルにおいて頻尿を改善する

○渡邉 信次郎[1]、松澤 亜可根[2]、小林 淳一[1]、藤森 芳和[1]

[1]キッセイ薬品工業・研究本部　研究統括部　創薬探索研究所、[2]キッセイ薬品工業・研究本部　薬理研究所

Transient receptor potential melastatin 8 (TRPM8) has a role in the abnormal sensory transduction of the bladder and is involved in the pathophysiology of hyperactivity bladder disorders. In the present study, we examined the effects of KPR-5714, a novel and selective TRPM8 antagonist, on voiding dysfunction induced by bladder afferent hyperactivity via mechanosensitive C-fibers in rats. In cystometry measurements, the intercontraction interval was decreased by intravesical instillation of 10 mM ATP in female rats. KPR-5714 dose-dependently prolonged the shortened the intercontraction interval provoked by ATP. In voiding behavior measurements, intratesticular injection of 3% acetic acid and water avoidance stress exposure decreased the mean voided volume and increased voiding frequency in male rats. KPR-5714 dose-dependently increased the mean voided volume and decreased voiding frequency without affecting the total voided volume in these rats. However, KPR-5714 did not influence the voiding behavior in normal rats. These results suggest that KPR-5714 improves voiding dysfunction by inhibiting the enhanced activity of mechanosensitive bladder C-fibers in rats with bladder overactivity and does not affect normal voiding behavior.

Improvement of muscle weakness by nalfurafine hydrochloride (TRK-820), a kappa opioid receptor agonist

κオピオイド受容体作動薬ナルフラフィン塩酸塩（TRK-820）による筋力低下改善作用

○上野 賢也、森山 正樹、大森 優、上田 寛、内田 将史

東レ・医薬研究所

Cancer cachexia is a progressive functional disorder that cannot be completely reversed with normal nutritional support. It is a multifactorial syndrome characterized by a persistent loss of skeletal muscle mass (with or without fat loss), and anamorelin is the only drug approved for the treatment of cancer cachexia in Japan. Recently, we found that nalfurafine hydrochloride (our development code TRK-820), which is used as a treatment for pruritus in dialysis and chronic liver diseases refractory to existing therapies, increased food intake in normal animals, increased body weight and prolonged life in a B16/F10 cell transplantation model mouse, and improved food intake, body weight gain and muscle weakness in an A549 cell transplanted non-small cell lung cancer model mice and may be a potential therapeutic agent for cancer cachexia. . In addition, genetic analysis of the hypothalamus of A549 cell transplantation model mice revealed that TRK-820 increased peptides involved in hyperphagia and suppressed dopamine-related genes. These results suggest that TRK-820 suppresses the effects of dopamine in the hypothalamus, causing activation of the hyperphagic system, leading to increased food intake and consequent weight gain. This presentation will discuss the therapeutic effects of TRK-820 on cancer cachexia and the details of the mechanisms currently known.

Effect of nalfurafine hydrochloride (TRK-820), a kappa opioid receptor agonist, in the treatment of cancer cachexia.

κオピオイド受容体作動薬ナルフラフィン塩酸塩（TRK-820）のがん悪液質治療効果

○大森 優、内田 将史、岩村 智勝、瀬戸 真由美、鈴木 知比古

東レ（株）・医薬研究所

Cancer cachexia is a progressive functional disorder that cannot be completely reversed with normal nutritional support. It is a multifactorial syndrome characterized by a persistent loss of skeletal muscle mass (with or without fat loss), and anamorelin is the only medicine approved for the treatment of cancer cachexia in Japan. We have previously found that nalfurafine hydrochloride (our development code TRK-820), which is used as a treatment for pruritus refractory to existing therapies for dialysis and chronic liver disease, increased food intake and body weight and improved muscle weakness in a mouse model of non-small cell lung cancer inoculated with A549 cells. These results suggest that TRK-820 is a potential therapeutic agent for cancer cachexia. This presentation will discuss the efficacy of TRK-820 in treating cancer cachexia in animal models and detail the mechanisms currently known.

Suppression of mitochondrial depolarization of neurons by KCC2 inhibitor VU0463271.

KCC2阻害剤VU0463271による神経細胞ミトコンドリア脱分極抑制作用

○原 友凜亜、平嶋 未佳、尾松 果奈、金城 俊彦、宇野 恭介、倉本 展行

摂南大・薬・機能

In neurons, massive calcium influx through NMDA receptor causes excitotoxicity by depolarizing mitochondria by opening mitochondrial permeation transition pore (mPTP). Through mPTP, potassium ions (K+) also pass through from the cytosol to mitochondrial matrix, and also causing depolarization. We hypothesized that the K+ concentration gradient between the cytosol and the matrix defines how damage the cells. Potassium chloride cotransporter 2 (KCC2) fundamentally play a role to exclude intracellular chloride ion (Cl-) out in mature neurons by using the driving force of potassium gradient. In this study, we experimentally investigated whether increasing the intracellular K+ concentration by inhibiting this transporter increases the degree of mitochondrial depolarization and the degree of cell death. Exposure of VU0463271, an inhibitor of KCC2 to primary cultured neurons did not change the MTT reducing ability. Contrary to expectations, pre-incubation of VU0463271 did not affect NMDA-induced neuronal cell death. Moreover, pre-incubation of VU0463271 lowered the degree of mitochondrial depolarization, which was induced by NMDA or valinomycin, a mitochondrial uncoupler. It was suggested that the K+ concentration in the cytosol was constantly regulated by buffering by mitochondria.

Determination of alteration of intracellular potassium level by using simple device.

簡易測定器を用いた神経細胞内K＋イオン濃度変化測定

○岡田 暉己、二股 有貴子、岩本 昂也、金城 俊彦、宇野 恭介、倉本 展行

摂南大・薬・機能

We have hypothesized that increased concentration of potassium ion (K+) in the cytoplasm determines the degree of mitochondrial depolarization and enhancement of cytotoxicity and is an important factor in determining neuronal cell death. The definition of intracellular ion concentration is possible using the patch clamp method, while the equipment is expensive. Fluorescence indicators for various ions are still in the development stage. In this study, we investigated whether it is possible to define intracellular and extracellular K + concentrations and chase alterations in intracellular K+ concentrations using a simple K+ meter. A calibration curve of K+ concentration-ppm was prepared with a potassium chloride solution. We could determine the K+ concentration in tissue homogenates from several brain regions, and it was found that the K+ concentration per tissue weight was almost constant. It was found that when the cell suspension or the medium of adherent cells was changed to a medium containing no K+, the intracellular K+ concentration decreased. Also in the medium containing a high concentration of K+, the intracellular K+ concentration increased. The medium of the cell suspension and adherent cells was changed to a medium containing valinomycin, which is a K+ ionophore, or VU0463271, which is a K+, Cl- cotransporter inhibitor, however no change in intracellular K+ concentration was observed. In the next, we will investigate using drugs that more directly change the intracellular K+ concentration (eg, K + channel opener).

A2BR contribution to form an appropriate sensory network in the cortex via suppression of mGluR5 a postnatal early development

アデノシンA2b受容体は発達期早期のmGluR5発現抑制を介した感覚刺激ネットワークの形成に寄与する

○宮川 美保[1]、田中 雅彬[1]、小泉 修一[1,2]

[1]山梨大・院医・薬理、[2]山梨大・山梨GLIAセンター

Metabotropic glutamate receptor 5 (mGluR5) is abundant in neonatal astrocytes but decreases with ages, and is almost absent in the healthy adult brain. Astrocytic mGluR5 is important in formation of excitatory synapses. We previously showed that A2b receptor (A2BR), being upregulated in astrocytes, is responsible for down-regulation of *Grm5* encoding mGluR5 during postnatal early development. Here, using A2BR knock out mice (A2BKO), we investigated effects of this loss of inhibitory control over mGluR5 during early development on adult brain functions. We firstly examined *Grm5* expression level in the healthy adult (7 months old) cortical astrocytes. The *Grm5* expression was twice as high in A2BKO mice as that of control wild-type mice (WT). Then, we performed two behavioral tests on A2BKO mice (9 to 11 weeks old). In von Frey test on naïve A2BKO mice, the percentage of maximum pain score of A2B mice was significantly higher than that of the WT mice. In temperature nociceptive threshold test, A2BKO mice exhibited lower threshold than WT mice. These results indicated that A2BKO mice are more sensitive to these sensory stimuli than WT mice, suggesting that astrocytic A2BR would contribute to form an appropriate sensory network in the cortex presumably via suppression of mGluR5-mediated synaptic remodeling.

Regulation of directional cell motility in alveolar macrophages by a formin family protein Fhod1

Forminファミリータンパク質Fhod1による肺胞マクロファージの方向性細胞運動の制御

○三浦 綾子[1]、實松 史幸[2]、武谷 立[1]

[1]宮崎大・医・薬理学、[2]尚絅大学・生活科学・栄養科学科

The actin cytoskeleton functions in various cellular events, including cell motility, morphogenesis, cytokinesis, and establishment and maintenance of cell polarity. Formin family proteins are structurally characterized by the presence of the formin homology domains (FH) 1 and 2, and play pivotal roles in actin filament assembly in a variety of cellular processes. Fhod3, a cardiac member of the family, is expressed abundantly in the heart and neurons, while Fhod1 is ubiquitously expressed. Fhod3 plays an essential role in cardiogenesis and neurogenesis. Fhod1 is known to contribute to the assembly of stress fibers, contractile actin structures found in cultured non-muscle cells. However, the *in vivo* role of Fhod1 remains poorly understood. Here we show that Fhod1 is abundantly expressed in the lung. Especially, alveolar macrophages isolated from bronchoalveolar lavage fluids were strongly stained with anti-Fhod1 antibodies. In Fhod1 KO mice, directional cell motility of alveolar macrophages was selectively impaired, suggesting the possibility that Fhod1-mediated actin assembly seems to promote cell polarization during cell migration. We will discuss roles of Fhod1 in the regulation of directed cell migration in alveolar macrophages.

Calcineurin B homologous protein 3 facilitates the skeletal muscle development

Calcineurin B homologous protein 3は骨格筋の分化・融合を促進させる

○古林 創史、野部 浩司

昭和大・薬

Calcineurin B homologous protein 3 (CHP3) is a calcium-binding protein and is highly expressed in heart. We previously found that this protein inhibits cardiomyocyte hypertrophy. However, the function in skeletal muscle is unknown. In the present study, we examined its role in the differentiation and fusion process of mouse skeletal muscle. CHP3 was barely expressed in adult rectus femoris muscle, but abundantly expressed in neonatal muscle. The expression level of CHP3 was increased in mouse C2C12 myoblasts during the differentiation into myocytes. Disruption of the CHP3 gene in the myoblasts with the CRISPR-Cas9 system decreased the expression level of myosin heavy chain (MHC), a marker of skeletal muscle differentiation. In addition, the myotube fusion index (number of myotubes with at least two nuclei per total myotubes) was decreased. On the other hand, over-expression of CHP3-mCherry increased MHC expression and myotube fusion. These results suggest that CHP3 regulates skeletal muscle development.

Prevention of endotoxic death by a nociceptor-derived anti-microbial peptide.

痛覚神経由来の抗菌ペプチドによる敗血症死の抑制

○近藤 豪[1]、杉澤 恵利香[2]、丸山 健太[2,3]

[1]北海道大・院医・医化学、[2]大阪大・iFReC、[3]生理学研

Sepsis is a high-mortality disease in which dysregulated production of pro-inflammatory cytokines cause multi-organ defects and lead to endotoxic shock. Despite the extensive use of anti-inflammatory treatments (e.g. TNF-α antibody or glucocorticoids) in patients undergoing endotoxic shock, its mortality rates remain high at approx.30%, indicating that the mechanism of endotoxic death is only partially explained by uncontrolled inflammation. In this study, we identified an anti-microbial peptide, Reg3γ, as a protective factor against endotoxic death. During endotoxemia, nociceptor-derived Reg3γ penetrates into the brain and suppresses the kynurenine pathway. Endotoxin-administered nociceptor-null mice and nociceptor-specific Reg3γ-deficient mice exhibit a high mortality rate accompanied by an aberrant level of quinolinic acid and a decreased ATP production in the brain despite normal peripheral inflammation. Strikingly, the central administration of Reg3γ protects mice from endotoxic death. These findings provide novel insights into the molecular machinery of tolerance to endotoxic death.

Research on histone deacetylase 2-selective inhibitors for the treatment of synovial sarcoma

滑膜肉腫治療を目指したHDAC2特異的阻害剤の研究

○権藤 花奈[1]、広川 貴次[2]、吉田 将人[1]、平尾 巧[2]、木越 英夫[1]、竹中 聡[3]、岩崎 憲治[4]

[1]筑波大院数理物質、[2]筑波大医学医療系、[3]大阪国際がんセ、[4]筑波大TARA

Synovial sarcoma is a malignant tumor of the soft tissues. Although it is clear that the SS18–SSX fusion protein generated by t(X;18)(p11.2;q11.2) drives synovial sarcoma, the underlying mechanism remains unknown, making drug discovery research difficult. However, it was recently reported that inhibition of histone deacetylase (HDAC) 2 leads to the degradation of SS18-SSX. Unfortunately, HDAC inhibitors usually inhibit multiple HDACs simultaneously, and there is only one example of an HDAC2-selective inhibitor. Therefore, the development of inhibitors with high isozyme selectivity would be valuable. In this study, we aimed to design small-molecule compounds with significant differences in binding affinity between HDAC1 and HDAC2, which have very high sequence identity. To achieve this aim, we performed molecular dynamics calculations, organic synthesis, measurement of dissociation kinetics, and measurement of growth-inhibitory effects on synovial sarcoma cells. The molecular dynamics calculations of the structures of HDAC1 and HDAC2 without drug revealed that the pocket volume of the drug binding site is clearly larger in HDAC2, suggesting that selective inhibitors could feasibly be developed. Based on the pocket volume revealed by MD pocket analysis, we are designing drugs with high affinity for HDAC2.

Elevated lipid peroxidation products that are produced during cell cycle progression are involved in the execution of ferroptosis

細胞周期の進行に伴う活性酸素生成はフェロトーシスの実行に重要である

○本間 拓二郎[1,2]、小林 翔[3]、藤井 順逸[2]

[1]大阪公立大学・院医・分子病態薬理学、[2]山形大・院医・生化学分子生物学、[3]山形大・農

Ferroptosis is a newly characterized form of cell death that is caused by the accumulation of intolerable levels of lipid peroxides in the cell via an iron-mediated Fenton reaction. The resulting lipid peroxides that are generated in membrane phospholipids cause ferroptotic cell death by disturbing the integrity of the plasma membrane. Glutathione (GSH), a tripeptide redox molecule that contains a cysteine (Cys) unit in the center, plays a pivotal role in protection against ferroptosis. Cys is metabolically produced by the transsulfuration pathway in conjunction with methionine (Met) metabolism and may fulfil the requirement in some organs under normal physiological conditions. In addition to protein synthesis, Met is the precursor for S-adenosylmethionine (SAM), which donates a methyl group to several acceptor molecules such as DNA, RNA, proteins, and phospholipids. Cell growth is arrested in Met-free medium, which characteristically occurs in cancer cells. Because Met is an essential amino acid, a defect in Met supply could impair the protein synthesis required for cell cycle progression.

Despite the significant roles of Met in Cys metabolism and cancer biology, the interplay between Met metabolism and ferroptosis in cancer cells has not been fully investigated. We had speculated that a combined deprivation of Met and cystine induces ferroptosis more effectively in vitro. In the current study, however, we found the Met/cystine double deprivation strongly prevented the execution of ferroptosis under conditions of intracellular Cys/GSH starvation, which led to the survival of HeLa cells as well as Hepa 1–6 cells. Supplementation of SAM resulted in the increased production of peroxidized lipids and induced ferroptosis in cells under double deprived conditions. On the other hand, SAM supplementation also increased DNA methylation and allowed cell cycle progression to resume. These collective results reveal the pivotal roles of lipid peroxides, the concentrations of which are elevated during cell cycle progression, in ferroptosis execution under Cys starvation conditions.

The transcription factor SOX4 regulates phenotypic changes in epithelial keratinocytes

上皮ケラチノサイトのフェノタイプ変化における転写因子SOX4の役割

○長岡 良礼[1]、武石 幸容[1]、高橋 千代[1,2]、武田 佳奈[1,2]、岡村 和彦[3]、姚 遠[4]、本村 香織[4]、大徳 浩照[4]、八田 光世[1]
[1]福岡歯科大・細胞分子生物学講座・分子機能制御学分野、[2]福岡歯科大・歯・矯正歯科学分野、[3]福岡歯科大・歯・病態構造学分野、[4]筑波大・生存ダイナミクス研究センター

SOX4 is a member of the SOX (Sex-determining region Y-related high-mobility group box) family of transcription factors, and known to associated with the promotion of epithelial cell tumorigenesis, invasion, and metastasis. However, the role of SOX4 in epithelial keratinocytes remains elusive. In this study, we aim to investigate the involvement of SOX4 in the phenotypic changes and functional regulation of a human keratinocyte cell line (HaCaT).Firstly, we generated a SOX4 overexpressing cell line (Tet on SOX4 HaCaT) in which SOX4 expression is induced in the presence of doxycycline (Dox). Dox treatment impaired intercellular contact and altered the cells to exhibit a protruding morphology. Moreover, phalloidin staining revealed an increase in filopodia (filamentous cell membrane extensions by fibrous actin bundles)-like structures. qRT-PCR and western blotting revealed that SOX4 decreased the expression of epithelial markers (KRT13, CLDN1) and increased the expression of mesenchymal markers (Vim, FN1). Because the gene profiles may have been significantly altered, we performed comprehensive RNA sequencing analysis. Gene clustering and gene ontology analyses of differentially expressed genes revealed that Dox treatment of Tet on SOX4 HaCaT increased the expression of genes related to the cytoskeleton such as actin fiber formation and actin skeletal regulation. These findings indicate that SOX4 induces EMT-like phenotypic and cytoskeletal changes in epithelial keratinocytes.

Involvement of ceramide kinase in the formation of autophagosomes

オートファゴソームの形成におけるセラミドキナーゼの関与

○布能 英樹

千葉大・院薬・薬効薬理学研究室

Autophagy is the principal intracellular degradation pathway that helps maintain cell homeostasis by degrading harmful protein aggregates and damaged mitochondria. It is known that abnormalities in autophagy cause serious diseases such as neurodegenerative diseases and cancer. Thus, autophagy is a fundamental and essential phenomenon for living organisms, and knowing its mechanism is indispensable for developing disease treatment and prevention methods. The most significant feature of autophagy is the creation of a new double-membrane organelle called the autophagosome. However, the involvement of sphingolipids in the formation of autophagosomes is entirely unknown. Therefore, we investigated the involvement of ceramide kinase (CerK) in the formation of autophagosomes.

CerK is an enzyme that produces ceramide-1-phosphate (C1P) from the ceramide, “the central molecule in sphingolipid metabolism.” This study found that CerK knockout cells had lower basal autophagy levels than WT cells. In CerK-KO cells, the treatment with rapamycin or bafilomycin A1 delayed formation of autophagosomes compared to WT cells. Furthermore, the knockdown of CerK in WT cells resulted in a decrease in the number of autophagosomes. These results indicate that CerK is involved in the formation of autophagosomes.

Duloxetine suppresses nitric oxide production induced by lipopolysaccharide in BV-2 microglia cells

BV−2ミクログリア細胞におけるデュロキセチンのリポポリサッカライド誘導NO産生に対する影響の検討

○中谷 善彦[1]、矢口 真菜美[1]、荻野 和樹[1]、野口 理沙子[1]、山本 直樹[1,2,3]、天野 託[4]

[1]国際医療福祉大・薬、[2]国際医療福祉大・基礎医学研究センター、[3]東京都立大学・神経科学、[4]栃木県立岡本台病院

It is well known that both selective serotonin and serotonin noradrenaline reuptake inhibitors can improve the symptoms major depressive disorder to increase the concentration for monoamine in the synaptic cleft based on the monoamine hypothesis. However, accumulating evidence has indicated that inflammation in the brain may be a key factor in the pathophysiological mechanisms of developing symptoms of major depressive disorder. In this study, we focused on whether duloxetine can show the ameliorative effect for inflammatory response induced by lipopolysaccharide (LPS) in BV-2 microglial cells. Our results indicated that duloxetine decreased the NO production induced by LPS in a concentration-dependent manner. The increase in the protein expression level of iNOS by LPS was decreased in a concentration-dependent manner by duloxetine treatment. Moreover, the increase of the protein expression levels of phosphorylated-I κ B α , phosphorylated-Akt and Akt by LPS were also decreased. Unexpectedly, the protein expression levels of other pro-inflammatory factors such as COX-2 and phosphorylation ratios for various molecules including I κ B α and Akt were not altered by the treatment of duloxetine. These findings suggest that duloxetine may also act as an anti-inflammatory agent, which could contribute to its therapeutic effectiveness.

Lactoferrin enhances neurite outgrowth via TrkA receptor in PC12 cells.

ラクトフェリンはPC12細胞のTrkA受容体を介して神経突起伸長を促進する

○水上 乃愛[1]、長嶋 大地[2,3]、古川 恵[3]、東方 優大[4]、日塔 武彰[4]、速水 耕介[1,2]、出雲 信夫[2,4]

[1]横浜薬科大・薬・機能性物質学研、[2]横浜薬科大・薬・総合健康メディカル研究セ、[3]横浜薬科大・薬・薬学教育セ、[4]横浜薬科大・薬・薬物治療学研

Lactoferrin (LF) is a multifunctional protein which have the antibacterial and immunomodulatory action in the milk of mammals. Recent studies have been investigated the effect of improvement of cognitive impairment in the central nerve system. However, the mechanisms underlying the effects of LF on neuron have not yet been elucidated. The aim of present study investigated the efficacy of LF on the cell outgrowth effect using by PC12 cells, and demonstrated to mechanisms of LF.

PC12 cells were treated with different concentration of LF (100 to 1000 μg/mL) for maximum of 72 h, and were treated with 50 ng/mL of nerve growth factor (NGF) as a positive control. Digital images of cells were taken with a phase-contrast inverted microscope with a camera at 24 and 72 hours after treatment of either LF or NGF. Total ERK and phosphorylated ERK expression levels were analyzed by western blotting, and the inhibitors were used AG879 and PD98059.

The cell outgrowth was significantly increased at 72 hours after NGF treatment. Treatment of 250 μg/mL LF was significantly increased all of the neurite length, neurite joint, and neurite branching (pass) compared to the non-treated group. The ratio of total ERK and phosphorylated ERK expression levels was maximized at 5 minutes after treatment of LF, and was persisted up to 10 minutes. These observed activities were inhibited by AG879 and PD98059.

This present study clarified that the role of LF may enhance the cell outgrowth via activation of phosphorylated ERK after 5 minutes, and it suggested to undergo nerve outgrowth via the same pathway of NGF.

Linalool, an essential oil component of *lavender*, inhibits the activation of nociceptive transient receptor potential ankyrin 1 (TRPA1) and voltage-gated Ca^{2+} channels in mouse sensory neurons

ラベンダー精油成分リナロールによる侵害受容性TRPA1チャネルと電位依存性カルシウムチャネルに対する抑制作用

○橋本 美穂、高橋 賢次、太田 利男

鳥取大・院・獣医薬理

Linalool, an essential oil component of *lavender* is commonly used in fragrances. It is known that linalool has anxiolytic, sedative, and analgesic actions. However, the mechanism of its analgesic action is not fully clear. Pain signals elicited by the activation of nociceptors on peripheral neurons are transmitted to the central nervous system. In this study, we focused the effects of linalool on TRP channels and voltage-gated channels, both of which are important for pain signaling via nociceptors in somatosensory neurons. For detection of channel activity, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using a Ca^{2+} imaging system and membrane currents were recorded by a whole-cell patch-clamp technique. In mouse dorsal root ganglion neurons linalool did not affect $[Ca^{2+}]_i$ responses to capsaicin and acids, TRP vanilloid 1 (V1) agonists. On the other hand, linalool suppressed the increases of $[Ca^{2+}]_i$ induced by allylisothiocyanate and carvacrol, TRPA1 agonists. In heterologously expressed channels, linalool suppressed $[Ca^{2+}]_i$ responses to TRPA1 agonists but not those to TRPV1 agonists. Linalool attenuated the $[Ca^{2+}]_i$ responses to high concentrations of KCl and voltage-gated Ca^{2+} currents but only slightly suppressed voltage-gated Na^+ currents. These results suggest that linalool exerts an analgesic action via the suppression of the nociceptive TRPA1 channel and voltage-gated Ca^{2+} channels.

TRPV4 activation prevents lipopolysaccharide-induced painful bladder hypersensitivity in rats.

Lipopolysaccharide誘発性膀胱炎の炎症制御におけるTRPV4の役割

○善積 克、渡辺 千寿子、溝口 広一

東北医科薬科大・薬

Although most studies have reported that activation of transient receptor potential vanilloid 4 (TRPV4) contributes to bladder pain and overactive bladder with a cardinal symptom of acute or chronic cystitis, its involvement in the protective response against bacterial infection in various cultured cells including urothelial cells has also been reported. In the present study, we investigated the potential benefit of intravesical TRPV4 agonist for painful bladder hypersensitivity produced by a rat model of LPS-induced cystitis and whether its effects modulate the LPS signal for cytokine release and macrophage phenotype change. The increased bladder pain-related behaviors and voiding frequency caused by LPS were suppressed by concurrent injection into the rat bladder of a selective TRPV4 agonist, GSK1016790A. Moreover, the production and secretion of proinflammatory cytokines (e.g., CXCL1, CXCL10), are suppressed by the presence of GSK1016790A. Furthermore, TRPV4 activation switched the LPS-stimulated pro-inflammatory M1-type macrophage to the anti-inflammatory M2-type macrophage. These results suggest that that activation of TRPV4 in bladder regulates the proinflammatory response by LPS, and TRPV4 functions may be a promising future therapeutic target for refractory chronic cystitis.

Roles of pannexin 1 in the trigeminal ganglion in orofacial mechanical allodynia following infraorbital nerve injury in rats

眼窩下神経の障害が誘発したラットの口腔顔面領域の機械的アロディニアにおける三叉神経節のpannexin 1の役割

○栗栖 諒子[1]、三枝 禎[2]、青野 悠里[2]、林 良憲[3]、人見 涼露[3]、前田 茂[4]、嶋田 昌彦[1]、岩田 幸一[3]、篠田 雅路[3]
[1]東京医科歯科大・病院・歯科ペインクリニック、[2]日本大・松戸歯・薬理、[3]日本大・歯・生理、[4]東京医科歯科大・院医歯・歯科麻酔・口腔顔面痛制御学

ATP and glutamate (Glu) are known to be released into the extracellular space through pannexin 1 (Panx1) channels in the cell membrane. Neuronal Panx1 contributes to the development and maintenance of peripheral inflammation-induced tactile hypersensitivity. In order to study mechanisms of orofacial neuropathic pain, we analyzed the role of Panx1 in the trigeminal ganglion (TG) of rats with infraorbital nerve injury (IONI). Male SD rats were used. We measured mechanical head-withdrawal threshold (MHWT) in IONI rats receiving an intra-TG Panx1 inhibitor or a metabotropic Glu receptor 5 (mGluR5) antagonist and in naive rats receiving intra-TG mGluR5 agonist administration post-IONI. Glu and Panx1 in TG were measured post-IONI. Panx1, mGluR5 and Glu synthetase expression in TG were analyzed immunohistochemically and changes in the number of mGluR5-$P2X_3$-expressed TG neurons were evaluated. MHWT was decreased after IONI and this decrease was reversed by the Panx1 inhibitor and the mGluR5 antagonist. The mGluR5 agonist decreased MHWT. IONI increased extracellular Glu in TG. Panx1 was expressed in satellite glial cells and TG neurons, and intra-TG mGluR5 antagonism decreased the number of mGluR5- and $P2X_3$-positive TG neurons post-IONI. The present results indicate that IONI facilitates Glu release via Panx1 that activates mGluR5 expressed in nociceptive TG neurons innervating the orofacial region. In turn, $P2X_3$ receptor-expressed TG neurons are enhanced via mGluR5 signaling, resulting in orofacial neuropathic pain.

Repagermanium (Ge-132) capable of trapping H_2S and ATP relieves paclitaxel-induced peripheral neuropathy in mice

H_2SおよびATP補捉作用を有するrepagermanium（Ge-132）はマウスにおけるパクリタキセル誘発性末梢神経障害を抑制する

○関口 富美子[1]、安達 義史[1]、島田 康弘[2]、中村 宜司[2]、川畑 篤史[1]

[1]近畿大・薬・病態薬理、[2]浅井ゲルマニウム研究所

We have reported that the enhanced activity of $Ca_v3.2$ T-type Ca^{2+} channels caused by endogenous H_2S participates in the paclitaxel (PCT)-induced peripheral neuropathy (PIPN), and that neuron-derived ATP promotes the PCT-induced macrophage (Mϕ) infiltration followed by extracellular release of HMGB1, a damage-associate molecular pattern protein, leading to PIPN development. On the other hand, we have found that repagermanium (i.e. Ge-132), once hydrolyzed into 3-(trihydroxygermyl)propanoic acid (THGP), can trap H_2S, in addition to ATP, and inhibit H_2S-induced enhancement of $Ca_v3.2$ activity and pain. Thus, we evaluated effects of THGP on PIPN in mice. In the mice treated with PCT repeatedly, daily i.p. administration of THGP at 100 mg/kg prevented PIPN development and Mϕ accumulation in the sciatic nerves. In Mϕ-like RAW264.7 cells, THGP at 10 mM inhibited the cell migration caused by ATP at 0.1 mM, but not extracellular HMGB1 release caused by ATP at 1 mM. Finally, a single i.p. administration of THGP at 100 mg/kg, as well as TTA-A2, a Ca_v3 inhibitor, at 1 mg/kg, reversed the established PIPN in mice. These data suggest that THGP prevents PIPN development by trapping ATP that promotes PCT-induced perineuronal Mϕ accumulation and reverses PIPN most probably by trapping H_2S that enhances $Ca_v3.2$ activity.

A TRPV1 antagonist AMG517 alleviates abnormal pain sensitivity and improves social deficits in the prenatal valproic acid-induced mouse model of autism

TRPV1アンタゴニストAMG517は胎生期バルプロ酸投与誘発の自閉症モデルマウスにおいて痛覚感受性の異常と社会性行動障害を改善する

○田原 孟[1]、今戸 瑛二[2,3]、川瀬 啓生[4]、樋口 桃子[4]、山川 英訓[5]、小川 公一[5]、古武 弥一郎[1]、田熊 一敞[6,7]、橋本 均[4,7,8,9,10]、浅野 智志[2]、吾郷 由希夫[2]

[1]広島大・院医・生体機能分子動態、[2]広島大・院医・細胞分子薬理、[3]広島大・院医・歯科麻酔、[4]大阪大・院薬・神経薬理、[5]塩野義製薬、[6]大阪大・院歯・薬理、[7]大阪大・院連合小児発達、[8]大阪大・データビリティフロンティア機構、[9]大阪大・先導的学際研究機構、[10]大阪大・院医・分子医薬

Individuals with autism spectrum disorders (ASDs) show neurobehavioral deficits, characterized by impairments in social interactions, repetitive behaviors, as well as wide sensory abnormalities. We have previously demonstrated that prenatal exposure to valproic acid (VPA) at embryonic day 12.5 (E12.5) causes autism-like behavioral abnormalities in male mouse offspring. We have also found that prenatal VPA exposure causes long-lasting mechanical allodynia and spinal microglial activation. In the present study, we aimed to investigate the role of the transient receptor potential vanilloid type 1 (TRPV1), a highly validated pain target, in abnormal pain sensitivity and social interaction deficits in prenatal VPA-treated mice. Behavioral analyses revealed that prenatal VPA-treated mice exhibited thermal hyperalgesia, mechanical allodynia and increased capsaicin-induced paw licking. A single administration of AMG517, a TRPV1 antagonist, alleviated both hyperalgesia and mechanical allodynia. Additionally, AMG517 reversed deficits in social behaviors in prenatal VPA-treated mice and also increased c-Fos expression, a marker for neuronal activity, in the nucleus accumbens. These findings suggest that prenatal exposure to VPA might alter TRPV1-mediated signaling and TRPV1 antagonists have a potential to treat certain cases of ASDs.

Inhibition of histone deacetylase *in utero* causes spinal microglial activation and mechanical allodynia in mice

胎生期のヒストン脱アセチル化酵素阻害は脊髄ミクログリアの活性化と機械的アロディニアを引き起こす

○今戸 瑛二[1,2]、Sun Samnang[3]、Huynh Ngoc Bao Tran[3]、中村 庸輝[4]、中島 一恵[4]、森岡 徳光[4]、木口 倫一[5]、浅野 智志[1,3]、吾郷 由希夫[1,3]

[1]広島大・院医・細胞分子薬理、[2]広島大・院医・歯科麻酔、[3]広島大・歯、[4]広島大・院医・薬効解析科学、[5]和歌山県医大・薬・生体機能解析

Valproic acid (VPA) is an anticonvulsant drug that is approved for use in epilepsy and bipolar disorder, but it also acts as a histone deacetylase (HDAC) inhibitor. We have previously demonstrated that prenatal exposure to VPA at embryonic day 12.5 (E12.5) causes autism-like behavioral abnormalities in mouse offspring. We have also found that prenatal VPA exposure causes long-lasting mechanical allodynia and spinal microglial activation. In the present study, we examined the effects of prenatal exposure to trichostatin A (TSA), a potent and specific inhibitor of HDAC class I/II, on tactile sensitivity and microglial morphology in the spinal cord. Pregnant ICR mice were intraperitoneally injected with either TSA (1 mg/kg) or vehicle on E12.5. Both male and female offspring of TSA-treated mothers (defined as TSA-treated mice) showed a significant decrease in withdrawal threshold in the von Frey test. The numbers of microglia in laminae I-IV and V-VI of the spinal cord dorsal horn in TSA-treated mice were increased compared with those in control mice. Increases in average intensity and cell area per microglia in laminae I-IV and V-VI were also observed in TSA-treated mice. These findings suggest that inhibition of HDAC during pregnancy causes mechanical allodynia associated with spinal microglial activation.

Disulfiram produces potent anxiolytic-like and antinociceptive effects in rodents

ジスルフィラムはモデル動物において強力な抗不安様作用・鎮痛作用を示す

○太田 有紗[1]、寺島 裕也[2]、山田 大輔[1]、中谷 百伽[1]、山内 つぐみ[1]、坂田 壮太[1,2]、松浦 航太[1,2]、藤塚 亮次[1,2]、重本 千宙[1]、吉岡 寿倫[1]、松島 鋼治[2]、斎藤 顕宜[1]

[1]東京理科大・薬・薬理学研究室、[2]東京理科大・生命医科学研究所・炎症・免疫難病制御部門

Disulfiram (DSF) is an FDA approved drug for the treatment of alcoholism. The drug acts by inhibiting aldehyde dehydrogenase, an enzyme essential to alcohol metabolism. However, a recent study has demonstrated that DSF also potently inhibits the cytoplasmic protein FROUNT, a common regulator of chemokine receptor CCR2 and CCR5 signaling. Several studies have reported that chemokine receptors are associated with the regulation of emotional and pain behaviors in rodents. Therefore, this study was performed to clarify these effects of DSF in rodents. The anxiolytic-like and antinociceptive effects of DSF were investigated using an elevated plus-maze (EPM) test and a formalin test, respectively. DSF significantly increased the amount of time spent in the open arms of the maze without affecting the total open arms entries in the EPM test. Moreover, DSF decreased the duration of pain-related behaviors in the formalin test. However, no effect in both EPM and formalin tests was seen following administration of the selective aldehyde dehydrogenase inhibitor cyanamide. These results suggested that DSF produces anxiolytic-like and antinociceptive effects in rodents. We propose that the inhibitory activity of DSF against FROUNT function provides an effective therapeutic option in anxiety accompanied with pain.

Protective role for calcitonin gene-related peptide in atherosclerosis progress in ApoE knockout mice

ApoEノックアウトマウスのアテローム性動脈硬化症の進行におけるカルシトニン遺伝子関連ペプチドの保護的役割

○井上 翔太[1]、川嶋 心[2]、橋川 直也[1]、橋川 成美[1]

[1]岡山理科大・大学院理学研究科、[2]岡山理科大・理

The calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide, and its various functions such as being a potent vasodilator, being a cytoprotectant, and inhibiting macrophage infiltration have been reported. However, its role in atherosclerosis remains unclear. We investigated whether CGRP has a role in atherosclerosis development in apolipoprotein E–deficient mice. We have previously reported double-knockout ApoE$^{-/-}$:CGRP$^{-/-}$ (DKO) mice, increased serum cholesterol levels, atherosclerotic plaque areas, and migration functions in peritoneal macrophages with increase in the level of inflammatory cytokine TNF α. Herein, we investigated whether inactivating TNF α improves atherosclerosis in DKO mice. We also investigated whether results similar with those of DKO could be obtained by administering a humanized monoclonal CGRP antibody, galcanezumab, to ApoE knockout (ApoE KO) mice. ApoE KO male mice and DKO male mice were fed a high-fat diet for 8 weeks, and effects on lesion size and macrophage functions were assessed after 2 weeks. The etanercept was intraperitoneally administered once a week (5 mg/kg), which caused significant reduction in the aortic root and atherosclerotic plaque area in DKO mice. ApoE KO mice were subcutaneously administered with galcanezumab once a week (50 mg/kg). Furthermore, TNF α inhibition reduced the macrophage migration. The galcanezumab increased atherosclerotic lesions similar in DKO mice. These results suggested that CGRP plays a critical role in inhibitory effect on atherosclerosis progression.

Chronic volume overload and aldosterone provide arrhythmogenic substrates in the rat atria via potentiation of proarrhythmic responses to cholinergic activation

慢性容量負荷とアルドステロンはコリン作動性の催不整脈応答増強を介してラット心房筋に催不整脈性基質を提供する

○相本 恵美、冨永 理紗、永澤 悦伸、高原 章

東邦大・薬・薬物治療

Cholinergic responses of atria have been known to play an important role in vagally-mediated atrial fibrillation (AF). We investigated influence of chronic volume overload and long-term exposure to higher plasma levels of aldosterone on electrophysiological responses to cholinergic activation in the rat atria. Rats were divided into three groups based on receiving sham surgery, aorto-venocaval shunt (AVS) surgery to deliver chronic volume overload, or AVS plus aldosterone administration (1.0 μg/h, i.p.) using an osmotic minipump. Four weeks later of the AVS surgery, hypertrophy as well as prolongation of atrial effective refractory period (AERP) were observed in the isolated heart. Carbachol at 0.1 and 1.0 μM shortened AERP, reflecting proarrhythmic response, which was potentiated by AVS and further enhanced by AVS plus aldosterone. Carbachol increased intra-atrial conduction velocity, reflecting antiarrhythmic response, which was also potentiated by AVS operation but attenuated by AVS plus aldosterone. These results suggest that chronic volume overload and aldosterone provide arrhythmogenic substrates in the rat atria via potentiation of proarrhythmic responses to cholinergic activation. Attenuation of antiarrhythmic response by AVS plus aldosterone may also modify the generation of arrhythmogenic substrates in the rat atria.

Involvement of the prostanoid EP_2 receptor stimuation in the glial cell-mediated vasodilation in the rat retina *in vivo*

ラット網膜におけるグリア細胞を介する血管拡張にはプロスタノイド EP_2 受容体刺激が関与する

○森 麻美、関 陽香、水越 聖、上園 崇、坂本 謙司

帝京大・薬・医薬品作用

We have previously found that nitric oxide (NO) derived from neuronal cells acts on glial cells and causes vasodilation in the rat retina via release of epoxyeicosatrienoic acids (EETs) and prostaglandins. The aim of this study was to identify the prostanoid receptors involved in the NO-induced glial cell-derived vasodilation in the rat retina.

We used male Wistar rats to examine the effects of intravitreal pretreatment of indomethacin, a cyclooxygenase inhibitor, PF-04418948, a prostanoid EP_2 receptor antagonist, and CAY10441, a prostanoid IP receptor antagonist, on the changes in the retinal arteriolar diameter induced by intravitreal injection of NOR3, an NO doner. The retinal arteriolar diameters were measured using the ocular fundus images captured with a high-resolution digital camera *in vivo*.

Increase in the retinal arteriolar diameter induced by intravitreal injection of NOR3 was significantly suppressed by intravitreal pretreatment of indomethacin and PF-04418948, but not CAY10441.

These results suggested that activation of arachidonic acid cascade and subsequent stimulation of prostanoid EP_2 receptors are involved in the rat retinal vasodilatory responses evoked by NO-induced glial cells stimulation. Thus, glial cells-derived prostaglandin E_2 may play important roles in the retinal vasodilatory mechanisms.

Actions of Myosin Modulators on Contractility and Ca^{2+} Transients in Healthy and Failing Human Hearts

ミオシンモジュレーターの健常並びに不全ヒト心臓における収縮力及びCa^{2+}トランジェントへの作用

Abi-Gerges Najah、Sweat Katrina、Truong Ky、Stafford Alexa、Miron Yannick、Roup Ana、Mai Christina、林 隆志、Page Guy、Ghetti Andre

AnaBios Corporation

Understanding the impact of myosin modulators (myotropes) on human heart biology is key to the successful development of drugs targeting heart failure (HF) and obstructive hypertrophic cardiomyopathy (OHCM). Omecamtiv mecarbil (OM; positive myotrope; Ph3 for HFrEF) increased contractility (EC_{50}=0.6μM) in isolated ventricular myocytes from organ donors, while negative myotropes exerted differential effects: Blebbistatin (BBS), N-benzyl-p-toluene sulphonamide and mavacamten (MAVA; approved for OHCM) decreased contractility (IC_{50}s 2.6, 16 and 0.2μM, respectively), hydroxy-BBS, (S)-3'-aminoBBS and para-amino-BBS caused biphasic responses. While none of the myotropes impacted peak amplitude of Ca^{2+} transients, controls increased (isoproterenol) and decreased (verapamil) peak Ca^{2+} amplitude. Moreover, OM generated similar increases in isometric force of ventricular trabeculae across healthy, HFrEF and HFpEF donor hearts. In contrast to OM, MAVA generated different force decreases between normal and HFpEF vs. HFrEF trabeculae. Thus, our data support the use of primary human heart preparations early in development to maximize clinical success of the most promising novel myotropes through evaluation of mechanism of action, structure-activity relationship stratification, and differential impact across related disease-states.

The Curcumin Analogue GO-Y022 Suppresses Pressure Overload-induced Systolic Dysfunction

クルクミン類似体GO−Y022は心機能低下を改善した

○平子 裕太[1]、砂川 陽一[1,2,3]、清水 果奈[1,2]、船本 雅文[1,2]、刀坂 泰史[1,2,3]、浜辺 俊英[1,2,3]、柴田 浩之[4]、小見山 麻紀[1]、長谷川 浩二[1,2]、森本 達也[1,2,3]

[1]静岡県立大・薬・分子病態学分野、[2]国立病院機構京都医療センター ・展開医療研究部、[3]静岡県立総合病院・臨床研究部、[4]秋田大・医学系研究科・臨床腫瘍学講座

【Introduction】We previously found that a natural p300 HAT inhibitor, curcumin (CUR), can inhibit cardiomyocyte hypertrophy and the development of heart failure *in vivo*. We focused on a CUR analog, GO-Y022, which shows stronger anti-cancer activity than CUR. The purpose of this study was to determine whether GO-Y022 inhibits p300-HAT activity and can be used as a therapeutic agent for heart failure.

【Methods & Results】*In vitro* HAT assay using recombinant p300-HAT domain showed that GO-Y022 inhibited p300-HAT activity as well as CUR. Primary cultured cardiomyocytes prepared from neonatal rats were treated with GO-Y022 or CUR and then stimulated with phenylephrine (PE) for 48 hours. One μM of GO-Y022 suppressed PE-induced histone H3K9 acetylation, hypertrophic response gene transcription, and cardiomyocyte hypertrophy to the same extent as 10 μM of CUR. C57BL/6j male mice were subjected to transverse aortic constriction (TAC) or sham operation. The TAC mice were randomly assigned to five groups: Vehicle, CUR at 1 or 50 mg/kg, or GO-Y022 at 0.2 or 1 mg/kg. After 8 weeks daily oral treatment, echocardiographic analysis showed that 1 mg/kg of GO-Y022 and 50 mg/kg of CUR improved a TAC-induced increase in left ventricular posterior wall thickness and a decrease in fractional shortening.

【Conclusion】These results indicate that GO-Y022 strongly inhibits both PE-induced hypertrophic responses and pressure overload-induced development of heart failure. These findings suggest that GO-Y022 may be a novel candidate agent for heart failure therapy.

Inhibition of transient outward K^+ current: potential prevention of reentrant arrhythmia developments in long QT syndromes

一過性外向きK^+チャネル電流の抑制：QT延長症候群におけるリエントリー性不整脈発生の予防可能性

○津元 国親[1]、島本 貴生[2]、青地 悠馬[2]、九田 裕一[1]、谷田 守[1]、姫野 友紀子[2]、天野 晃[2]、倉田 康孝[1]

[1]金沢医科大・医、[2]立命館大・生命科学部・生命情報学

Excessive prolongation of cardiac action potential duration (APD) is a risk factor for lethal ventricular arrhythmias. Previously, we have elucidated the underlying mechanisms for the development of early afterdepolarization (EAD)-mediated premature ventricular complexes (PVCs), leading to the occurrence of reentrant ventricular tachycardia in congenital or acquired long QT syndromes. However, a method to prevent EAD-mediated PVC developments has not yet been established. This study aims to theoretically determine the effect of inhibition of the transient outward K^+ channel current (I_{to}) on the development of EAD-mediated PVCs. Our previous study (doi:10.1254/jpssuppl.95.0_3-P-226) investigated the relationship between EAD and PVC initiation using a 6 × 6 cm myocardial sheet model consisting of 360,000 human ventricular myocyte model units (Kurata et al., Biophys J, 2005). In the present study, we examined the effect of I_{to} inhibition on PVC initiation under PVC onset conditions by performing simulations of excitation propagation. Intriguingly, only 10% inhibition of I_{to} prevented the development of reentrant arrhythmias evoked by EAD-mediated PVCs. Inhibition of I_{to} by 30% or more completely suppressed EAD developments, resulting in the prevention of PVC initiation. Based on our results, I_{to} inhibitions may prevent EAD development-mediated reentrant arrhythmias in the long QT syndrome.

Comparison of voluntary exercise and renin angiotensin inhibitor administration in a mouse model of dilated cardiomyopathy

拡張型心筋症モデルマウスにおける自発運動とレニン・アンギオテンシン阻害剤投与の比較

○杉原 匡美[1]、柿木 亮[2,5]、村山 尚[3]、三井田 孝[1,6]、櫻井 隆[3]、森本 幸生[4]、呉林 なごみ[3]

[1]順天堂大・医・臨床検査医学、[2]順天堂大・スポーツ健康科学部、[3]順天堂大・医・薬理学、[4]国際医療福祉大・福岡保健医療学、[5]城西国際大・経営情報学部、[6]順天堂大・医療科学・臨床検査

Dilated cardiomyopathy (DCM) is one of major causes of heart failure (HF). Although exercise is regarded as one of therapies for HF, the effects of exercise on patients with DCM have not been established. A knock-in mouse model of human inherited DCM, TNNT2 ΔK210, shows similar characteristics to DCM patients. We have showed that one of angiotensin receptor blockers (ARB) is effective on cardiac function and electrical remodeling and found that voluntary exercise also improves cardiac function in homozygote model mice (DCM mice). In this study, we examined combination therapy in DCM mice. The DCM mice showed enlarged heart and frequent sudden death with t1/2 of ~70 days. Male DCM mice were divided into 4 groups based on the administration of ARB and voluntary exercise: without drug or exercise (control), oral administration of ARB (ARB), daily exercise (ex), and combined ARB and exercise (comb). The ex and comb groups started wheel running at 1 month of age. Cardiac function was measured with echocardiography at 2.5 months of age. After sacrifice, weights of body, heart, lung, and lower extremity muscles were measured. Gene expressions of HF- and arrhythmia-related genes in myocardium were quantified by qPCR analysis. On echocardiography, the ejection fraction was significantly improved in only comb-group (Cont: 21.7±6.1%(n=6), ARB: 26.9±5.1% (n=6), ex: 27.2±4.5%(n=5), comb: 31.9±3.6%(n=6)). On the other hand, the heart weight/body weight ratio decreased in the ARB and the comb groups. Our results indicate a synergistic effect between ARB and voluntary exercise to cardiac function in DCM.

Comparative Study of Transcriptome in the Hearts Isolated from Mice, Rats, and Humans

マウス、ラットおよびヒトにおける心臓トランスクリプトームの比較研究

○岡本 洋介[1]、岡田 大瑚[2]、小林 大礎[1]、尾野 恭一[1]、石井 邦明[3]

[1]秋田大学大学院、[2]京都大・院医・ゲノム医学センター、[3]山形大・医・薬理学講座

The heart is a critical organ for maintaining life in mammals and has long been one of the most important targets of scientific research, and the basic molecular mechanisms of heart beat appear to have already been established. However, few studies have focused on species differences, and challenges remain in studying genes that have universal functions across species and genes that determine species differences. Here, we analyzed transcriptome data from mouse, rat, and human atria, ventricles, and sinus node (SA) and calculated and compared specificity measure (SPM) values that account for species differences among the three cardiac regions. SA has the largest species differences and we searched for a gene, which by our criteria was SHOX2; the SPM value for SHOX2 was prominently high across species. Similarly, SPM values identified 3 atrial-specific markers, 11 ventricular-specific markers, and 17 SA-specific markers. Ontology analysis identified 70 cardiac region- and species-specific ontologies. These results suggest that reanalysis of existing data by calculating SPM values may identify novel tissue-specific and species-dependent gene expression. This study demonstrates that SHOX2 is an SA-specific transcription factor, a novel cardiac region marker, and that species-dependent ontology is important. No COI.

Mode of prolongation in the early (J-T_{peak}) and late (T_{peak}-T_{end}) repolarization can predict onset pattern and probability of spontaneous termination of following torsade de pointes attack

早期（J–T_{peak}）および後期（T_{peak}–T_{end}）再分極時間の延長様式が引き続き発生するtorsade de pointes発作の開始様式と自然停止確率を予測する

○神林 隆一[1]、後藤 愛[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: I_{Kr} inhibitors can prolong the repolarization, and may develop trigger (premature ventricular contraction: PVC) and substrate (spatial dispersion of repolarization) for the onset of torsade de pointes (TdP). We investigated how the mode of preceding changes in the early (J-T_{peak}) and late (T_{peak}-T_{end}) repolarization represent the characteristics of TdP.

Methods: Well-known torsadogenic drug hydroxychloroquine in doses of 1, 3 and 10 mg/kg/10 min was intravenously administered to the chronic atrioventricular block dogs under the monitoring of Holter electrocardiogram (n=4 for each dose).

Results: While the low or middle dose of hydroxychloroquine did not induce TdP, the high dose induced TdP in each animal at 10.0 (#1), 8.7 (#2), 9.3 (#3) and 11.3 (#4) min after the start of administration. The R on T-type PVCs were frequently induced in the animal #1, #2 and #3 before the onset of TdP, which was not observed in the animal #4. The TdP spontaneously terminated in the animal #1, while it degenerated into ventricular fibrillation in the others. The changes of J-T_{peak} just before the onset of PVC (#1, #2, and #3) or TdP (#4) were +37, +36, +23 and +1 ms, whereas those of T_{peak}-T_{end} were +12, +20, +22 and +89 ms, respectively.

Discussion: The prolongation of J-T_{peak} may trigger R on T-type PVCs possibly through Ca^{2+} overload, whereas the prolongation of T_{peak}-T_{end} will develop the substrate for maintaining spiral reentry.

Involvement of regional specificity of glial cells in neuronal alpha-synuclein expression and neurodegeneration in parkinsonian models

パーキンソン病モデルにおけるα−シヌクレイン発現と神経変性へのグリア細胞部位特異性の関与

○宮崎 育子[1]、菊岡 亮[1]、磯岡 奈未[1]、十川 千春[2]、十川 紀夫[3]、北村 佳久[4]、浅沼 幹人[1]

[1]岡山大・院医歯薬・脳神経機構、[2]広島工業大・生命・生体医工、[3]松本歯科大・院歯・遺伝子工学・分子創薬、[4]就実大・薬・薬物治療

Exposure to pesticides, such as rotenone or paraquat, increases the risk of Parkinson's disease (PD). Various studies revealed the link between pesticide toxicity and cellular pathology in PD such as alpha-synuclein (alpha-Syn) aggregation and neuronal death. Recently, we demonstrated region-specific astrocyte-microglia interaction promoted rotenone-induced non-cell-autonomous dopaminergic neurodegeneration. In this study, we examined effects of regional difference in glial cells on neuronal alpha-Syn expression and neurotoxicity. We prepared mesencephalic neuronal culture and glial cell culture (astrocyte+microglia) from mesencephalon or striatum of SD rats embryos (E15). Treatment with conditioned media from mesencephalic, but not striatal, glial cell culture upregulated alpha-Syn expression in mesencephalic neurons, which were dopaminergic, serotonergic and GABAergic neurons. Conditioned media from rotenone-exposed mesencephalic, but not striatal, glial cells increased alpha-Syn expression in dopaminergic and serotonergic neurons; however, neuronal damage was observed only in the dopaminergic neurons. These results suggest that regional specificity of glial cells could contribute to neuronal alpha-Syn expression, and that some factors in addition to alpha-Syn accumulation are involved in dopaminergic neurodegeneration.

Mechanisms underlying mutant astrocyte-mediated demyelination in Alexander disease

アレキサンダー病において変異アストロサイトが引き起こす髄鞘脱落のメカニズム

○久保田 友人[1,2]、繁冨 英治[1,2]、齋藤 光象[1,2]、篠崎 陽一[1,2]、小林 憲司[1,2]、田中 謙二[3]、池中 一裕[4]、大野 伸彦[5]、小泉 修一[1,2]

[1]山梨大・院医・薬理、[2]山梨大・院医・GLIAセンター、[3]慶應大・医・先端医脳科学、[4]生理学研・分子神経生理、[5]自治医大・医・解剖

Alexander disease (AxD), a rare neurodegenerative disease, is caused by the mutation of *GFAP* gene (Brenner et al., *Nat Genet.*, 2001) encoding glial fibrillary acidic protein (GFAP) which is enriched in astrocytes. Thus, AxD is a primary astrocyte disease. AxD patients mainly show severe neurological symptoms such as psychomotor developmental delay, motor deficits etc. with white matter disorders (leukodystrophy). However, molecular pathogenesis that leads from astrocytic mutations to leukodystrophy and further to these clinical manifestations are not well understood. Here, we show that AxD astrocytes directly cause demyelination in corpus callosum (CC) using AxD model mice carrying human mutant GFAP with R239H (Tanaka et al., *GLIA*, 2007). First, we found by immunohistochemical analysis that myelinated area stained with either myelin basic protein or proteolipid protein in CC was markedly decreased in AxD compared to wild-type (WT) mice. Second, the number of astrocytes was increased in CC of AxD compared to WT. In addition, AxD astrocytes with Rosenthal fiber, a hallmark of AxD, were highly accumulated in demyelinated area. Such astrocytic accumulation was not observed in CC of WT. These spatial correlation of AxD astrocytes with demyelination of CC would suggest that pathological astrocytes may be directly involved in local demyelination in CC. Third, we performed RNAseq analysis of AxD astrocytes and found that Galectin-3 and lipocalin 2 (Lcn2) were the top 10 most up-regulated genes (Saito & Shigetomi et al., *GLIA*, 2018). Interestingly, both Galectin-3 (Morizawa et al, *Nat Commun.*, 2017) and Lcn2 (Wan et al., *Nat Commun.*, 2022) could positively control astrocytic phagocytosis. Together, all these findings suggest that AxD astrocytes may cause demyelination by acquiring their abnormal phagocytic ability in CC, thereby leading to leukodystrophy and various neurological symptoms.

Astrocytes in the PVN are involved in corticotropin-releasing factor-induced sympathetic activation in rats

室傍核アストロサイトは脳内コルチコトロピン放出因子による交感神経系活性化に関与する

○山口 奈緒子、岡田 尚志郎

愛知医大・医・薬理

Corticotropin-releasing factor (CRF) plays a key role in stress responses in the brain. CRF activates not only the hypothalamic-pituitary-adrenal axis but also the sympathetic nervous system. We previously reported that both central administration of CRF and exposure to acute restraint stress (RS) increase plasma catecholamine levels and activate presympathetic neurons in the paraventricular hypothalamic nucleus (PVN). However, it is unclear whether glial cells in brain regions including the PVN are involved in CRF- or stress-induced sympathetic activation. In this study, we examined roles of astrocytes in the CRF-induced elevation of plasmas catecholamine levels (noradrenaline and adrenaline) using fluorocitrate which blocks glial metabolic function. Intracerebroventricular pretreatment of fluorocitrate suppressed the CRF-induced elevation of plasma levels of noradrenaline and adrenaline. On the other hand, microinjection of fluorocitrate into the PVN suppressed the CRF-induced elevation of adrenaline, but not of noradrenaline. Furthermore, we tried to examine effects of inhibition of astrocytes on RS-induced sympathetic activation. Our results suggest that astrocytic function in the brain is important for the CRF-induced sympathetic activation and the effects might be brain region-dependent.

Long-term stimulation of α7 nicotinic acetylcholine receptor selectively suppresses thrombin-polarized M1 microglia

α7ニコチン性アセチルコリン受容体の長期刺激はトロンビンによって分極したM1ミクログリアを選択的に抑制する

○大西 正俊、町田 葵、井上 敦子

福山大・薬

The effect of nicotine on impaired M1 and protective M2 microglial polarization was investigated using BV-2 cell line. Alpha 7 nicotinic acetylcholine receptor (α7 nAchR) expression was transiently increased by nicotine treatment and then it was gradually decreased for 14 days. Treatment with nicotine for 14 days slightly polarized naïve M0 microglia to CCL1-positive M2b and inducible NO synthase (iNOS)-positive M2d subtypes. Conversely, arginase1-positive M2c microglia was decreased. On the other hand, exposure of thrombin recruited iNOS- and interleukin-1β-double positive M1 microglia. Long-term treatment with nicotine significantly decreased thrombin-induced iNOS mRNA and oppositely showed the tendency to increase the arginase1 mRNA level. From the effect of nicotine on M0 microglia, it is unlikely that nicotine has shifted from iNOS-positive M1 to arginase1-positive M2 subtype. Thus, the selective cell death of M1 microglia was presumed. The mitogen-activated protein kinase (MAPK)s pathway has previously shown to be involved in the survival of thrombin-activated microglia. Treatment with nicotine for 14 days suppressed thrombin-phosphorylated p38 MAPK through the α7 nAchR. These results suggest that long-term stimulation of α7 nAchR causes suppression of thrombin-activated p38 MAPK followed by possible apoptosis in M1 microglia.

Suppressive effects of glatiramer acetate on methamphetamine-induced preference with microglial osteopontin upregulation in mice

グラチラマー酢酸塩のミクログリアのオステオポンチン発現を伴ったメタンフェタミン場所嗜好性行動の抑制

○泉尾 直孝、玉谷 隆典、中島 卓海、高橋 晃、浅野 昂志、新田 淳美

富山大・薬・薬物治療

[Background] Methamphetamine (METH) is a widely distributed addictive drug in the world. Since there is no effective treatment for METH addiction, novel therapeutic agents are required. Previously, we reported that microinjection of osteopontin (OPN), immune-modulating extracellular matrix protein, to the nucleus accumbens reduced METH preference behavior (*Sci. Rep.* 2017;7(1):13084) The study has offered a possibility that upregulation of osteopontin in the brain is a therapeutic strategy for the drug addiction. Here, we focused on the glatiramer acetate (GA), which is reported to increase OPN in the brain (*Brain Behav. Immun.* 2017;67:163-180) and examined the effects of GA on METH preference. [Methods] Mice (C57BL/6J, 8-9 weeks, male) were received subcutaneous GA (0.1 mg) administration for 14 days before and during behavioral experiments. METH (1 mg/kg) was treated to these mice during behavioral experiments. OPN-positive cells were detected in the flowcytometry experiments. [Results] GA suppressed METH-induced conditioned place preference, not hyperlocomotion. Number of OPN-positive cells increased by GA administration, and most cells were microglia. [Discussion] GA ameliorated preference behavior induced by METH, which was possibly mediated by microglial OPN upregulation. GA could be a candidate of a therapeutic agent for drug addiction.

Evaluation of oligodendrocyte precursor cell properties in a 3-dimension (3D) culture system

三次元培養環境におけるオリゴデンドロサイト前駆細胞の機能評価

○中野 静香[1,2,3]、植田 尭子[1]、松永 行子[2,3]、村松 里衣子[1]

[1]精神神経セ・神経研・神経薬理、[2]東大院・工・バイオ、[3]東大生研

Axons in the central nervous system (CNS) are wrapped with myelin formed by oligodendrocytes. CNS damage causes demyelination which leads to neurological dysfunction. Remyelination is a regenerative process, which is mediated by oligodendrocyte precursor cells (OPCs) development. Mechanisms underlying OPCs development have been extremely investigated with in vitro models using 2-dimension (2D) culture systems. Although 3D culture easily offers model mimicking physical and mechanical properties of cell adhesion or microenvironment in vivo, little is known about the difference of OPCs properties between in 2D and in 3D culture. Here, we characterized the phenotypic difference of OPCs cultured in between 2D and a collagen-based 3D culture system. OPCs isolated from murine mixed glial culture were embedded in type I collagen gel (3D, final concentration: 2.4 mg/mL) or plated on glass substrate (2D), respectively. Immunocytochemical analysis showed a significantly low level in the percentage of $Ki67^+$ cells in $Olig2^+$ cells in 3D compared with 2D, suggesting that OPCs have less proliferative capacity in 3D. When differentiation was induced by triiodothyronine, a low level of the expression of myelin basic protein was observed in 3D compared to 2D, suggesting that OPCs have less differentiation capacity in 3D. Collagen gel can easily control the stiffness by change in concentration, therefore, these results provide crucial insights to understand OPCs response in 3D culture, which contributes to develop a 3D model mimicking pathological conditions.

Postischemic voluntary running exercise promotes the survival of astrocytes born after cerebral ischemia and alters astrocytic gene expression

脳梗塞後の自発運動は脳梗塞後に新生したアストロサイトの残存を促進し、遺伝子発現を変化させる

○山口 菜摘、澤野 俊憲、中谷 仁、田中 秀和

立命館大・院生命科学

Cerebral ischemia causes neuronal damage and functional impairment. Dendritic spine dynamics is involved in functional recovery. We have previously reported that voluntary running exercise after focal cerebral ischemia ameliorates the ischemia-induced dendritic spine loss in the peri-infarct motor cortex layer 5. Environmental change surrounding neurons affects the neuronal morphologic plasticity. The aim of this study is to reveal the effect of postischemic exercise on the glial phenotypes. We found that voluntary running exercise promoted the survival of astrocytes born after middle cerebral artery occlusion (MCAO) until postoperative day 15. Transcriptome analysis of astrocytes detected 10 upregulated genes and 70 downregulated genes in exercise group compared with non-exercise group. Gene ontology analysis showed that the downregulated genes were related to apoptosis and neuronal morphology. Exercise tended to decrease the expression of *Lipocalin 2* known to reduce dendritic spines in astrocytes after MCAO. Our data suggest that voluntary running exercise after cerebral ischemia alters astrocytic gene expression, which contributes to the amelioration of the ischemia-induced dendritic spine loss.

Role of astrocytes in nicotine-induced motor excitement symptoms

ニコチンによる運動興奮症状におけるアストロサイトの関与

○國澤 直史、加藤 将貴、小田 明奈、白川 美波、坂口 茉鈴、清水 佐紀、大野 行弘
大阪医科薬科大・薬・薬品作用解析学

Nicotine elicits motor excitement symptoms such as tremor and convulsive seizures. We performed behavioral studies in combination with the brain mapping analysis of glial fibrillary acidic protein (GFAP), a marker of astrocytic activation, to clarify the role of astrocytes in nicotine-induced tremor and convulsive seizures. Intraperitoneal (i.p.) injection of nicotine induced straub tail and tremor at 0.3 or 1 mg/kg, and convulsive seizures at 3 mg/kg in mice. Immunohistochemical examination revealed that nicotine region-specifically elevated GFAP expression in the piriform cortex (PirC) at 1 mg/kg (i.p.), and CA3 area and dentate gyrus (DG) of hippocampus at 0.3-3 mg/kg (i.p.). We next evaluated the effect of fluorocitrate (FC), an astrocyte inhibitor, on nicotine-induced tremor and convulsive seizures. Intracerebroventricular injection of FC (1 nmol) reduced GFAP expression in PirC, basolateral amygdala, CA2, CA3 and DG. Whereas FC did not affect the generation of nicotine tremor, it caused a significant decrease in seizure intensity and a decreased incidence of seizures. These results suggest that astrocytic activation by nicotine in PirC, CA3 or DG is involved in nicotine-induced convulsive seizures.

Microglial replacement ameliorates Rosenthal fiber accumulation in Alexander disease, a primary astrocyte disease.

ミクログリア置換によって、一次性アストロサイト疾患であるアレキサンダー病のローゼンタール線維の蓄積が改善される。

○小林 憲司[1,2]、繁冨 英治[1,2]、檀上 洋右[1,2]、Parajuli Bijay[1,2]、久保田 友人[1,2]、齋藤 光象[1,2]、田中 謙二[3]、池中 一裕[4]、小泉 修一[1,2]

[1]山梨大・院医・薬理学、[2]山梨大・院医・GLIAセンター、[3]慶應義塾大・医・先端研・脳科学、[4]生理学研・分子神経生理

Alexander disease (AxD) is a rare neurodegenerative disease caused by the pathogenic variants of *GFAP*, an astrocyte-specific intermediate filament. This resulted in the formation of aberrant inclusions within astrocytes called Rosenthal Fibers (RFs), one of the main pathological hallmarks. In addition, activation of microglia (MG) is also observed in the AxD brain. We previously reported a beneficial role of MG using the AxD model mice, overexpressing mutant human GFAP. Here, we enhanced this beneficial function of MG by replacing MG using PLX5622 (PLX), a CSF-1R antagonist. Turning PLX ON and OFF causes MG removal and repopulation respectively (Rep), which allows us to replace old MG with repopulated MG (Rp-MG) in the AxD mice. Interestingly, the RFs were significantly reduced by Rep, indicating that Rep rescued the AxD pathology. We further analyzed the bulk RNA-sequencing data and found that lysosomal and phagocytic pathways were highly enhanced by Rep. Additional histological analysis revealed that Rp-MG strongly expressed CD68 and engulfed RFs, suggesting Rep could increase MG phagocytic capacity to remove RFs. Overall, the MG replacement could restore the AxD pathology by increasing their phagocytic capacity against RFs.

Establishment of a screening method for the developmental neurotoxicity of chemicals using the image analysis of the dynamics of neurons, astrocytes, and microglia in zebrafish

ゼブラフィッシュのニューロン、アストロサイト、およびミクログリア動態イメージングを利用した神経発達毒性評価系の確立

○弓削 瑞葵[1]、若井 恵里[1]、小岩 純子[1]、白水 崇[1]、駒田 致和[2]、西村 有平[1]

[1]三重大・院医・統合薬理学、[2]近畿大・理工・発生生物学

A wide variety of substances that may affect the development of the central nervous system (CNS) in human exist in environment. Evaluating how chemical teratogens adversely affect the developing brain may provide important insights not only for the management of industrial chemicals but also for the prevention and treatment of CNS diseases. However, a system for efficiently and comprehensively evaluating the developmental neurotoxicity (DNT) of chemicals has not yet been sufficiently established. Here, we try to establish the assessment of DNT analyzing the dynamics of neurons, astrocytes, and microglia in developing zebrafish CNS, with particular focus on the property of microglia reflecting neuroinflammation. We generated a transgenic zebrafish line expressing different fluorescent proteins; Cerulean for neurons, mCherry for astrocytes, and mVenus for microglia. Subsequently, we exposed various neurotoxicants to zebrafish embryo and performed in vivo fluorescence imaging. We are trying to quantitatively measure the total amount, distribution, and morphology of neurons, astrocytes, and microglia in the larval zebrafish brain by imaging analysis and detect DNT comparing with already reported findings.

Miyako Bidens Pilosa extract regulates microglia polarization in a mouse model of amyotrophic lateral sclerosis at advanced-stage

宮古ビデンス・ピローサエキス末は、進行期の筋萎縮性側索硬化症モデルマウスにおいてミクログリア極性を制御する

○鶴田 こむぎ、設樂 尊人、高橋 愛、宮岸 寛子、小菅 康弘

日本大・薬

Bidens pilosa is generally used as ethnomedicine and functional food worldwide. We have reported previously that Miyako Island Bidens pilosa extract (MBP) suppresses glial activation and prolongs the life span in the $SOD1^{G93A}$ transgenic mouse model of Amyotrophic Lateral Sclerosis (G93A). However, the therapeutic mechanisms of MBP remain unclear. In the present study, we investigated the microglia activation and polarization profile in the spinal cord of G93A. Real-time PCR revealed that oral administration of MBP at 2 g/kg/day for 7 days was inhibited the induction of M1-microglial markers (CD11c, IFN-γR) and inflammatory cytokines (TNF-α, IL-1β, IL-6). In contrast, the increased expression of M2-microglial markers (IL-13R, Ym1) and anti-inflammatory cytokines (TGF-β, IL-10) was not affected. Moreover, we determined the effect of MBP on cell proliferation using BV2 microglia cell line. Exposure of BV2 cells to MBP, resulted in a reduction of MTT reduction activity without increase the number of ethidium homodimer-1 stained dead cells. We also found that MBP suppressed the LPS-induced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) production. These results suggest that MBP regulates neuroinflammation through suppressing the microglia polarizing into M1 type.

GPR143 expressed in the dorsal striatum positively regulates behavioral responsiveness to dopamine D2 receptor agonist quinpirole.

背側線条体に発現するL-DOPA受容体GPR143は、ドーパミン D2 受容体作動薬キンピロール応答を正に制御する。

○大瀧 百々代、増川 太輝、北村 慧、井上 美優、田近 伶、荒井 柾美、五嶋 良郎

横浜市立大・院医・分子薬理神経生物学

L-3,4-dihydroxyphenylalanine (L-DOPA) by itself has been believed to be an inert amino acid that exerts action and effectiveness in Parkinson's disease through its conversion to dopamine by aromatic L-amino acid decarboxylase. We propose that L-DOPA is a neurotransmitter. We recently identified a G-protein coupled receptor 143 (GPR143), as a receptor for L-DOPA. GPR143 is distributed in some specific brain regions including striatum and substantia nigra in which dopamine receptors are highly expressed. To investigate the role of GPR143 in dopaminergic transmission, we examined the effect of quinpirole, a dopamine D2 receptor (D2R) agonist, on mouse behavior. Quinpirole (0.03 mg/kg, i.p.) decreased locomotor activity in wild-type (WT) mice. We found that this behavioral response to quinpirole was attenuated in Gpr143 gene-deficient (Gpr143-/y) mice when compared to WT mice. In contrast, SKF81297 (2.5 mg/kg, i.p.), a D1R agonist, increased locomotor activity in Gpr143-/y mice to a similar extent to that in WT mice. To validate GPR143 function after its depletion, we performed rescue experiment of GPR143 using adeno-associated virus encoding GPR143-P2A-enhanced green fluorescent protein (EGFP) or EGFP in Gpr143-/y mice. The decreased responsiveness to quinpirole in Gpr143-/y mice was rescued by re-expression of GPR143 in the dorsal striatum. These results indicate that GPR143 positively regulates D2R function in the dorsal striatum.

Characteristics of α-synuclein uptake at the blood-brain barrier

血液脳関門におけるαシヌクレインの取り込み特性

○道具 伸也[1]、横谷 みき[1]、高田 芙友子[1]、岩尾 卓朗[1]、松本 純一[2]、佐野 和憲[3]

[1]福岡大・薬・応用薬剤、[2]福岡大・薬・生物薬剤、[3]福岡大・薬・生体機能制御

Parkinson disease (PD) is characterized by widespread distribution of aggregated α-synuclein (α-Syn) protein in inclusions known as Lewy bodies. α-Syn is secreted from neurons and transferred to neighboring cells. This cell-to-cell transmission is thought to underlie the progress of PD. In addition, it is known that blood α-Syn levels in patients with PD are elevated and blood-borne α-Syn can penetrate into the brain across the blood-brain barrier (BBB). Therefore, another explanation for elevated brain levels of α-Syn is the increased transport of α-Syn from blood to brain across the BBB. Here, we investigated how monomeric α-Syn is taken up by brain endothelial cells which constitute the BBB. We assessed uptake of α-Syn by brain endothelial cells as cell/medium ratio using primary cultures of rat brain endothelial cells (RBECs). Increasing concentrations of α-Syn resulted in the increased cellular accumulation of α-Syn in RBECs. The cell/medium ratio did not show significant changes with the increased concentration of extracellular α-Syn ranging from 0.05 to 10 μg/mL, suggesting that α-Syn uptake by RBECs is independent of a saturable transport system. However, the α-Syn uptake by RBECs showed a temperature-dependent manner, suggesting that α-Syn uptake by RBECs is mediated by an energy-dependent transport system. This uptake was inhibited by mannan. These results suggest that α-Syn transport at the BBB would be mediated by a mannan-sensitive transport system including mannose receptor-mediated transcytosis.

Role of hyperpolarization activated cyclic nucleotide-gated (HCN) channel 1 in modulating seizure susceptibility to pilocarpine

ピロカルピン誘発けいれんの感受性調節におけるHCN1チャネルの役割

○石﨑 悠斗[1]、清水 佐紀[1]、毎田 愛莉[1]、森山 芽衣[1]、佐納 匠[1]、宮越 樹里[1]、庫本 高志[2]、大野 行弘[1]

[1]大阪医薬大・薬・薬品作用解析学、[2]東京農業大・農・動物栄養

Hyperpolarization activated cyclic nucleotide-gated (HCN) channel 1 is abundantly expressed in cerebral cortex, hippocampus, and cerebellum, regulating spontaneous rhythm and neural oscillation. Previous study reported that human mutations in the *HCN1* gene are associated with early infantile epileptic encephalopathy and genetic generalized epilepsy (Brain, 141, 3160-3178, 2018), however, the functional mechanism underlying seizure generation is still unknown. In this study, we examined the effects of HCN1 channel inhibitor (ZD7288) and the *HCN1* gene deletion on pilocarpine-induced seizure to elucidate the role of HCN1 channel in modulating seizure susceptibility. Treatment of mice with ZD7288 significantly increased the incidence of status epilepticus and the frequency of seizures as compared with control mice. *Hcn1*-KO rats showed a higher seizure susceptibility to pilocarpine than control (F344) rats. Thus, pilocarpine elicited status epilepticus in all *Hcn1*-KO rats tested, but in none of control rats. In addition, we performed the immunohistochemical analysis of c-Fos expression following pilocarpine-induced seizure. *Hcn1*-KO rats showed a significantly higher c-Fos expression than control rats, notably in the cerebral cortex and amygdala. These results suggest that HCN1 channels play an important role in modulating the susceptibility to epileptic seizures, implying that dysfunction of HCN1 channels cause neuronal hyperactivation of the cerebral cortex and amygdala.

p-Hydroxyamphetamine induced prepulse inhibition disruptions is modulated by serotonergic and adrenergic receptors

p-Hydroxyamphetamine誘発性プレパルスインヒビション障害はセロトニンおよびアドレナリン受容体により調節される

○小野木 弘志[1]、中川西 修[2]、根本 亙[2]、三反崎 聖[3]、丹野 孝一[2]、只野 武[4]

[1]東北福祉大・健康科学・保健看護、[2]東北医科薬科大・薬・薬理、[3]高崎健康福祉大・薬・分子神経科学、[4]金沢大・院医薬保健・環境生態医・公衆衛生

Amphetamine metabolite *p*-hydroxyamphetamine (*p*-OHA) has been shown to have many pharmacological effects, including causing psychostimulant-induced behaviors such as hyperlocomotion and head-twitch response. We have previously reported that *p*-OHA induces prepulse inhibition (PPI) disruptions in mice, and the PPI disruptions are involved in the dopaminergic system. In this study, we investigated the involvement of the serotonin (5-HT) and noradrenaline (NA) neurotransmission in *p*-OHA-induced PPI disruptions in mice. As a result, *p*-OHA-induced PPI disruptions were attenuated by pretreatment with ketanserin (a $5\text{-HT}_{2A/2C}$ receptor antagonist), MDL100,907 (a selective 5-HT_{2A} receptor antagonist), 5,7-dihydroxytryptamine (a 5-HT neurotoxin), *p*-chlorophenylalanine (a 5-HT synthesis inhibitor) and prazosin (a selective α_1 receptor antagonist), but were not attenuated by pretreatment with DSP-4 (a NA neurotoxin), fuzaric acid (a NA synthesis inhibitor). These results suggest that the 5-HT neurotransmission is strongly involved in *p*-OHA-induced PPI disruptions, with ancillary involved of α_1 receptors.

Neuroprotective effects of leukotriene receptor blockers on the secretory phospholipase A_2-induced neuronal apoptosis

分泌型ホスホリパーゼA_2によるアポトーシスに対するロイコトリエン受容体遮断薬の神経保護効果

○矢上 達郎、山本 泰弘、高馬 宏美

姫路獨協大・薬

Neurological diseases *e.g.* brain ischemia are associated with mammalian secretory phospholipase A_2 ($sPLA_2$). The group IB $sPLA_2$ ($sPLA_2$-IB) induced neuronal cell death via apoptosis, which were accompanied with chromatin condensation and DNA fragmentation. Previously, we had established the $sPLA_2$-IB-induced neuronal apoptosis as the *in vitro* model for cerebral ischemia. Prior to neuronal apoptosis, $sPLA_2$-IB generates leukotrienes (LTs) via 5-lipoxygenase (LOX), which are increased in the ischemic brain. Recently, we reported that LOX inhibitors prevented neurons from the toxicity of $sPLA_2$-IB, suggesting an involvement of LTs to the neurotoxicity of $sPLA_2$-IB. In the present study, we evaluated protective effects of receptor antagonists for LTB_4 (LY293111) and cysteinyl LTs (ONO-1078) in the primary culture of rat cortical neurons. The two LT receptor antagonists suppressed the neurotoxicity of $sPLA_2$-IB in a concentration-dependent manner. $sPLA_2$-IB shrank neuronal cell bodies and shortened neurites Both LY293111 and ONO-1078 ameliorated morphological degenerations such as shrank neuronal cell bodies and shortened neurites in the $sPLA_2$-IB-treated neurons. Furthermore, these LT receptor blockers prevented neurons from the $sPLA_2$-IB-condensed chromatin and fragmented DNA, suggesting an involvement of LTs in the $sPLA_2$-IB-induced neuronal apoptosis.

Purinergic receptor-dependent induction of long-term potentiation of inhibitory synaptic transmission in the rat insular cortex

ラット島皮質抑制性シナプス伝達におけるプリン受容体を介した長期増強

○小助川 聖史[1]、山本 清文[1]、小林 真之[1]

[1]日本大・歯・薬理学講座、[2]日本大・歯・歯科矯正学講座

Adenosine triphosphate (ATP) is released from glial cells in the central nervous system, and induces plastic changes in synaptic transmission. Despite the recent progress in terms of the roles of purinergic receptors in cerebrocortical excitatory synaptic transmission, their contribution to inhibitory synaptic transmission has been unknown. Setting the ultimate goal to clarify the role of ATP in neuroplastic changes in inhibitory synaptic transmission in the cerebral cortex, this study aimed to elucidate the effects of ATP and α,β-methylene ATP ($\alpha\ \beta$ mATP), a selective agonist of P2X receptors (P2XR), on inhibitory synaptic transmission in the insular cortex (IC). We performed multiple whole-cell patch-clamp recordings from pyramidal cells (PYR) in IC of the VGAT-Venus transgenic rats. Administration of $\alpha\ \beta$ mATP (100 μM) increased the amplitude of unitary IPSC (uIPSC). Interestingly, $\alpha\ \beta$ mATP increased the amplitude of miniature IPSCs (mIPSCs), which sustained even after washout of $\alpha\ \beta$ mATP. The P2XR-dependent potentiation was blocked by intracellular application of an NMDA receptor (NMDAR) antagonist, MK801. These results suggest that the activation of the P2XR-NMDAR pathway induces long-term potentiation of IPSCs in the IC.

Brain cannabinoid CB_1 receptors suppress centrally administered bombesin-induced facilitation of the rat micturition

ボンベシン脳室内投与による排尿促進に対し脳内カンナビノイドCB_1受容体は抑制作用を示す

○清水 孝洋[1]、鄒 瑣[1]、山本 雅樹[2]、清水 翔吾[1]、清水 信貴[3]、東 洋一郎[1]、吉村 直樹[4]、齊藤 源顕[1]

[1]高知大・医・薬理、[2]高知大・医・小児思春期医学、[3]高知大・医・骨盤機能セ、[4]ピッツバーグ大・医・泌尿器科学

To clarify roles of brain cannabinoid CB_1 receptors in regulation of the micturition, we examined the effects of intracerebroventricularly (icv) administered rimonabant (Rimo, a CB_1 antagonist) and JZL195 (JZL, an inhibitor of enzymes related to degradation of endogenous CB receptor ligands) on icv administered bombesin (BB, a stress-related neuropeptide)-induced facilitation of the micturition in urethane-anesthetized (0.8 g/kg, ip) male Wistar rats. A catheter was inserted into the bladder to perform cystometry. Three hours after the surgery, JZL (10 or 30 nmol/rat) or Rimo (100 or 300 nmol/rat) was icv administered 30 min before BB administration (0.01 or 0.03 nmol/rat, icv). BB (0.03 nmol) shortened the intercontraction interval (ICI), an indicator of micturition frequency, while JZL significantly suppressed the BB-induced response. BB at a lower dose (0.01 nmol) showed no significant effect on ICI, while the BB significantly shortened ICI after pretreatment with Rimo. In addition, JZL-induced suppression of the BB (0.03 nmol)-induced response was abolished in the presence of Rimo. These results indicate that brain CB_1 receptors suppress the centrally administered BB-induced facilitation of the rat micturition.

Construction of the ELISA assay to quantify Semaphorin 3A in the adult brain

成体脳におけるセマフォリン3A発現を定量するELISAシステムの確立

○古川 涼音[1]、小林 瑞季[1]、林 克儀[2]、中村 史雄[3,4]、櫻井 隆[5]、五嶋 良郎[3]、山下 直也[1,3,5]

[1]神奈川工科大・応用バイオ、[2]カイオム・バイオサイエンス、[3]横浜市立大・医、[4]東京女子医科大・医、[5]順天堂大・医

Extracellular soluble signals that control several aspects of neuronal development are known to play a critical role in maintaining neuronal function and homeostasis in the mature nervous system. Abnormal expression and/or secretion of these molecules are therefore thought to be associated with the onset of various types of neurological disorders. It has been reported that the expression of Semaphorin 3A (Sema3A), a secreted type of repulsive axon guidance molecule, is impaired in several neurodegenerative disorders. However, due to the lack of a reliable Sema3A antibody, our knowledge about Sema3A expression in the adult brain is still limited. Here we report the identification of a pair of Sema3A monoclonal antibodies for the sandwich ELISA assay using the Autonomously Diversifying Library system. Both Sema3A monoclonal antibodies recognize the Sema domain of human Sema3A and can measure recombinant Sema3A in the range of 0-100 pM by ELISA. The specificity of this assay was confirmed by using the embryonic brains from *sema3A* deficient mice as a negative control. Moreover, this assay could measure Sema3A concentration in Tris buffered saline-and SDS-soluble lysate obtained from the adult mice brains. These data suggested that our ELISA assay is a reliable tool for the validation of Sema3A as a biomarker in neurodegenerative disorders.

The effect of neurotrophin-3 overexpression in the hippocampus

海馬におけるニューロトロフィン−3過剰発現の影響

○笠倉 奈々美、村田 優花、染谷 僚太、田端 遼、瀬木（西田） 恵里
東京理科大・院先進工学・生命システム工学

Neurotrophin-3 (NT-3) is a type of neurotrophic factor expressed in the hippocampus. It has been reported that NT-3 was upregulated by stress and corticosterone administration in the hippocampus of adult mice. Since stress suppresses neurogenesis in the hippocampus, we examined whether increasing NT-3 expression suppresses neurogenesis. We also investigated the effects of increased NT-3 expression on mature neuron function and stress-induced behavior. We generated NT-3 overexpressing mice in the hippocampus by administering adeno-associated virus carrying NT-3 gene. Expression of NT-3 in the hippocampus was higher more than 35-fold higher than in the control group. After 4weeks of NT-3 carrying virus administration, the number of proliferating cells and immature neurons in the hippocampal dentate gyrus was decreased in the NT -3 overexpression group. On the other hand, calbindin expression was increased in mature hippocampal neurons. In behavioral experiments, locomotor activity and anxiety-like behavior tended to be increased in the NT-3 overexpression group. These results suggest that high-dose NT-3 may be involved in the suppression of stress-induced neurogenesis and anxiety-like behavior.

Low serum oxytocin levels and autism spectrum disorder-like behaviors in mice lacking the cannabinoid CB_1 receptors

カンナビノイドCB_1受容体遺伝子欠損マウスにおける血清オキシトシンの低値と自閉スペクトラム症様行動の発現

○縄田 陽子[1]、安作 美香[1]、西奥 剛[1]、山口 拓[2]

[1]長崎国際大・薬・薬理、[2]長崎国際大・薬・薬物治療

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disability that demonstrates impaired social interactions, social communication deficits, and restrictive/repetitive behaviors. It is reported that children with ASD and some animal models of ASD show the abnormality of endocannabinoid (eCB) systems. To determine the causal role of the eCB systems in the ASD, we have investigated the relationship between the eCB system and ASD-like symptoms, using the cannabinoid CB_1 receptor knockout (CB1KO) mice. We found that male CB1KO mice demonstrated reduced sociability (3-chambered social approach task) and elevated repetitive grooming behaviors (hole-board test). Moreover, CB1KO mice showed resistance to change a learned pattern of behavior (reversal learning task using T-maze). On the other hands, the serum oxytocin, reported as lower levels in autistic children, significantly decreased in CB1KO mice. These findings suggest that CB1KO mice show abnormal behavioral phenotypes and endocrine system including social deficits, repetitive behaviors, cognitive inflexibility and low serum oxytocin levels, which have face and construct validity as an animal model for ASD. Therefore, the CB1KO mice will be a valuable tool for the exploration of pathological mechanisms and development of novel therapeutics in the ASD.

Study of the molecular mechanism of autophagy activation *via* nicotine receptors in a cellular model for Parkinson's disease

パーキンソン病細胞モデルにおけるニコチン受容体を介したオートファジー活性化の分子機構に関する研究

○滝沢 進之佑、藤牧 綾香、大内 一輝、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] Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) have increasingly been realized to have common cellular and molecular mechanisms including protein aggregation and inclusion body formation. In the present study, to examine whether the activation of a7 nicotinic receptor has neuroprotective effect against PD-associated mutant a-synuclein-induced aggregation and neurotoxicity in a cellular model, we used nicotine and PNU 282987, an a7 nicotinic receptor agonist.

[Methods] To investigate the effect of nicotine and PNU 282987 against mutant a-synuclein aggregates, we evaluated the protein level of a-synuclein in a cellular model of PD.

[Results] We succeeded in generating a new α-Syn stably expressing cell line using a piggyBac transposon system. Using this system, we investigated the neuroprotective effect of the nicotine and PNU 282987 on a-synuclein toxicity. We found that PNU 282987 provided significant protection against a-synuclein-related neurotoxicity. These results suggest that nicotine and PNU 282987 reduced intracellular aggregate *via* the activation of autophagy.

[Conclusions] Nicotine as well as PNU 282987 against a-synuclein-induced aggregation *via* the activation of autophagy are an efficient source of new treatment and prevention for synucleinopathies.

Docosahexaenoic acid (DHA) selectively inhibits prostanoid TP receptor-mediated contractions of guinea pig tracheal smooth muscle

ドコサヘキサエン酸（DHA）はプロスタノイドTP受容体を介したモルモット気管平滑筋の収縮反応を選択的に抑制する

○小原 圭将、稲葉 理花子、川北 美礼、De Dios Regadera Montserrat、植竹 智美、村田 梓、西岡 菜々子、黒木 孝太、追川 俊哉、吉岡 健人、田中 芳夫

東邦大・薬・薬理

Docosahexaenoic acid (DHA) is an n-3 polyunsaturated fatty acid abundant in fish oil. Chronic administration of DHA has been reported to improve asthma due to its anti-inflammatory effects. However, the immediate effects of DHA on isolated tracheal smooth muscle (TSM) contractility have not been studied. In this study, we investigated the potential inhibitory effects of DHA on the guinea pig TSM contractions. DHA (3×10^{-5} M) significantly inhibited TSM contractions induced by U46619 (a thromboxane A_2 (TXA_2) mimetic, 10^{-8} M) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 5×10^{-7} M). The TSM contractions induced by U46619 (10^{-8} M) and $PGF_{2\alpha}$ (5×10^{-7} M) were significantly inhibited by SQ 29,548 (a prostanoid TP receptor antagonist, 10^{-6} M). DHA (4×10^{-5} M/6×10^{-5} M) shifted the concentration-response curves (CRCs) for U46619 and $PGF_{2\alpha}$ to the right in a concentration-dependent manner. However, the slope of the regression line in the Schild plot of DHA *vs.* U46619/$PGF_{2\alpha}$ was larger than unity. In contrast, DHA (4×10^{-5} M) did not significantly affect the CRCs for acetylcholine, histamine, and leukotriene D_4. These findings indicate that DHA selectively inhibit prostanoid TP receptor-mediated TSM contractions. DHA may have preventive and ameliorative effects on asthma attacks associated with TSM hyper-contraction caused by TXA_2 and $PGF_{2\alpha}$.

The ROCK signaling regulates the pulmonary vascular permeability via maintenance of lung homeostasis.

ROCKによる肺血管透過性調節機構の解明

○赤嶺 孝祐、寺林 健、佐々木 隆子、石崎 敏理

大分大・医・薬理

Rho-associated kinase (ROCK), including the ROCK1 and ROCK2 isoforms, is a protein kinase involved in signaling from Rho to actin cytoskeleton. Many studies on ROCK functions in cultured cells have been reported. However, functions of ROCK in vivo remain incompletely understood, because targeted disruption of the ROCK1 and ROCK2 genes leads to embryonic death. In this study, using a tamoxifen-induced Cre-loxP system, we deleted ROCK1 and ROCK2 in adult mice (ROCK double conditional knockout mice: ROCK DcKO) and explore the in vivo functions of ROCK.

ROCK DcKO mice exhibited poor survival and were observed pulmonary leakage of blood cells after tamoxifen injection. To confirm the increase of vascular permeability in ROCK DcKO, we carried out vascular permeability assay using Evans blue dye. As a result, massive leakage of Evans blue dye was observed in the lung tissue of ROCK DcKO compared with that of control mice. In addition, the number of neutrophils was increased in broncoalveolar lavage fluid (BALF) in ROCK DcKO mice. In the lung tissue of ROCK DcKO mice, the intensity of phalloidin staining and VE-cadherin staining was obviously reduced. These results indicated that pulmonary vascular permeabilities were increased in ROCK DcKO mice. Furthermore, we found that deletion of ROCKs led to induce the proinflammatory cytokine gene expression through NF-κB and AP-1 by microarray analysis and qPCR.

Thus, our study suggest ROCK might be involved in maintenance of lung homeostasis though actin cytoskeleton rearrangement.

The effects of dasatinib on corticosteroid insensitive airway inflammation in mice induced by tobacco smoke

マウスのタバコ主流煙誘発ステロイド治療抵抗性気道炎症に対するdasatinibの効果

○西本 裕樹[1]、安藤 大稀[1]、入江 孝祐[1]、開沼 郁美[1]、片山 侑紀[1]、佐藤 しおり[1]、鈴木 智大[1]、原田 真衣[1]、吉田 翼[1]、木村 元気[1]、Ito Kazuhiro[2]、木澤 靖夫[1]

[1]日本大・薬・機能形態学、[2]NHLI, Imperial College London, United Kingdom

Chronic obstructive pulmonary disease (COPD) is characterized by corticosteroid insensitive airway inflammation. Tobacco smoke (TS) induces oxidative stress and activation of Src, which cause airway inflammation. Recently, we reported that dasatinib (DAS), a Src inhibitor, suppressed airway inflammation in mice induced by lipopolysaccharide. Then, in this study, we determined the effects of DAS alone and the combination with quercetin, an antioxidant agent, on TS induced airway inflammation.

A/J mice were exposed to TS for 11 days, followed by intranasal treatments with DAS, quercetin and fluticasone propionate (FP) twice daily for 3 days. Bronchoalveolar lavage fluid (BALF) was collected one day after the last drug treatment, and the numbers of inflammatory cells in BALF were measured by hemocytometers and a flow cytometer.

TS induced the significant increases in the numbers of neutrophils and macrophages in BALF, and FP had no effect on these increases. DAS improved the corticosteroid insensitive airway inflammation in mice induced by TS. In addition, the combination of DAS with quercetin showed further suppressive effects on airway inflammation in mice than DAS alone. These results suggested that the combination of Src inhibitors with antioxidant agents may be a novel therapeutic strategy for the treatment of COPD.

RAMP1 signaling attenuates acute lung injury by inhibiting cytokine production and neutrophil recruitment

RAMP1シグナルは，サイトカイン産生と好中球誘導を阻害することで急性肺傷害(ALI)を抑制する

○山下 敦[1,2]、伊藤 義也[1]、松田 弘美[2]、長田 真由子[1]、田邉 美奈[1]、古江 明子[1]、細野 加奈子[1]、畑中 公[1]、辻川 和丈[3]、馬嶋 正隆[1,4]、岡本 浩嗣[2]、天野 英樹[1]

[1]北里大・院医療・分子薬理、[2]北里大・医・麻酔科、[3]大阪大・院薬・細胞生理、[4]神奈川工科大・健康医療

Neuroimmune interactions have emerged as critical regulators of inflammation. Neuropeptide calcitonin gene-related peptide (CGRP) regulates cytokine production in immune cells through signaling for CGRP receptor, receptor activity-modifying protein 1 (RAMP1). Here, we examined the role of RAMP1 signaling in LPS-induced lung injury. Acute lung injury was induced by intratracheal injection of LPS in wild-type mice (WT) and RAMP1 knockout mice (RAMP1 KO). Compared with WT, RAMP1 KO exhibited decreases in survival rate and increases in lung injury score, and total protein concentrations and pro-inflammatory mediators including IL-6 and CXCL2 in BALF at 72 h after LPS administration. In WT, CGRP and RAMP1 levels in the lung were increased after LPS administration, and RAMP1 was expressed in alveolar macrophages. After LPS administration, the numbers of alveolar macrophages in both types of mice were diminished, and reached nadir at 6h, and restored to half levels of pre-values in WT thereafter, but remained low in RAMP1 KO until up to 72 h. By contrast, the numbers of neutrophils were increasing with time after LPS administration in two genotypes, and those in RAMP1 KO were larger than WT at 72 h. These results suggested that RAMP1 signaling attenuated LPS-induced acute lung injury by inhibiting cytokine production, neutrophil accumulation, and pulmonary vascular permeability.

Anti-allergic rhinitis activity of tea tree (*Melaleuca alternifolia*) essential oil in mice sensitized and challenged with Japanese cedar pollen

マウススギ花粉症モデルの鼻炎様症状に対するtea tree (*Melaleuca alternifolia*) 精油の効果

○牧野 春香[1]、山下 道生[2]、安藤 祐介[3]、笠井 菜穂子[1]、田中 淑媛[1]、星野 楓月[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

Effects of tea tree (TT) essential oil 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 TT (*i.n.*) 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: the TT treatments did not affect the increase in Cry J1-specific antibodies. 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 the pretreatments with TT. Thus, TT might be useful for the treatment of AR induced by JCP.

Inhibition of Japanese cedar pollen-induced nasal allergic response by lemon grass (*Cymbopogon citratus*) essential oil in mice

マウスにおけるスギ花粉誘発鼻アレルギー反応に対するlemon grass (*Cymbopogon citratus*) 精油の抑制効果

○松尾 香寿美[1]、山下 道生[2]、安藤 祐介[3]、笠井 菜穂子[1]、田中 淑媛[1]、星野 楓月[1]、牧野 春香[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

Current study determined effects of lemon grass (LG) essential oil on allergic rhinitis (AR) induced by Japanese cedar pollen (JCP). Male ICR 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 LG (*i.n.*) 30 min before each JCP challenge. Frequency of sneezing was counted for 20 min after each nasal challenge. Sneezing induced by histamine (Hist, *i.n.*) was also counted on days 18 and 25. In the JCP sensitized and repeatedly challenged mice, both serum levels of IgG and IgE specific for Cry J1, a major allergen of JCP, were significantly increased. The*i.n.* JCP challenge to sensitized mice caused a significant increase in sneezing, indicating an induction of nasal allergic response. An increase in sneezing was also induced by the *i.n.* application of Hist. The Hist-induced sneezing was further increased significantly on day 25, indicating an induction of nasal hyperresponsiveness (NHR) after the repeated JCP challenges. Both the JCP-induced nasal allergic response and NHR were inhibited by the pretreatments with LG. It is thus possible that aromatherapy using LG is effective for the treatment of Japanese cedar pollinosis.

Identification of betuletol from Brazilian Green Propolis that suppresses IL-33 gene expression in Swiss3T3 cells

ブラジル産プロポリスに含まれるIL−33遺伝子発現抑制成分としてのベツレトールの単離・同定

○水口 博之[1]、Shaha Aurpita[2,3]、Islam Rezwanul[2,4]、北村 嘉章[5]、武田 憲昭[5]、福井 裕行[1,6]

[1]大阪大谷大・薬、[2]徳島大・院医歯薬・分子情報薬理学、[3]The Hormel Inst., Univ. Minnesota、[4]Dept. Biomed. Sci., Charles E. Schmidt Coll. Med., Florida Atlantic Univ.、[5]徳島大・院医歯薬・耳鼻咽喉科学、[6]錦秀会

IL-33 is involved in the pathogenesis of chronic inflammations through the induction of Th2 cytokines and eosinophils. We showed that IL-33 gene expression level was correlated with the blood eosinophils number of patients with pollinosis. It suggests that suppression of IL-33 gene expression could ameliorate IL-33-induced eosinophilic chronic inflammation. It was reported that Brazilian Green Propolis (BGP) has many pharmacological properties. We showed that BGP suppressed the expression of histamine H_1 receptor and IL-9 genes. Here, we sought to investigate the effect of BGP on the suppression of IL-33 gene expression and identify the responsible active compound. The BGP ethanolic extract suppressed PMA-induced IL-33 gene up-regulation in Swiss3T3 cells. It was fractionated using various solvents. The active fraction, then, was further fractionated by silica gel column chromatography, followed by Sephadex LH-20 column. A single compound was detected in TLC, and FTIR and NMR analyses identified the compound as betuletol. Betuletol suppressed PMA-induced IL-33 gene up-regulation. Immunoblot analysis showed that betuletol suppressed PMA-induced ERK phosphorylation. In conclusion, betuletol was identified as an active compound, and it suppresses IL-33 gene expression through the inhibition of ERK phosphorylation.

Restoration of liver sinusoidal endothelial cells after monocrotaline-induced liver injury

モノクロタリン肝障害後の肝類洞内皮細胞再生

○伊藤 義也[1]、大高 史聖[3]、田邉 美奈[2]、長田 真由子[2]、山下 敦[2]、古江 明子[2]、細野 加奈子[1,2]、畑中 公[1]、天野 英樹[1,2]
[1]北里大・医、[2]北里大・院医療・分子薬理学、[3]北里大・医・消化器内科学

Objective: Hepatic sinusoidal obstruction syndrome (SOS) induced by chemotherapy or hematopoietic stem cell transplantation causes severe liver injury. Although the pathology of SOS is characterized by damage to liver sinusoidal endothelial cells (LSECs), the processes underlying LSEC repair are incompletely understood. The purpose of this study was to clarify the regeneration process of damaged hepatic sinusoidal endothelial cells in SOS.

Methods and Results: A SOS model was created by intraperitoneal administration of monocrotaline (MCT) to male C57BL/6 mice. ALT levels peaked at 48 h post-MCT administration and necrosis occurred around the central veins, but recovered to normal at 120 h. The hepatic expression of EC damage markers increased after 48 h and was also accompanied by intrahepatic hemorrhage. Thereafter, EC damage markers decreased with hepatic repair, and the hepatic sinusoidal structure was reconstructed. LYVE-1 mRNA expression, an LSEC marker, increased during the liver repair phase. LYVE-1 expression around the central veins was weak before treatment and further decreased at 48 h, but increased at 96 h and 120 h. Bone marrow-derived cells did not incorporate into the restored LYVE-1-positive cells.

Conclusions: These results suggested that the regenerating endothelium was thought to be due to endothelial proliferation in the vicinity of the injury, and that bone marrow-derived cells are unlikely to be involved.

Administration of a SIRT1 activator preserves autophagic activity and improves age-related sarcopenia in mice.

骨格筋におけるSIRT1の活性化はオートファジー活性を維持して加齢に伴うサルコペニアを改善する

○細田 隆介、久野 篤史、中島 龍汰、岩原 直敏、野島 伊世里、堀尾 嘉幸

札幌医科大・医

[Background] Sarcopenia is characterized by loss of skeletal muscle function and mass associated with aging. Autophagy is positively regulated by an NAD^+-dependent protein deacetylase SIRT1 and has been reported to maintain skeletal muscle. In this study, we examined the effect of an activator of SIRT1, resveratrol (RSV), on muscle autophagy and age-related sarcopenia.

[Method and Results] Ddy mice were fed a normal diet or a diet containing RSV (0.4 g/kg diet) for 37 weeks starting at 23 weeks of age. Although rotarod running time gradually shortened with aging in both groups, it was longer in the RSV-fed group at 50 weeks of age. At 60 weeks of age, we sampled tibialis anterior muscles for further analyses in control mice (60 wo) and RSV-treated mice (60 wo+RSV). Compared with mice at 20 weeks of age (20 wo), myofiber diameter determined by HE staining was reduced in 60 wo; however, it was maintained in 60 wo+RSV. Western blot analysis using anti-acetyl-lysine antibody showed increases in acetylated proteins in 60 wo compared to 20 wo, suggesting suppression of SIRT1 activity in 60 wo. However, RSV blocked the aging-induced protein acetylation. LC-II/LC3-I ratio, a marker of autophagic activity, was lower and protein level of p62, which degraded by autophagy, was higher in 60 wo than those in 20 wo, suggesting aging-associated suppression of autophagy. These aging-associated changes were attenuated by RSV treatment.

[Conclusion] These results suggest that activation of SIRT1 in skeletal muscle preserves autophagic activity and attenuates age-related sarcopenia.

The role of calcitonin gene-related peptide (CGRP) in regulation of intestinal fat absorption

腸管脂肪吸収における神経ペプチドCGRPの役割解明

○細野 加奈子[1,2]、伊藤 義也[1,2]、別當 朋広[3]、畑中 公[1,2]、馬嶋 正隆[1,4]、天野 英樹[1,2]

[1]北里大・医、[2]北里大・院医療・分子薬理、[3]北里大・医・消化器内科、[4]神奈川工科大・健康医療科学

Lacteals, lymphatic vessels located at the center of each intestinal villus, play an important role in fat absorption. Their abnormalities in the structure and function may cause impaired lipid circulation, leading to obesity and abnormal lipid metabolism. We examined whether CGRP (calcitonin gene-related peptide) and its receptor, RAMP1 (receptor activity modifying protein 1), are involved in fat absorption via lacteals.

RAMP1 knockout (RAMP1 KO) and wild-type (WT) mice were fed a high-fat diet (HFD) or a normal diet (ND) for 8 weeks from 4 to 12 weeks of age. RAMP1KO mice given HFD increased body weight, adipose tissue mass, serum levels of total cholesterol, triglycerides, and blood glucose, and developed severe obesity compared to WT mice. In addition, the length of the lacteals tended to be shorter in HFD-fed RAMP1KO mice than in HFD-fed WT mice. On the other hand, no difference was observed when eating ND. HFD-fed RAMP1KO mice had decreased expression of lymphatic endothelial markers and pro-lymphangiogenic factors. After oral administration of BODIPY-FL-labelled C_{16} fatty acids, the fluorescence intensity in blood in HFD-fed RAMP1KO mice was higher than that in HFD-fed WT mice.

These results suggest that CGRP/RAMP1 signaling is involved in the regulation of dietary fat absorption by the lacteals.

In vivo imaging techniques to evaluate fatty liver in a diet-induced NAFLD model using PXB mice

PXBマウスを用いた食餌誘発性NAFLDモデル脂肪肝のin vivoイメージングによる評価

○堀本 泰弘[1,2]、西方 龍太郎[1,2]、笹木 祐司[3]、林田 健一郎[1]、沼田 洋輔[1]、角崎 英志[4]

[1]新日本科学・薬効薬理研究部、[2]新日本科学・実験動物管理部、[3]新日本科学・病理研究部、[4]新日本科学・前臨床カンパニー

Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common liver disease in the world. Nonalcoholic steatohepatitis (NASH), the most advanced form of NAFLD, can progress to cirrhosis and hepatocellular carcinoma. Computed tomography (CT) and magnetic resonance imaging (MRI) are being used as non-invasive diagnosis for the fatty liver in NAFLD patients. The present study utilized CT and MRI to examine liver fat mass in human liver chimeric mice (PXB mice, PhoenixBio, Co, Ltd.) fed a choline-deficient, L-amino-acid-defined, high-fat-diet (CDAHFD, A06071302, Research Diets, Inc.) for 28 and 85 days in order to produce a diet-induced NAFLD. Serum chemistry and liver histopathology were also conducted. CT and MRI revealed that the CDAHFD-fed PXB mice (model mice) had higher levels of fat content in the liver compared to the control diet-fed PXB mice from 14 to 56 day. In serum, the model mice had higher levels of total bilirubin compared to the control mice. Histopathological examinations revealed that Picro-Sirius Red-positive collagen fibers were observed at 28 and 85 days in the control mice and model mice. These results indicate that CDAHFD can induce fatty liver in PXB mice as seen in human NAFLD and that CT and MRI may be useful non-invasive methods for evaluation of liver fat in mice.

Effects of lactoferrin on a choline-deficient methionine-defined High-Fat Diet (CDAHFD60)-induced NASH model mice

高脂肪コリン欠乏メチオニン減量飼料により誘発した非アルコール性脂肪肝炎（NASH）モデルマウスに対するラクトフェリンの効果

○古川 恵[1]、青木 亮憲[1]、石戸 健太郎[2]、日塔 武彰[2]、大野 恵[3]、出雲 信夫[2,4]

[1]横浜薬科大・薬・薬学教育セ、[2]横浜薬科大・薬・薬物治療学研、[3](株)NRLファーマ、[4]横浜薬科大・薬・総合健康メディカル研究セ

Inflammation is critical step for nonalcoholic steatohepatitis (NASH) development that progress cirrhosis and liver cancer. Lactoferrin (LF) is known a multifunctional protein such as anti-allergy, anti-inflammatory and anti-obesity effects. However, there is unclear understanding of how LF affects NASH. Therefore, in this study, we investigated the effect of LF on inflammation in NASH model mice.

NASH model mice were generated by fed a choline-deficient methionine-defined high-fat diet (CDAHFD60) in male C57BL/6J mice. Mice were randomly assigned to 4 groups: standard diet; CDAHFD60; CDAHFD60 plus 2400 mg/kg LF; CDAHFD60 plus 4800 mg/kg LF. LF was mixed with drinking water and allowed to be freely taken for 15 days. At the end of experiment, the serum samples and liver tissues were collected. AST and ALT in serum were measured by assay kit and inflammatory cytokines (TNF-α, IL1β and IL-6) in liver were examined by RT-qPCR.

The increase in AST and ALT induced CADHFD60 was significantly reduced by LF. Furthermore TNF-α, IL-1β and IL-6 expression levels in liver were increased by CADHD60, LF significantly suppressed that. Our results indicated that LF inhibited NASH development by suppressing inflammatory cytokines.

Immunohistochemical characterization of TRPV2 and TRPV1 in trinitrobenzene sulfonic acid (TNBS)-induced colitis in ratsl with visceral hypersensitivity

TNBS誘起ラット大腸炎における温度感受性TRPV2およびTRPV1の免疫組織学的解析と内臓痛覚過敏への関与

加藤 伸一、松本 健次郎、安田 浩之、○宮本 早也佳

京都薬科大・薬・薬物治療学

TRPV2 and TRPV1 are originally identified as heat sensitive TRP channels. We investigated the expression patterns of TRPV2 and TRPV1 in the rat distal colon and extrinsic primary afferent neurons, and their roles in visceral hypersensitivity in TNBS-induced colitis rats. Both TRPV2 and TRPV1 expressions in colon, DRG, and nodose ganglion (NG) were significantly upregulated in TNBS-induced colitis. TRPV2-expressing cell bodies colocalized with the intrinsic primary afferent marker NeuN and the inhibitory motor neuronal marker nNOS in myenteric plexus. TRPV2 expressions also colocalized with the resident macrophage marker ED2 in the mucosa while no TRPV1-expressing cell bodies were detected in the myenteric plexus. Both TRPV2- and TRPV1-expressing cell bodies in DRG and NG were double-labeled with the neuronal retrograde tracer fluorescent fluorogold. Large- and medium-sized TRPV2-positive neurons were labeled with the A-fiber marker NF200, CGRP, and substance P in DRG while small-sized TRPV1-positive neurons were labeled with the C-fiber markers IB4, CGRP, and substance P. TRPV2- and TRPV1-positive NG neurons were

labeled with NF200 and IB4. TNBS treatment increased p-ERK1/2-positive cells in TRPV2 and TRPV1 neurons but did not affect the subpopulations in the DRG and NG. Both TRPV2 and TRPV1 antagonists significantly alleviated visceral hypersensitivity in TNBS-induced colitis model rats. These findings suggest that intrinsic/extrinsic TRPV2- and extrinsic TRPV1-neurons contribute to visceral hypersensitivity in an experimental colitis.

Analysis of drug-induced appendicitis using the Japanese Adverse Drug Event Report (JADER) database

自発副作用報告データベース（JADER）を用いた薬剤性虫垂炎の解析

○京谷 陽司、趙 晶、中平 毅一、吉栖 正典

奈良県立医科大・医

[Introduction] Appendicitis is one of the most frequent diseases affecting people of all ages. Several drugs were reported as suspects for appendicitis in Japanese Adverse Drug Event Report (JADER) database, but appendicitis is not listed in the package inserts of these drugs except barium sulfate. We investigate the association of these drugs with appendicitis.

[Methods] We assessed the JADER database between Jan. 2004 and Dec. 2021. Signal was detected when the lower limit of the 95% CI of the reporting odds ratio (ROR) was greater than 1. Time-to-onset profile and ROR adjusted for age and sex were detected by weibull plot and logistic regression analysis, respectively.

[Results] The total number of adverse event reports was 1,551,265, of which 591 were reports of appendicitis. Signals were detected in 22 drugs except barium sulfate. Among them, 8 drugs showed significant shape parameters (ß) in weibull plot and drug-dependent patterns for changes in the risk of appendicitis over time. Adjusted RORs indicated significant association for 5 drugs.

[Conclusion] Our results suggest that several drugs are associated with appendicitis. Among them, the pharmacological mechanism of clozapine is still unclear, and further investigation of the mechanism of clozapine might contribute to establish the management and control of appendicitis.

L-DOPA suppresses bladder smooth muscle contraction induced by carbachol in rats

コリン作動性膀胱収縮に対するDOPAの抑制作用

○ZOU SUO[1]、清水 孝洋[1]、濱田 知里[1]、清水 翔吾[1]、東 洋一郎[1]、増川 太輝[2]、五嶋 良郎[2]、齊藤 源顕[1]

[1]高知大・医・薬理、[2]横浜市立大・院医・薬理

L-3,4-Dihydroxyphenylalanine (L-DOPA) has been recognized as a precursor of dopamine. Recently, L-DOPA has been proposed as a neurotransmitter and is reported to sensitize vasomotor response to sympathetic tone through activation of L-DOPA receptor GPR143. In this study, to clarify roles of L-DOPA/GPR143 signaling in regulation of parasympathetic tone, we examined effects of L-DOPA on muscarinic receptor-mediated bladder contraction in wild-type (WT) and *Gpr143* gene-deficient (*Gpr143*$^{/y}$) rats. Bladder strips were prepared from nine-week-old male rats, and effects of pretreatment with L-DOPA (10^{-8}, 10^{-7} and 10^{-6} M) on carbachol (CCh, 10^{-8} to 3×10^{-4} M)-induced bladder strips contraction were investigated by organ bath study. In bladder strips from WT rats, L-DOPA alone showed no contraction or relaxation, while pretreated L-DOPA dose-dependently suppressed CCh-induced contraction. The L-DOPA-induced suppression was also observed in bladder strips from *Gpr143*$^{/y}$ rats. These results suggest that L-DOPA induces suppression of the muscarinic receptor-mediated rat bladder contraction independently of GPR143 signal transduction.

Inhibition of CD38 promotes muscle regeneration

○夜久 圭介[1]、Nawaz Allah[1]、中川 崇[1,2]

[1]富山大・医・分子医科薬理、[2]富山大・未病研究センター

Sarcopenia is aging-related progressive loss of skeletal muscle mass and strength, which is associated with decreased quality of life and increased mortality. Aging-related decline of muscle regeneration is a risk factor for the onset of sarcopenia.

Nicotinamide adenine dinucleotide (NAD^+) is considered as an anti-aging molecule which can suppress the onset of aging-related diseases. NAD^+ is an essential molecule for all living organisms, which is associated with multiple biological processes, including energy production, DNA repair, and protein modifications. NAD^+ levels in tissues decrease with aging, which leads functional loss of tissues. On the other hand, NAD^+ repletion is considered to have therapeutic potential against aging-related diseases. For example, treatment of NAD^+ precursor is reported to suppress loss of muscle function caused by aging, which is lead by the increase in NAD^+ levels.

Increased degradation of NAD^+ is the cause of decreased NAD^+ levels during aging. CD38 is the enzyme which degrades NAD^+, and expression levels of CD38 increase with aging. NAD^+ decline caused by CD38 leads multiple aging signatures, such as delayed metabolism, cell senescence, and tissue fibrosis. Importantly, inhibition or genetic loss of CD38 can remarkably increase NAD^+ levels in tissues. Additionally, inhibition of CD38 improves muscle function in aged animals. Therefore, CD38 is an attractive target against the onset of sarcopenia.

In this research, we investigated the effect of inhibition or genetic loss of CD38 on muscle regeneration. Genetic deletion of CD38 reduced muscle fibrosis and improved recovery of muscle after the treatment of cardiotoxin. Furthermore, inhibition of CD38 increased NAD^+ levels and promoted myoblast differentiation. These results suggest that inhibition of CD38 has therapeutic potential against the onset of sarcopenia.

Angiotensin II-induced contraction of superior mesenteric arteries from streptozotocin-induced diabetic rats

ストレプトゾトシン誘発糖尿病ラット上腸間膜動脈におけるアンジオテンシン II による収縮反応

○寺田 侑加[1]、日比野 凪沙[1]、井上 滉太[1]、藤田 義人[2]、稲垣 暢也[2]、屋山 勝俊[1]

[1]神戸学院大・薬・循環器薬理、[2]京都大・院医・糖尿病・内分泌・栄養内科学

Diabetes is a major risk factor for vascular diseases, and often associated with vascular dysfunction. However, the mechanisms of vascular dysfunction in diabetes are not fully understood. Inactivation of myosin light chain phosphatase thorough myosin phosphatase targeting subunit 1 phosphorylation by Rho kinase is important for angiotensin II (Ang II)-induced contraction. We have previously reported that Rho kinase activation by Src, epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase 1 and 2 (Erk1/2) is involved in protein tyrosine phosphatase inhibitor-induced contraction. We therefore investigated the effects of inhibitors of these factors on Ang II-induced contraction in diabetic rat superior mesenteric arteries.

Wistar male rats were induced diabetes by intraperitoneal injection of streptozotocin (40 mg/kg). After 8 weeks, superior mesenteric arteries were excised and performed organ chamber experiments. Protein levels were measured by Western blotting.

The contraction of superior mesenteric arteries from diabetic rats increased in the response to Ang II in comparison with those from the control rats. However, we did not observe any differences in Ang II-induced contraction between the groups in the presence of Rho kinase, EGFR, and Erk1/2 inhibitors. Moreover, protein level of Erk1/2 was significantly increased in diabetic rats. These data suggest that increased activity of Rho kinase, EGFR, and Erk1/2 might be involved in excessive contraction by Ang II in superior mesenteric arteries of diabetic rats.

Endothelium-dependent and -independent Vasodilator Effects of SGLT2 inhibitors

SGLT2阻害薬の内皮依存性および内皮非依存性血管弛緩作用

○金田 剛治[1]、大山 唯花[1]、佐々木 典康[2]、金田 寿子[1,3]

[1]日本獣医生命科学大・獣医・獣医薬理研究室、[2]日本獣医生命科学大・獣医・獣医生化学研究室、[3]帝京大・医・解剖学講座

SGLT2 inhibitors (SGLT2i) are new agents for patients with heart failure (HF) irrespective of diabetes. However, the effects of in vasoconstriction remain unclear. This study examined the mechanism of vasorelaxation induced by SGLT2i in endothelium-intact and -denuded rat aorta and mesenteric artery. 1) Dapagliflozin and empagliflozin inhibited phenylephrine (PE)-induced contraction in a dose-dependent manner. However, this relaxation was lower in the absence of the endothelium in aorta and mesenteric artery. 2) Increase in dapagliflozin and empagliflozin-induced relaxation in the presence of the endothelium was attenuated by preincubation in L-NAME (100 μM) and the removal of the endothelium, but not indomethacin (10 μM) in aorta and mesenteric artery. 3)Remove of glucose from PSS did not affect dapagliflozin and empagliflozin -induced relaxation in endothelium-denuded aorta. 4) In fura 2-loaded endothelium-denuded aorta, empagliflozin inhibited high K^+- or PE-induced muscle tension and increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) level. These results may suggest that dapagliflozin and empagliflozin causes vasorelaxation by increasing cGMP content in correlation with the release of NO from endothelial cells and by decreasing $[Ca^{2+}]_i$) level via inhibiting voltage-dependent Ca^{2+} channel.

ATP-evoked relaxation of esophageal smooth muscle via potassium channels in rats

ATPによるラット食道平滑筋の弛緩反応に対するカリウムチャネルの関与

○椎名 貴彦、志水 泰武

岐阜大・応用生物・獣医生理

The external muscle layer of the mammalian esophagus consists of striated muscle fibers and smooth muscle fibers. Striated muscle is mainly regulated by cholinergic signaling, whereas smooth muscle is regulated by cholinergic and non-cholinergic signaling in the esophagus. ATP is a representative non-cholinergic extracellular transmitter, which control smooth muscle motility in the blood vessels and gastrointestinal tracts via purinergic receptors. We have demonstrated that exogenous application of ATP evokes relaxation of smooth muscle in the muscaris mucosa of the rat esophagus. In the present study, the aim was to clarify involvement of potassium channels in purinergic relaxation of the esophageal smooth muscle. An isolated segment of the rat esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. After contraction of esophageal smooth muscle was induced by carbachol, we applied ATP, which evoked relaxation of smooth muscle. On the other hand, ATP did not affect high-potassium induced contraction. Pre-application of an antagonist of ATP-dependent potassium channels (K_{ATP} channels) blocked ATP-induced relaxation, but not voltage-gated potassium channel blockers. These findings indicate that K_{ATP} channels might be involved in ATP-induced relaxation of the esophageal smooth muscle in rats.

Effect of cisplatin on the expressions of myosin heavy chain isoforms in skeletal muscle of mouse

シスプラチン誘発筋萎縮時のミオシン重鎖アイソフォームの発現低下

○酒井 寛泰[1]、Xu Xinran[1]、宮内 優[1]、千葉 義彦[2]、今 理紗子[1]、五十嵐 信智[1]、亀井 淳三[1,3]、細江 智夫[1,4]
[1]星薬科大・薬、[2]星薬科大・薬・分子生物学、[3]順天堂大・企画調査室、[4]星薬科大・薬・生物制御科学

We previously reported that cisplatin induced skeletal muscle atrophy in mice. Myosin heavy chain (MyHC) is one of the structural proteins essential for the contractile response of skeletal muscle, and several isoforms exist. However, the kinetics of its expression in cisplatin-induced muscle atrophy is unknown. In this study, we investigated the expression changes of MyHC isoforms in cisplatin-induced muscle atrophy. Mice were treated with cisplatin intraperitoneally once daily for 4 days, and quadriceps muscles were isolated 1 day after the last dose; C2C12 myotubes were also treated with cisplatin. Cisplatin significantly reduced protein expression of MyHC isoforms (I, IIa, IIx, IIb) in mice compared to controls. However, only MyHC-IIa was downregulated when changes in MyHC gene expression were examined. Similar to the results in mice, cisplatin attenuated protein expression of all MyHC isoforms in C2C12 myotubes, and gene expression of MyHC-IIa was downregulated. Furthermore, the downregulation of protein expression of all MyHC isoforms was inhibited by treatment with MG-132, a proteasome inhibitor. These results indicate that cisplatin downregulates MyHC protein expression. Thus, we suggest that proteasomal degradation of MyHC proteins is one of the causes of cisplatin-induced muscle atrophy.

Establishment of muscle strength measurement system using aged mice

高週齢マウスを用いた筋力測定系の確立

○真壁 大地、森田 枝美、緒里 真一、吉原 佐江子、清水 広夢、田代 貴士、片山 誠一、廣中 直行、西 勝英

㈱LSIM安全科学研究所・熊本研究所・薬理研究部

Japan has a very high aging rate among developed countries. The increase of medical expenses in a super-aged society has become serious. It is important to maintain good quality of life (QOL) throughout life. One of the factors that lowers QOL of the elderly is a decline in mobility accompanied by a decrease in muscle strength such as sarcopenia. Many food and drug suppliers are developing products to prevent muscle weakness. In this study, we tried to establish a test system to evaluate effects of therapeutic agents on muscle weakness using aged mice. First, we compared the muscle weakness of 12, 40, 53, 79, 92, and 105-week-old C57BL/6J male mice using a small animal muscle strength measuring device (Aurora Scientific). Next, we measured blood and organ concentrations of AGEs, NT-proBNP, and MURF1, which are indicators of aging. Muscle pathology was also examined. Compared with 12-week mice, aged mice (79 to 92 weeks) showed significantly lower muscle strength. Finally, we tested the effects of nicotinamide mononucleotide (NMN, a precursor of NAD), which is recently attracted attention as a dietary supplement with potential anti-aging effects. Oral administration of NMN for 12 weeks showed preventive effect on muscle weakness in aged mice. The present test system would be useful to evaluate potential efficacy of newly developed therapeutic agents on muscle weakness in the elderlies.

Suppressive effect of the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ activator, SKA-121 on IL-10 and IL-8 expression in THP-1-derived M_2 macrophages

THP−1由来M_2マクロファージにおけるカルシウム活性化カリウムチャネル$K_{Ca}3.1$活性化薬によるIL−10およびIL−8発現抑制

松井 未来、梶栗 潤子、鬼頭 宏彰、遠藤 京子、○大矢 進

名古屋市立大・院医

The human monocytic leukemia cell line, THP-1-differentiated macrophages are a useful tool to investigate the physiological significance of tumor-associated macrophages (TAMs). In the tumor microenvironment (TME), TAMs with the M_2-like phenotype play a critical role in the promotion of cancer progression and metastasis by inhibiting the immune surveillance system. We examined the involvement of Ca^{2+}-activated K^+ channel $K_{Ca}3.1$in the expression of pro-tumorigenic cytokines and angiogenic growth factors in THP-1-derived M_2 macrophages. THP-1 cells into M_0 macrophages were induced by a treatment with PMA treatment for 24 hr, and then cells were treated with IL-4 and IL-13 for 72 hr to induce the polarization of M_2 macrophages. The expression levels of IL-8 and IL-10 were significantly decreased by treatment with the selective $K_{Ca}3.1$ activator, SKA-121 in THP -1-derived M_2 macrophages. Furthermore, under in vitro experimental conditions that mimic extracellular K^+ levels in the TME, IL-8 and IL-10 levels were both significantly elevated, and these increases were reversed by treatment with SKA-121. Among several signaling pathways potentially involved in the transcriptional regulation of IL-8 and IL-10, respective treatments with ERK and JNK inhibitors significantly repressed their transcriptions, and treatment with SKA-121 significantly reduced the phosphorylated ERK, JNK, c-Jun, and CREB levels. These results strongly suggest that the $K_{Ca}3.1$ activator may suppress IL-10-induced tumor immune surveillance escape and IL-8-induced tumorigenicity and metastasis by inhibiting their production from TAMs through both ERK-CREB and JNK-c-Jun cascades.

Amino acid transporter LAT1 (SLC7A5) on cancer cell-derived exosomes as a potential prognostic- and diagnostic biomarker.

がん細胞由来エクソソーム上におけるアミノ酸トランスポーターLAT1（SLC7A5）の発現：予後および診断バイオマーカーとしての潜在的価値

○大垣 隆一[1,2]、Liu Yumiao[1]、徐 旻憓[1]、岡西 広樹[1]、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部門

L-type amino acid transporter 1 (LAT1/SLC7A5), which transports large neutral amino acids, is highly upregulated in various cancer cells and supports their enhanced growth and proliferation. Previous immunohistochemical studies have reported significant correlations between the high LAT1 expression and the poor prognosis of patients in multiple cancer types. Furthermore, several LAT1-selective inhibitors are currently under evaluation as novel anti-cancer drugs. Detecting the LAT1 expression levels in tumor lesions with minimally invasive methods, therefore, would be beneficial for prognosis and diagnosis of cancers, including the companion diagnosis in the upcoming LAT1-targeted therapy. Molecules on exosomes, small extracellular vesicles, released from cancer cells are emerging as a novel class of biomarkers. In this study, we found that LAT1 is detectable in exosomes isolated from the cell culture supernatants of cancer cells. Notably, the abundance of LAT1 on exosomes was associated with its expression level in the cancer cells from which they derived. LAT1 was also present in exosomes isolated *in vivo* from peritoneal washing fluid of intraperitoneal tumor-bearing mice. These results indicate that LAT1 on cancer cell-derived exosomes holds a significant potential as a biomarker for diagnosis and prognosis of cancers.

The effect of acute treatment with cyclophosphamide in post-weaning on stress response and hippocampal stem/progenitor cell proliferation in the adulthood

離乳後のクロホスファミドの急性適応が成体期のストレス反応性および海馬神経幹・前駆細胞の増殖能に与える影響

○尾中 勇祐、山口 太郎、米山 雅紀

摂南大・薬

Chemotherapy for childhood cancer can cause late-appearing side effects in survivors that affect multiple organs, including the brain. Cyclophosphamide is used in childhood cancer and has the blood brain barrier permeability. The present study aims to reveal whether an acute treatment with cyclophosphamide after weaning affects the brain function and hippocampal neurogenesis in the adult mouse. Cyclophosphamide were intraperitoneally once injected to 3 weeks old male ddY mice. Five weeks after injection, restraint stress loading or the behavioral test battery was performed. After the test battery, mice were received 2 consecutive injections of 5-bromo-2′ -deoxyuridine (BrdU) with a 12-h interval. BrdU positive cells in the hippocampal dentate gyrus were counted after immunostaining for BrdU. Treatment with cyclophosphamide had the ability to enhance restraint stress-induced increase of plasma corticosterone, and decrease the number of BrdU-positive cells in the dentate gyrus 5 weeks afterward. However, cyclophosphamide failed to change emotional and cognitive functions. These data suggest that the acute treatment with cyclophosphamide in post-weaning exhibits prolonged changes in stress response and the suppressive effect on hippocampal neurogenesis.

L-type amino acid transporter 1 inhibitor JPH203 as a new therapeutic target for castration resistant prostate cancer treatment

去勢抵抗性前立腺癌におけるアミノ酸トランスポーターLAT1選択的阻害薬JPH203の効果

○齋藤 心平[1,2]、坂本 信一[2]、濱口 紀江[1]、齊藤 将太[1]、裵 祥存[1,2]、靈園 良恵[1]、平山 友里[1]、橋本 弘史[1]、市川 智彦[2]、安西 尚彦[1]

[1]千葉大・院医薬・薬理学教室、[2]千葉大学医学部附属病院・泌尿器科

L-type amino acid transporter 1 (LAT1) plays a role in transporting essential amino acids including leucine, which regulates the mTOR signaling pathway. Here, we studied the expression profile and functional role of LAT1 in prostate cancer using JPH203, a specific inhibitor of LAT1. LAT1 was highly expressed in castration-resistant prostate cancer (CRPC) cell lines including C4-2 and PC-3, while poorly expressed in castration sensitive LNCaP cells. 5 μ M of JPH203 significantly blocked 14C leucine uptake in CRPC cells, while not affected in LNCaP cells. JPH203 inhibited cell migration at 30 μ M in CRPC cells, while not affected in LNCaP cells. Combination of JPH203 30 μ M and Enzalutamide 10 μ M additively blocked the cell proliferation in CRPC cells. RNA sequence identified CD24 as a novel downstream target of JPH203 in C4-2 cells. SiCD24 blocked cell migration in C4-2 cells via blocking phosphorylation of GSK3β and activating phosphorylation of β catenin. JPH203 25mg/kg significantly inhibited tumor growth in the C4-2 xenograft nude mouse model. Targeting LAT1 by JPH203 may represent a novel therapeutic option in CRPC.

Eribulin modulates stathmin dynamics and enhances paclitaxel sensitivity in ovarian cancer cells

卵巣がん細胞においてエリブリンはスタスミン動態を調節し、パクリタキセルの感受性を高める

○安曇 麻奈、吉江 幹浩、高野 航瑠、草間 和哉、田村 和広

東京薬科大・薬・内分泌薬理

Ovarian cancer is the major cause of death from gynecologic malignancies owing to poor prognosis. Stathmin, a microtubule-destabilizing protein, is expressed in a stage-dependent manner in ovarian cancer. Eribulin is a microtubule dynamics inhibitor used to treat breast cancer and sarcoma. The present study explored the antitumor efficacy of eribulin and the involvement of stathmin in the action of eribulin in ovarian cancers. In a xenograft model of ovarian cancer, eribulin treatment reduced the tumor weight accompanied with an increased level of phosphorylated stathmin, and a decreased expression of proliferating cell nuclear antigen (PCNA). Eribulin abolished the formation of tumor blood vessels with large lumens. In cultured ovarian cancer cell lines, eribulin stimulated stathmin phosphorylation and decreased stathmin protein expression. A protein phosphatase 2A (PP2A) activator FTY720 attenuated the eribulin-induced phosphorylation of stathmin. The levels of PP2A subunits were downregulated by eribulin. SiRNA-mediated stathmin knockdown attenuated the inhibitory effects of eribulin on cell viability. In addition, eribulin enhanced the antiproliferative effects of paclitaxel on ovarian cancer cells, which was accompanied by a decreased stathmin expression. These results suggest that eribulin may suppress ovarian cancer tumorigenesis partly by regulating the stathmin dynamics, and that combined treatment of eribulin with paclitaxel could be useful for ovarian cancer.

Differentiation-inducing factor-1 inhibited cancer cell adhesion to vascular endothelial cells via curtailing VCAM-1 protein synthesis

細胞性粘菌分化誘導因子−1はVCAM−1のタンパク質合成の抑制を介してがん細胞の血管内皮細胞への接着を阻害した

○有岡 将基、石兼 真、笹栗 俊之、高橋 富美

産業医科大・医・薬理学

We previously reported that differentiation-inducing factor-1 (DIF-1) inhibited lung colony formation in mouse models of cancer metastasis by suppressing cancer cell proliferation and motility. Although adhesion of circulating tumor cells to vascular endothelial cells is an essential process for the initiation of tumor metastasis formation, the effects of DIF-1 on this process has not been elucidated. In this study, therefore, we investigated the effect of DIF-1 on the adhesion ability of tumor cells (human melanoma cells A2058, mouse melanoma cells B16BL6 and human colon cancer cells HCT116) to human umbilical vein endothelial cells (HUVECs). Treatment with DIF-1 significantly inhibited these cancer cells adhesion to HUVECs. To elucidate the mechanism of DIF-1 action, we analyzed adhesion-related genes in HUVECs. We found that the expression of vascular endothelial cell adhesion molecule-1 (VCAM-1) was suppressed by DIF-1 and the protein synthesis inhibitor cycloheximide abolished this inhibitory effect. We further evaluated the effect of DIF-1 using the lung metastasis model. Surprisingly, only 3 days administration of DIF-1 prior to inoculation of B16BL6 melanoma cells significantly inhibited lung colony formation. These results suggest that the main mechanism of DIF-1 anti-metastatic effect might be attenuation of adhesion between circulating tumor cells to blood vessels.

The BDNF/TRKB pathway promotes EMT to induce parotid gland cancer cell aggressiveness via interaction with CAF

BDNF/TRKB経路は癌関連線維芽細胞との相互作用を介して耳下腺癌細胞のEMTを促進しその悪性度を高める

○森脇 一将[1]、桑原 宏子[2]、綾仁 悠介[3]、東野 正明[3]、寺田 哲也[3]、河田 了[3]、朝日 通雄[1]

[1]大阪医科薬科大・医・薬理、[2]大阪医科薬科大・医・病理、[3]大阪医科薬科大・医・耳鼻咽喉科

The molecular features of parotid gland cancer (PGC) are not fully understood enough to develop an effective drug therapy because of the rarity. Given the poor prognosis of many human cancers in which TRKB is highly expressed, we investigated the involvement of the BDNF/TRKB pathway in PGC tissue using clinical specimens and observed high expressions of TRKB and BDNF in both tumor cells and stromal cells such as cancer-associated fibroblasts (CAFs). Therefore, to obtain more detail information of BDNF/TRKB signaling in PGC, we established primary co-culture system of patient-derived PGC cells and CAFs. In the culture system, PGC cells co-cultured with CAFs exhibited significant upregulation of BDNF and epithelial-mesenchymal transition (EMT). Similar results were observed in PGC cells treated with conditioned medium (CM) from co-culture of PGC cells and CAFs. TRK inhibitors suppressed BDNF- or CM-induced Snail upregulation and cell migration in PGC cells. Importantly, immunohistochemical and clinicopathological analyses of tumors from the patients with PGC revealed that TRKB expression levels in PGC cells were significantly correlated with aggressive features, including vascular invasion, nodal metastasis, and poor prognosis. Collectively, these data suggest that the BDNF/TRKB pathway regulates PGC cell aggressiveness via cross-talk with CAFs and is a potential therapeutic target for PGC harboring invasive and metastatic features.

Correlational changes in insular cortical neuronal activity and cardiac signals

島皮質の神経活動と心拍動の変化の相関

○木下 航輔[1]、久我 奈穂子[2]、佐々木 拓哉[2,3]

[1]東北大・薬・薬理、[2]東北大・院薬・薬理、[3]東京大・院薬・薬品作用

The insular cortex serves as a hub cortical region that is bidirectionally connecting to an extensive cortical and subcortical brain areas and plays a crucial role in interoception, the sensation of internal states of the body, such as heartbeats, hunger, and blood pressure. However, neurophysiological mechanisms and insights supporting this hypothesis remain to be clarified. To address this issue, we performed simultaneous recordings of multiunit spike patterns and local field potential (LFP) signals from the insular cortex, an electrocardiogram signal, and a peripheral blood glucose concentration from freely moving rats. Recordings were daily obtained for seven hours. No pronounced temporal correlational changes were found between ongoing heart rate and blood glucose fluctuations. Insular cortical LFP power in some frequency bands, such as the delta and theta bands, showed apparent temporal correlational changes with ongoing heart rates when the rats were resting. At single-cell levels, a subset of insular cortical neurons increased or decreased their spike rates in response to changes in heart rates and blood glucose levels. These results highlight insular cortical neurons as a detector of temporal changes in interoceptive signals.

Alteration in BAK (BCL2 antagonist)1 and Bcl-2-associated athanogene (BAG) 3 in Hypothyroid Embryonic Chick Cerebellum

甲状腺ホルモン低下状態の鶏胚小脳におけるBAK(BCL2 antagonist)1およびBCL2関連athanogene (BAG) 3の変化

○三部 篤、猪俣 結衣、髙橋 慎太郎、玉田 さち、夏堀 陽子、東尾 里英子

岩手医科大・薬

Hypothyroid conditions during fetal development can induce severe developmental defects such as mental retardation. Thyroid dysfunction and hypothyroid conditions induced by anti-thyroid drugs may influence neuronal development during embryogenesis. Little is known, however, about the details of the mechanisms underlying the adverse effects of thyroid dysfunction on neuronal development. In this study, we examined the gene expression levels of Bcl-2 family members and Bcl-2-associated athanogene (BAG) 3 in chick cerebellum under hypothyroid conditions using a fertilized chicken egg/chick embryo model. The hypothyroid chicks exhibited a marked reduction in BAG3 protein levels concomitant with a marked increase in mitochondrial Bak1 protein levels in cerebellum while no alteration in gene expression of Bak1 was seen. Since evidence of apoptotic cell death was observed in the cerebellum under hypothyroid conditions, these results suggest that reduction in BAG3 as well as enhancement of Bak1 protein may play an important role in the apoptosis of neuronal cells under hypothyroid conditions .

Antioxidative activity of remifentanil as a direct free radical scavenger

フリーラジカルスカベンジャーとしてのレミフェンタニルの抗酸化作用

○徳丸 治[1]、山崎 玲音[2]、尾方 和枝[1]、松本 重清[3]、北野 敬明[3]

[1]大分大・福祉健康科学・生理、[2]大分大・医・学生、[3]大分大・医・麻酔

Purpose

Remifentanil is an ultra-short acting opioid used for general anesthesia. Recently, it is reported that remifentanil is preventive against oxidative stress including ischemia/reperfusion injury and inflammation. However, detailed mechanism is not fully described. We aimed to study free radical scavenging activity of remifentanil against multiple free radicals.

Methods

Free radical scavenging activity of remifentanil was evaluated against nine species of free radicals by electron spin resonance spectroscopy with the spin-trapping method. From dose-response curves, reaction rate constants with free radicals examined were estimated. Antioxidative activity against lipids was assessed by TBARS assay.

Results

Remifentanil significantly scavenged the following five free radical species in dose-dependent manners; hydroxyl radical, *tert*-butoxyl radical, ascorbyl free radical, singlet oxygen and nitric oxide. Remifentanil did not scavenge superoxide anion, *tert*-butyl peroxyl radical, DPPH and tyrosyl radical. Remifentanil significantly inhibited oxidation of lipids in mice brain tissue.

Conclusions

Remifentanil dose-dependently scavenged multiple free radicals including hydroxyl radical. It is speculated that the direct free radical scavenging activity of remifentanil might contribute to its antioxidative activity *in vitro* and *in vivo*.

Involvement of Mas-related G protein-coupled receptor X1 (MrgprX1) in the chronic itch

慢性そう痒におけるMas-related G protein-coupled receptor X1 (MrgprX1) の関与

泉本 直樹、○今野 光洋、森山 正樹、湯沢 夏美、岩村 智勝、成見 英樹

東レ・医薬研究所・創薬薬理研究室

Atopic dermatitis (AD) is a common dermatologic disease that is accompanied by severe chronic pruritus. Recently, it has been reported that mas-related G protein-coupled receptor (Mrgpr) involves in the control of histamine-independent itch and the modulation of Mrgpr might be a promising target for the treatment of chronic itch. In the present study, we used the transgenic mice expressed human MrgprX1 in the sensory nerve and Mrgpr cluster KO (Mrgpr-KO) mice and evaluated the scratching behaviors in the acute itch model evoked by BAM8-22. In addition, the modulation of Mrgpr and the effect of steroid drug in the MC903-induced chronic itch model in both transgenic mice were examined. Intradermal BAM8-22 did not induce the scratching behaviors in the Mrgpr-KO mice over vehicle-treatment, on the other hand, it evoked scratching behaviors in the hMrgprX1 transgenic mice compared to the vehicle-treated animals. In the chronic itch model evoked by the repetitive application of MC903 produced the scratching behaviors in both Mrgpr-KO and hMrgprX1 transgenic mice over vehicle-treatment, but the ones in the hMrgprX1 mice evidently outweighed them in the Mrgpr-KO mice. In addition, dexamethasone, a steroid drug, significantly attenuated the scratching behaviors evoked by MC903 in both Mrgpr-KO and hMrgprX1 transgenic mice to the same extent. From these observations, it is suggested that the itch in the AD patients might be able to be inhibited completely by the combination of steroid and hMrgprX1 antagonist.

Mechanisms for the allodynia caused by complement anaphylatoxin C5a in mice

マウスにおける補体アナフィラトキシンC5a誘起アロディニアの発現メカニズムについて

○田島 和樹[1]

[1]近畿大・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 thrombin-thrombomodulin (TM) complex generates activated forms of protein C (APC) and thrombin-activatable fibrinolysis inhibitor (TAFIa), known as carboxypeptidase B (CPB), and degrades high mobility group box 1 (HMGB1), a pronociceptive molecule. We have shown that soluble TM (TM α) prevents chemotherapy-induced peripheral neuropathy (CIPN) in rodents, and that APC and TAFIa/CPB, as well as HMGB1 degradation, contribute to the anti-CIPN effect of TM α. Given our recent evidence for the involvement of complement C5a, degradable by TAFIa/CPB, in CIPN, we analyzed the mechanisms for peripheral C5a-mediated pain in mice. Intraplantar (i.pl.) injection of C5a at 30-300 ng induced dose-dependent mechanical allodynia, as assessed by von Frey test, an effect abolished by i.p. administration of DF2593A, a mouse C5a receptor (C5aR) antagonist, TAFIa/CPB, TM α or an anti-HMGB1-neutralizing antibody. Systemic (i.p.), but not local (i.pl.), administration of liposomal clodronate, a macrophage depletor, partially reduced the C5a-induced allodynia. In macrophage-like RAW 264.7 cells, C5a caused HMGB1 release in the presence, but not absence, of a sub-effective concentration of lipopolysaccharide. Together, C5a-induced allodynia is considered to involve C5aR-dependent HMGB1 release from monocyte-derived macrophages and non-macrophage cells.

In vitro assessment of drug-induced peripheral pain in DRG neurons at single cell level using CMOS-MEA

CMOS-MEAを用いた一細胞レベルでの末梢神経痛みのin vitro評価

○韓 笑波、柴田 未可子、松田 直毅、鈴木 郁郎

東北工業大学・大学院工学研究科

Drug-induced peripheral neuropathy occurs as a major adverse effect of chemotherapy. However, a highly accurate assessment platform for drug-induced peripheral neuropathy and pain has not been established yet. In the present study, we introduced a CMOS-MEA system with high spatiotemporal resolution, to detect electrophysiological activity of cultured DRG neurons.

The CMOS-MEA that consists of 236,880 electrodes, makes it possible to identify each single soma of active DRG neurons. Therefore, the spike pattern of each single neuron is able to be demonstrated before and after cumulative additions of TRP channel agonist (i.e., capsaicin, AITC and menthol). And we found that paclitaxel, a wildly used anticancer drug, could sensitive DRG response to capsaicin burning by spike pattern analysis. After identifying the axonal conduction pathway, we also calculated the velocity of axonal conduction from each neuron, and the result showed to be in a reasonable range. The mean conduction velocity could be increased by administration of vincristine, which is known to cause peripheral neuropathy.

Using CMOS-MEA system with high spatiotemporal resolution, we succeed in evaluating spike pattern at single neuron level and measuring axonal conduction velocity in cultured DRG neurons. And changes in these parameters of DRG neuron response to anti-cancer drugs could also be detected, suggesting that this system is effective for assessment of drug-induced peripheral neuropathy and pain.

Intracochlear macrophages are involved in the development of sensorineural hearing loss by decreasing inner hair cell synapses

蝸牛内マクロファージは内有毛細胞シナプス数を減少させることにより感音難聴発症に関与する

○山口 太郎

摂南大・薬

The development of sensorineural hearing loss is a serious problem because it significantly reduces the quality of life. Age-related, noise-induced, and drug-induced sensorineural hearing loss has been reported to decrease inner hair cell synapses (IHC synapses) in its early stages. This study aimed to determine the cause of this decrease in IHC synapses. We prepared the mice in which repeated exposure (1-h exposure per day, 5 times) to moderate noise (8-kHz octave band noise, 90 dB sound pressure level) induced gradual hearing impairment along with a loss of IHC synapses. Treatment with PLX3397 (macrophage-depletion drug, 290 mg/kg) significantly improved reduced the number of synapses and hearing impairment caused by repeated noise exposure. Treatment with minocycline (macrophage-activation inhibitor, 50 mg/kg) significantly improved reduced the number of synapses and hearing impairment caused by repeated noise exposure. These results suggest that intracochlear macrophages are activated by noise exposure and may negatively regulate the number of IHC synapses.

Contractility assessment using hiPSC-CMs with alignment

配向性を有するヒトiPS細胞由来心筋細胞の収縮評価

○佐塚 文乃、林 紗代、柳田 翔太、諫田 泰成

国衛研・薬理部

Evaluation of cardiomyocyte contraction using hiPSC-CMs holds great promise to predict drug-induced heart failures in vitro. However, it has been difficult to detect the positive inotropic effects of drug using hiPSC-CMs. Due to the immature property, hiPSC-CMs do not show alignment. In this study, we evaluated whether hiPSC-CM with alignment can detect the drug-induced positive inotropic effects. We used hiPSC-CM (iCell Cardiomyocyte, FCDI), which were cultured using commercially available plates for alignment. After long-term culture, we found that the aligned culture improved the basal contraction velocity, which were measured by motion vector analysis (SI8000, Sony). To understand the molecular mechanisms of the contraction improvement, we performed next-gene sequencing analysis. Gene expression levels of ion channels and metabolism-related factors were up-regulated by alignment. In addition, we observed that isoproterenol enhanced contraction velocity in hiPSC-CMs with alignment in a dose-dependent manner. These results suggests that the aligned culture of hiPSC-CMs facilitates the maturation and the responses to isoproterenol.

The interaction between MMP-9 and TNF-α contributes to the exacerbation of capillary degeneration in a rat model of NMDA-induced retinal injury

ラットNMDA網膜傷害モデルにおいてMMP−9とTNF−αの相互作用は血管変性の増悪に関与する

○小嶋 美帆香、浅野 大樹、森田 茜、柏原 俊英、中原 努

北里大・薬・分子薬理

Matrix metalloproteinases (MMPs) and tumor necrosis factor (TNF)-α contribute to the pathogenesis of several ocular diseases. In this study, we aimed to determine the role of interaction between TNF-α and MMP-9 in capillary degeneration. In rats, retinal injury was induced by intravitreal injection of *N*-methyl-D-aspartic acid (NMDA) at postnatal day 7. We examined 1) the effects of blocking MMP-9 and TNF-α signaling pathway on capillary degeneration, 2) changes in protein levels and distribution of MMP-9 and TNF-α, and 3) the interaction between MMP-9 and TNF-α in regulating the expression level of each protein in retinas of NMDA-injected eyes. Intravitreal injection of GM6001, an MMP inhibitor, or TNF-α neutralizing antibody (anti-TNF-α Ab) attenuated capillary degeneration in retinas of NMDA-injected eyes. Protein levels of TNF-α increased 2 h after NMDA injection, whereas those of MMP-9 increased 4 h after the injection. Anti-TNF-α Ab suppressed activation of MMP-9 in retinas of NMDA-injected eyes, whereas GM6001 diminished the TNF-α protein expression. Incubation of recombinant TNF-α with supernatants of homogenized retina increased protein levels and activity of MMP-9. These results suggest that TNF-α and MMP-9 collaboratively contributes to the progressive capillary degeneration in injured retinas.

Transcription factor ERG controls endothelial cell function and vascular normalization in tumor angiogenesis

転写因子ERGは血管内皮機能調節と腫瘍血管正常化に関与する

○亀井 竣輔[1]、荒田 佳菜子[1]、宮村 優里[1]、荒木 喜美[2]、久保田 義顕[3]、南 敬[1]

[1]熊本大・生命資セ・分子血管、[2]熊本大・生命資セ・疾患モデル、[3]慶應義塾大・医・解剖

Tumor microenvironment, in particular tumor angiogenesis, forms a niche that facilitates rapid tumor growth and metastasis. We have reported ERG, an ETS family transcription factor downregulated within the tumor endothelial cells (ECs), is critical for increasing H3K27ac enrichment and mRNA transcription on EC-specific genes (Nuc Acids Res 2016, PLoS Genet 2018). However, the role of ERG in tumor angiogenesis is unclear. Here we successfully generated EC-specific mouse ERG-transgenic mouse (*Erg*^{iEC}/Cdh5-Cre-ERT2;*Erg*) and evaluated the tumor growth and angiogenesis in Lewis Lung Carcinoma (LLC)-transplantation model. We observed differential angiogenesis patterns: a decrease in blood vessel number, a 1.5-fold increase in pericytes coverage, and a 7 % expansion of blood vessel diameter within the tumor in *Erg*^{iEC} mice. And *Erg*^{iEC} mice showed a 15 % decrease in tumor size in basal conditions, meanwhile, a significant 50 % decrease with a combination of anti-cancer drug cisplatin-treated conditions. RNA-sequencing analysis additionally revealed differential gene expression patterns related to inflammation, cell cycle, and cell growth on the smaller tumor tissues in the cisplatin-treated *Erg*^{iEC} mice than in the cisplatin-treated control mice. These results suggested that the transcriptional regulation of ERG was crucial for modulating EC function and the normalization of tumor blood vessel environment via preventing ERG transcriptional decrease was a novel molecular target for developing cancer drugs.

Toxicity assessment of tyrosine kinase inhibitors using human-induced pluripotent stem cell-derived cardiomyocytes

ヒトiPS細胞由来心筋細胞を用いたチロシンキナーゼ阻害薬の毒性評価

○林 紗代、佐塚 文乃、柳田 翔太、諫田 泰成

国立医薬品食品衛研・薬理部

BCR-ABL tyrosine kinase inhibitors (TKIs) have contributed to the improvement of the survival of patients with chronic myelogenous leukemia (CML). Growing evidence suggests that cancer therapy-related cardiac dysfunction has become important as the most undesirable side effect of chemotherapy. For example, one of BCR-ABL TKIs, nilotinib, has been reported to have a risk of QT prolongation associated with Torsades des Pointes and cardiac failure. We have previously reported that nilotinib causes QT prolongation and early afterdepolarization (EAD) using human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). In this study, we evaluated the effect of BCR-ABL TKIs on contractility using hiPSC-CMs.

We used iCell Cardiomyocyte 2.0 (Cellular Dynamics International). To assess the chronic cardiotoxicity of the several BCR-ABL TKIs including nilotinib, we analyzed the contractility velocity recorded by motion vector analysis (SI8000, Sony).

We found that treatment with BCR-ABL TKIs decreased beating rate in hiPSC-CMs in a concentration-dependent manner. In addition, nilotinib and other BCR-ABL TKIs decreased the relaxation velocities.

These results suggest that BCR-ABL TKIs affect contractility in hiPSC-CMs. We are planning to analyze the adverse drug event reporting database using BCR-ABL TKIs to conform the in vitro data.

The mechanism for impairing vasorelaxation response enhancement by perivascular adipose tissue in metabolic syndrome

メタボリックシンドロームにおける血管周囲脂肪組織による血管弛緩反応の増強作用消失のメカニズム

○籠田 智美[1,2]、懐 理紗[1]、麓（丸山） 加菜[1]、篠塚 和正[1]

[1]武庫川女大・薬・薬理2、[2]武庫川女大・バイオサイエンス研

Sex differences in the modulatory function of arterial tone by perivascular adipose tissue (PVAT) are reported. We demonstrated that sex difference in enhancing vasorelaxation response by PVAT in renal arteries differs between metabolic syndrome (MetS) model strains, SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SPZF) and SHR/NDmcr-cp rats (CP), at the same age. The underlying mechanism of this discrepancy was investigated.

Systolic blood pressure (sBP) was measured using a tail-cuff method. Ring preparations with and without PVAT were made from male and female SPZF and CP renal arteries at 23 weeks of age. Vasorelaxation and mRNA transcript levels in PVAT were examined using organ bath methods and quantitative real-time polymerase chain reaction, respectively.

sBP was higher in SPZF than CP but lower in females than males. PVAT increased acetylcholine-induced relaxations in renal arteries in CP females only. There were no significant differences in mRNA levels of angiotensin II type 1 receptor (AT1R) and AT1R-associated protein (ATRAP) among groups, but the AT1R/ATRAP ratio, which indicates AT1R activity, was lower in CP than SPZF. The AT1R/ATRAP ratio in PVAT was negatively correlated with enhancing the effects of PVAT on acetylcholine-induced relaxations while positively correlated with sBP.

This study suggests that overactivation of AT1R signaling in PVAT, probably resulting from high blood pressure, induces the decline in compensatory PVAT effects in MetS. Negative regulation of AT1R signaling is beneficial for sustained favorable PVAT effects.

Administration of nicotinamide mononucleotide attenuates cardiotoxicity and skeletal muscle atrophy induced by doxorubicin in mice.

ニコチンアミド・モノヌクレオチドはドキソルビシンによる心毒性や筋萎縮を抑制する

○久野 篤史、細田 隆介、岩原 直敏、野島 伊世里

札幌医科大・医

Background: Doxorubicin (DOX), an anti-cancer drug, induces cardiotoxicity and skeletal muscle atrophy. We recently reported that resveratrol, an activator of NAD^+-dependent deacetylase SIRT1, prevented DOX-induced cardiotoxicity. In this study, we examined effects of nicotinamide mononucleotide (NMN), a precursor of NAD^+, on DOX-induced cardiotoxicity and skeletal muscle atrophy.

Methods: Male mice were randomly divided into three groups: vehicle, DOX, and NMN+Dox groups. In the DOX group, mice received DOX (5 mg/kg, IP) once a week for four times. NMN (500 mg/kg, IP) was administered to mice in the NMN+DOX group 30 min before and 2 days after DOX injection. Echocardiography was performed at 1 week after the final DOX injection. Heart weight and tibialis anterior (TA) muscle weight were measured at 1 week after final DOX.

Results: Body weight was gradually decreased in the DOX group compared with the vehicle group. NMN partially blocked the decrease in body weight by DOX. Left ventricular fractional shortening (FS), an index of cardiac contraction, was lower in the DOX group than the vehicle group but was preserved in the NMN+DOX group. Heart weight-to-tibia length (TL) ratio and TA weight-to-TL ratio were lower in the DOX group, indicating myocardial and skeletal muscle atrophy by DOX. NMN treatment blocked the reductions heart weight and TA weight induced by DOX.

[Conclusion] These findings suggest that supplementation of NAD^+ by administration of NMN could be a therapeutic strategy for prevention of cardiotoxicity and skeletal muscle atrophy induced by DOX.

A comparative study of the effects of cigarette smoke extracts (CSE) from combusted or heated cigarettes on contractile function, spontaneous beating rate, and intracellular Ca^{2+} dynamics in rat ventricular myocytes.

紙巻きタバコおよび加熱式タバコのタバコ煙抽出液がラット心室筋細胞の収縮機能、自動拍動数および細胞内Ca^{2+}動態に及ぼす影響の比較検討

○安田 純平[1]、松村 早希子[1]、陳 以珊[1]、納富 拓也[1]、堀之内 孝広[2]、西谷（中村） 友重[1]

[1]和歌山県立医科大・医・薬理学講座、[2]北海道大・院医・細胞薬理学

Smoking is known as a risk factor for cardiovascular diseases. Recently, the use of heated cigarettes, which are expected to be a less toxic, has increased in Japan. However, little is known about their direct effects on cardiomyocytes. In the present study, we compared the effects of heated and a combustible cigarette smoke extracts (CSEs) on the cell viability, contractile function and intracellular Ca^{2+} dynamics of freshly isolated or cultured rat cardiomyocytes. We used Ploom X (PX) and IQOS (IQ) as heated CSEs, and 1R6F (RF) as a combustible CSE. The cell viability (MTS) assay using cultured myocytes showed that the order of toxic effects was RF>IQ>PX. Functional analysis using the Cell Motion Imaging System showed that all types of 1% CSE reduced cardiomyocyte contractility with their impairing activity RF≒IQ>PX. Intracellular Ca^{2+} dynamics analysis showed that all types of 1% CSE decreased the Ca^{2+}-transient amplitude, which may be the mechanism of reduced contractility. In addition, RF treatment induce Ca^{2+} leakage (possibly from sarcoplasmic reticulum), which may be the cause of the increased diastolic Ca^{2+} levels. Furthermore, the spontaneous beating rate of cultured cardiomyocytes was markedly reduced by RF. Taken together, these results indicate that heated cigarette extracts are also toxic to cell viability and contraction of cardiomyocytes. In addition, each CSE had different effects on intracellular Ca^{2+} dynamics, suggesting that mechanism of action is different.

Inhibitory effects of eicosapentaenoic acid (EPA) on contractions in pig basilar and coronary arteries

ブタ脳底動脈および冠動脈の収縮反応に対するエイコサペンタエン酸 (EPA) による抑制効果

○吉岡 健人、小原 圭将、追川 俊也、上村 洸平、山口 明奈、藤澤 和輝、花澤 瞳、藤原 実貴、遠藤 太尊、鈴木 太智、De Dios Regadera Montserrat、伊藤 大地、斎藤 昂、中込 佑尚、山下 冬馬、木口 真由、齊藤 祐花、仲尾 友里、宮路 陽南子、欧 光瀚、徐 可悦、田中 芳夫

東邦大・薬・薬理

Eicosapentaenoic acid (EPA) is an n-3 polyunsaturated fatty acid (PUFA) found in fish oil. We recently showed that docosahexaenoic acid (DHA), another n-3 PUFA, potently inhibited pig basilar and coronary artery contractions induced by U46619 (a TP receptor agonist) and prostaglandin (PG) $F_{2\alpha}$. We also showed that prostanoid TP receptors are potential targets for DHA. In this study, we investigated whether EPA, like DHA, suppresses contractions of pig basilar and coronary arteries induced by U46619 and $PGF_{2\alpha}$ through inhibition of the TP receptor. EPA suppressed both U46619- and $PGF_{2\alpha}$-induced pig basilar and coronary contractions in a concentration-dependent manner without affecting 80 mM KCl-induced contractions. U46619-/$PGF_{2\alpha}$-induced contractions in both arteries were completely/largely suppressed by SQ 29,548 (a TP receptor antagonist). In addition, EPA suppressed U46619-/$PGF_{2\alpha}$-induced increases in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in human TP receptor-overexpressing 293T cells, whereas it showed only a slight effect on $PGF_{2\alpha}$-induced $[Ca^{2+}]_i$ increases in human FP receptor-overexpressing 293T cells. These findings suggest that EPA strongly suppresses TP-receptor-mediated contractions of pig basilar and coronary arteries, which can be partially attributed to its inhibitory effects on the TP receptor.

5-HT_{1A} receptor activation induces oligodendrocyte transcription factors via the Gab1/GSK3β signaling pathway in stress-maladaptive mice

5−HT_{1A}受容体刺激はストレス非適応マウスにおいてGab1/GSK3βシグナリングを介してオリゴデンドロサイト転写因子を誘導する

○黒川 和宏[1]、髙橋 浩平[1]、宮川 和也[1]、持田(斎藤) 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

Our previous study demonstrated that 5-HT_{1A} receptor activation by chronic administration of flesinoxan, a 5-HT_{1A} receptor agonist, reduces the abnormal emotionality in stress-maladaptive mice and promotes oligodendrogenesis and myelination. However, the effects of 5-HT_{1A} receptor activation on oligodendrocyte transcription factors under the stress-maladaptive situations and the underlying mechanisms remain unknown. In the present study, we investigated whether activation of 5-HT_{1A} receptor by flesinoxan could act on oligodendrocytes to induce oligodendrocyte transcription factors in stress-maladaptive mice. Western blot analysis revealed that administration of flesinoxan significantly increased the expression levels of Gab1 and p-GSK3β (Y216) in the hippocampus of stress-maladaptive mice, whereas decreased the expression level of p-GSK3β (Ser 9) in the same brain regions. Under this condition, the significant increase in mRNA expression levels of oligodendrocyte transcription factors, i.e. olig2, SOX10, MYF, NKX2.2 and Zfp24, in the hippocampus were observed by real-time PCR analysis. The present findings suggest that 5-HT_{1A} receptor activation may induce oligodendrocyte transcription factors via the hippocampal Gab1/GSK3β signaling pathway and reduce abnormal emotionality due to maladaptation to excessive stress.

The critical role of PACAP/PAC1 signaling from the rhinal cortex to the hippocampal astrocytes on the working memory

嗅内皮質−海馬神経回路のPACAPはアストロサイトPAC1受容体を介して作業記憶に寄与する

○神戸 悠輝、グエン トウ、栗原 崇、宮田 篤郎

鹿児島大・院医歯

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor (PAC1) are widely distributed in the central nervous system. Although it has been reported that PACAP-PAC1 signaling contributes to learning and memory, it is not clear what mechanism is involved in neuroplasticity. In this study, we investigated the involvement of PACAP-PAC1 signaling in astrocytes for working memory. First, to confirm the expression of PAC1 in hippocampal astrocytes, we obtained astrocyte-specific mRNA using the Ribo-tag method, and RT-qPCR revealed that astrocytes expressed PAC1 six times higher than all cell types. A selective knockout of hippocampal astrocytic PAC1 resulted in the impaired working memory of the Y-maze test. To investigate the origin of PACAP neurons projecting to hippocampal astrocytes, we infected the hippocampus of PACAP-Cre mice with adeno-associated virus (AAV), which is retrogradely transported and expresses mCherry in a Cre-dependent manner, and observed mCherry-positive cells. We found some mCherry-positive cells in the rhinal cortex. Furthermore, we infected the rhinal cortex of PACAP-cre mice with AAV, which expresses a synaptophysin-EGFP chimeric protein cre-dependently. We found many synaptophysin-EGFP positive punctate around hippocampal astrocytes, suggesting PACAP neurons in the rhinal cortex sent axons to the hippocampal astrocytes. These findings suggested that PACAP from the rhinal cortex might act on hippocampal astrocytic PAC1 and contribute to the working memory.

Analysis of *in vivo* astrocytic Ca^{2+} activity in mouse models of neurodegenerative diseases

神経変性疾患モデルマウスにおける*in vivo*アストロサイトCa^{2+}活動の解析

○茂木 優貴、飯野 正光、金丸 和典

日本大・医・生理学分野

Astrocytic contribution to neurodegenerative diseases is attracting attention as a potential target for drug discovery and therapeutics. Intracellular Ca^{2+} signaling in astrocytes is affected by bioactive substances from damaged brain cells including neurons. This can trigger theenhancement and/or attenuation of Ca^{2+}-dependent processes in astrocytes, which lead to an alteration of gene expression profiles and secretion of neuroprotective and neurotoxic molecules. Thus, analysis of astrocytic Ca^{2+} activities may provide clues to regulatingneurodegenerative diseases. However, it remains elusive how the pathological conditions affect astrocytic Ca^{2+} activities. Therefore, we are trying to establish a new method to analyze astrocytic Ca^{2+} activities in neurodegenerative diseases. We applied drug-induced or geneticmanipulation-induced neurodegenerative disease models to transgenic mouse linesexpressing a Ca^{2+} sensor protein or a Ca^{2+} signal-suppressing enzyme in astrocytes. Using macroscopic *in vivo* Ca^{2+} imaging and optical clearing method-assisted volumetric immunohistochemical analysis, we found correlations between astrocytic Ca^{2+} activities and neurodegenerative phenotypes. These results and further analysis may contribute to the development of therapeutic strategies for neurodegenerative diseases.

Impact of astrocytic lactate release on brain ischemic tolerance

脳虚血耐性におけるアストロサイト乳酸放出の影響

○平山 友里[1]、小泉 修一[2,3]、安西 尚彦[1]

[1]千葉大・院医・薬理、[2]山梨大・院医・薬理、[3]山梨大・院医・GLIAセンター

Brain ischemic tolerance is an endogenous neuroprotective mechanism, whereby an experience of non-lethal ischemic episode (preconditioning; PC) produces resilience to subsequent lethal ischemia. We previously showed that PC caused activation of astrocytes and a subsequent upregulation of P2X7 receptors (P2X7Rs), activation of which induced ischemic tolerance. However, the downstream signals of P2X7Rs responsible for the ischemic tolerance remain unknown. Here we show that astrocytic P2X7R-dependent lactate release has an indispensable role for this event. Using a middle cerebral artery occlusion model, we found that extracellular lactate levels during lethal ischemia were significantly increased in mice that experienced PC, and the increase depended on P2X7Rs. In the *in vitro* experiments, although stimulation of astrocytes with P2X7R agonist BzATP had no effect on the protein levels of MCT1 and MCT4, which were responsible for lactate efflux in astrocytes, BzATP induced the plasma membrane translocation of these MCTs via their chaperone CD147. Importantly, CD147 was increased in activated astrocytes after PC, and CD147 blocking antibody abolished astrocyte-mediated lactate release and ischemic tolerance. Taken together, our findings suggest that astrocytes induce ischemic tolerance by P2X7R-dependent lactate release.

Functional expression of choline transporters in microglia and their regulation of microglial M1/M2 polarization

ミクログリアにおけるコリントランスポーターの機能発現とミクログリアM1/M2極性化の制御

○稲津 正人[1,3]、岡田 寿郎[2]、武藤 瑛祐[2]、山中 力[3]、内野 博之[2]

[1]東京医大・医総研、[2]東京医大・麻酔科学分野、[3]東京医大・分子予防医学

Microglia are key cells of the immune system in the central nervous system and are suggested to be deeply involved in the development of neurodegenerative diseases. It is well known that microglia have functional plasticity, with an inflammatory M1 phenotype and an anti-inflammatory M2 phenotype. Inhibition of choline transport in macrophages has been reported to suppress the secretion of inflammatory cytokines. However, the role of the choline transport system in regulating microglial M1/M2 polarization has not been fully elucidated to date. In this study, we investigated the mechanism of choline uptake in mouse microglial cell line SIM-A9, and its association with microglial M1/M2 polarization. Choline transporter-like protein 1 (CTL1) were highly expressed in SIM-A9 cells and were localized in the plasma membrane. Functional analysis of choline uptake demonstrated the existence of Na^+-independent, pH-dependent, and intermediate-affinity transport systems. Choline uptake was concentration-dependently inhibited by hemicholinium-3 (HC-3). Expression of the mRNA of M1 microglia markers IL-1β and IL-6 was increased by LPS, and their effects were suppressed by choline deprivation and HC-3. In contrast, mRNA expression of the M2 microglial marker arginase-1 was increased by IL-4, and the effect was enhanced by choline deprivation and HC-3. Our results suggest that inhibition of CTL1-mediated choline uptake in microglia preferentially induces M2 microglia polarization, which is a potential therapeutic approach for inflammatory brain diseases.

The astrocytic TRPA1 channel plays a protective role in vascular cognitive impairment

アストロサイトのTRPA1チャネルは血管性認知障害に対して保護的に機能する

○抱 将史[1]、中島 弘貴[1]、戸堀 翔太[1]、川下 綾香[1]、宮之原 遵[1]、森嶋 美沙[1]、永安 一樹[1]、繁冨 英治[2,3]、小泉 修一[2,3]、森 泰生[4]、白川 久志[1]、金子 周司[1]

[1]京都大・院薬、[2]山梨大・医・薬理、[3]山梨大・グリアセンター、[4]京都大・エ・分子生物化学

Vascular cognitive impairment (VCI) is a syndrome defined as cognitive decline caused by vascular disease, which is associated with Alzheimer's disease and vascular dementia. Since chronic cerebral hypoperfusion (CCH) is commonly present in various types of dementia and induces VCI, we used bilateral common carotid artery stenosis (BCAS) mice as a CCH-induced VCI model.　Transient receptor potential ankyrin 1 (TRPA1), the most redox-sensitive TRP channel, is functionally expressed in the brain and seems to function as a polymodal sensor in vascular disease. To clarify the involvement of TRPA1 in CCH-induced VCI, we used genetically engineered mice: TRPA1-knockout (TRPA1-KO) and cell-specific conditional TRPA1-KO mice. We showed that TRPA1 deficiency exacerbated BCAS-induced cognitive impairment and white matter injury during early-stage CCH. TRPA1 stimulation with cinnamaldehyde ameliorated BCAS-induced outcomes. We also revealed that BCAS increased a cytokine in astrocytes. Moreover, TRPA1-stimulated primary astrocyte cultures expressed the cytokine, and culture medium derived from these TRPA1-stimulated cells promoted oligodendrocyte precursor cell myelination. Overall, TRPA1 stimulation in astrocytes plays a protective role in CCH-induced VCI through the cytokine production.

Expression and function of equilibrative nucleoside transporters in cultured astrocytes

培養アストロサイトにおける平衡型ヌクレオシド輸送体の機能と発現

○田中 康一[1,2,3]、井澤 琢人[1]、冨田 和男[1,2]、五十嵐 健人[1,2]、北中 順惠[3]、北中 純一[1]、佐藤 友昭[2]、西山 信好[1]

[1]兵庫医科大・薬・薬理、[2]鹿児島大・院医歯・歯科薬理、[3]兵庫医科大・医・薬理

We have found that cultured differentiated astrocytes pretreated with N^6, $2'$-O-dibutyryladenosine 3',5'-cyclic monophosphate (DBcAMP), a permeable analogue of cAMP, incorporate thymidine, but not uridine, via nucleoside transporters including equilibrative nucleoside transporters (ENTs) into TCA insoluble fraction for repair on DNA injury in the presence of hydrogen peroxide (H_2O_2) at an early time, and these phenomena are specific in differentiated astrocytes, but not undifferentiated astrocytes and neurons.

We studied expression and function of ENTs and LIMPII (Lysosomal Integral Membrane Protein II). We could confirm ENT1, that is hypersensitive nucleoside transporter, and ENT2, that is low-sensitive nucleoside transporter in cultured astrocytes by RT-PCR and western blot analysis and [^{3}H]thymidine incorporation experiment. Astrocytes were double-stained by anti-GFAP antibody and anti-ENT3 antibody. We could confirm ENT3, that is assumed to be presented in lysosome, in cultured astrocytes co-stained by GFAP. We found the expression of ENT3 and LIMPII by RT-PCR and western blot analysis, and the coexpression of ENT3 and LIMPII in cultured astrocytes by immunocytochemistry. We clarified that ENT3 is localized in astrocytic lysosome.

These results indicate that ENTs expressed and function in cell membrane and lysosome could relate with H_2O_2-induced thymidine incorporation and DNA repair and disassembly in cultured astrocytes.

Glial-derived secreted protein Tinagl1 regulates neuronal survival

グリア由来分泌タンパク質Tinagl1は神経生存を制御する

○小椋 正人[1]、小野塚 星矢[1]、八巻 淳子[1]、和田 郁夫[2]、本間 美和子[1]

[1]福島県立医科大・医・生体物質、[2]福島県立医科大・医・細胞科学

Tubulointerstitial nephritis antigen-like 1 (Tinagl1), as a secreted matricellular protein, are implicated in the modulation of diverse processes including cell survival. We previously created transgenic mice expressing a phosphorylation-defective mutant of succinate dehydrogenase A ($SDHA^{Y215F}$) in astrocytes for evaluation of the effects of mitochondrial ROS on astrocyte reactivity and showed that reactive astrocytes induce dopaminergic neuronal loss though the expression of Tinagl1 in substantia nigra. In the present study, we investigate the effect of glial-derived Tinagl1 on neuronal survival. Conditioned medium prepared from primary astrocytes expressing either $SDHA^{Y215F}$ or Tinagl1 significantly decreased the number of microtubule associated protein 2-positive cells as compared with control medium in primary neuron culture. Furthermore, recombinant Tinagl1 (rTinagl1) also significantly induced neuronal apoptosis in a dose-dependent manner. Signaling analysis revealed that rTinagl1 inhibits Akt activation, whereas promotes MKK4 activation in primary neuron culture. These results suggest that glial-derived Tinagl1 may regulate neuronal survival through modulation of Akt and MKK4 signaling. Exact molecular targets for Tinagl1 will be discussed.

Proepileptic roles of astrocytes in mesial temporal lobe epilepsy with hippocampal sclerosis.

海馬硬化を伴う内側側頭葉てんかんにおけるアストロサイトの寄与

○木下 慎一[1]、池谷 裕二[1,2]、小山 隆太[1]

[1]東京大・院薬・薬品作用学教室、[2]東京大・Beyond AI研究推進機構

About 30% of patients with mesial temporal lobe epilepsy (mTLE), in which the hippocampus is the epileptic focus, is drug-resistant to existing antiepileptic drugs that target the modulation of neuronal activity. mTLE is commonly accompanied by the hippocampal sclerosis, which is primarily characterized by neuronal cell loss and chronic astrogliosis in hippocampal CA3 areas. It has been suggested that the changes in astrocytic function associated with hippocampal sclerosis contribute to epileptogenesis, but the underlying cellular and molecular mechanisms remain unclear. Here, we found that during the development of hippocampal sclerosis, some astrocytes migrate from CA3 to the dentate gyrus (DG) and upregulate the gene expression that could modulate neural activity related to the epileptic state. In intrahippocampal kainic acid (KA)-injected mTLE model mice, we found that some astrocytes migrate from CA3 to DG during the early-stage of hippocampal sclerosis after KA-injection. Then, we investigated the transcriptional profiles of the migrating astrocytes, finding an increased expression of genes that could result in hyperactivation of neurons. Thus, our findings reveal previously unknown changes in CA3 astrocytes during hippocampal sclerosis and propose astrocytes as a therapeutic target for mTLE.

HASTR/ci35, conditionally immortalized human astrocytes, can be a useful tool for studies in characterization of human reactive astrocytes

HASTR/ci35は活性化アストロサイトモデルとして有用である

○根岸 由佳、長谷川 理歩、山下 雅子、馬場 知代、森尾 花恵、降幡 知巳

東薬大・薬・個別化薬物治療学

Reactive astrocytes play important pathophysiological roles in various brain diseases in a context-dependent manner, and they have thus gained significant attention as a target for development of new drugs. Therefore, we aimed to characterize human astrocytes/conditionally immortalized, clone 35 (HASTR/ci35) as an *in vitro* reactive human astrocyte model. The results of quantitative PCR and RNA-sequencing showed that, upon exposure to pro-inflammatory cytokines, HASTR/ci35 cells exhibited significant up-regulation of mRNA levels of various inflammation associated genes, such as interleukin-6, intercellular adhesion molecule 1, and pentraxin 3. The results of the protein array confirmed their induction at protein levels. Qualitatively, the response profile of HASTR/ci35 cells appeared to be similar to that observed in human primary astrocytes. To summarize, HASTR/ci35 cells show an unequivocal reactive response to inflammatory stimuli, indicating that they can serve as a human reactive astrocyte model. While further characterization is currently underway, it can be expected that they have a considerable potential to be used in reactive astrocyte-targeted drug development studies.

Loss of the sustained antidepressant effect of (*2R,6R*)-hydroxynorketamine in NMDA receptor GluN2D subunit knockout mice

NMDA受容体GluN2Dノックアウトマウスにおける(*2R,6R*)-ヒドロキシノルケタミンの持続的抗うつ効果の消失

○山岸 愛実[1,2]、井手 聡一郎[1]、池窪 結子[1]、三品 昌美[3]、池田 和隆[1]

[1]東京都医学総合研・依存性物質プロジェクト、[2]新潟大・院医歯・先端分子病態学、[3]立命館大・総合科学技術研究機構

Background: The NMDA receptor antagonist ketamine exhibits acute and sustained antidepressant effects and is rapidly metabolized. We have reported that GluN2D plays an important role in the sustained antidepressant effect of (*R*)-ketamine, an optical isomer of ketamine. Meanwhile, the pharmacological mechanism of major metabolites of ketamine, (*2R,6R;2S,6S*)-hydroxynorketamine (HNK), is still unclear. In this study, we investigated the role of GluN2D in the antidepressant effect of (*2R,6R;2S,6S*)-HNK.

Methods: We investigated the acute and sustained antidepressant effects of (*2R,6R;2S,6S*)-HNK on 4 h restraint stress-induced depression in wild-type and GluN2D-KO mice using the tail-suspension test (TST). After behavioral tests, mouse brains were removed and brain regions considered to be related to the antidepressant effect were collected and examined for protein expression of neuroplasticity-related molecules using the Western Blotting (WB).

Results: (*2R,6R*)-HNK, but not (*2S,6S*)-HNK, reduced immobility time in the TST 10 min after administration in wild-type and GluN2D-KO mice. Further, the sustained antidepressant effect at 96 h post-treatment was observed only in wild-type mice and disappeared in GluN2D-KO mice. We analyzed the changes in protein expression levels of molecules assumed to be related to neuroplasticity by the WB.

Conclusion: Among the major metabolites of ketamine, (*2R,6R*)-HNK has acute and sustained antidepressant effects and GluN2D plays an important role in the sustained antidepressant effects of (*2R,6R*)-HNK.

Resveratrol prevents dextran sulfate sodium-induced colitis-like symptoms and depressive-like behavior by activation of AMP-activated protein kinase pathways in the brain-gut

レスベラトロールは脳腸AMPK経路の活性化によりデキストラン硫酸ナトリウム誘発性腸炎様所見並びにうつ様行動を抑制する

○高橋 浩平[1]、黒川 和宏[1]、洪 麗花[1]、宮川 和也[1]、持田（斎藤） 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

Patients with inflammatory bowel disease (IBD) have higher rates of psychiatric pathology including depression. The dextran sulfate sodium (DSS)-treated mouse is a well-characterized animal model of colitis that exhibits both IBD- and depressive-like symptoms. Recently, we found that DSS-treated mice decreased phosphorylation of AMP-activated protein kinase (AMPK) in the hippocampus and rectum. AMPK is known to be associated with the regulation of inflammation, autophagy, and neurogenesis. Hence, we examined whether AMPK activator resveratrol (RSV) prevents the DSS-induced colitis and depressive-like behavior in mice. DSS-treated mice exhibited the colitis-like symptoms, and depressive-like behavior in the tail-suspension test. Moreover, DSS-treated mice showed the hippocampal and rectal decrease in p-AMPK and LC3 II / I levels as well as increase in p-p70S6K (a downstream target of mTORC1), p62, pro-inflammatory cytokines and cleaved caspase -3 levels, and the reduction of cell proliferation in the dentate gyrus of hippocampus. Treatment with RSV prevented these abnormalities in DSS-treated mice, and induced LC3-positive puncta in the hippocampus. These findings indicate that activation of AMPK in the hippocampus-gut may be involved in the antidepressant and anti-inflammatory effects of RSV in DSS-treated mice.

Calcitonin gene-related peptide as an anxiety regulator in the mouse hippocampus: Mechanisms for transcriptional regulation of monoamine oxidase B by calcitonin gene-related peptide administration

CGRP投与によるMAOBの転写調節と海馬における不安様行動の発現について

○藤原 享志朗[1]、渡邉 杏香[2]、橋川 直也[1,2]、橋川 成美[1,2]

[1]岡山理科大・大学院理学研究科・臨床生命科学専攻、[2]岡山理科大・理・臨床生命科学

Calcitonin gene-related peptide (CGRP) is a neuropeptide that affects anxiety; however, how it evokes anxiety-like responses remains unclear. Here, we show that intracerebroventricular (i.c.v.) administration of CGRP (0.5 nmol) increased monoamine oxidase B (MAOB) and decreased dopamine in the mouse hippocampus. CGRP administration revealed anxiety-like behavior in open field, hole board, and plus-maze tests. CGRP decreased dopamine but increased the transcriptional regulator of MAOB, Krüppel-like factor 11 (KLF11), and the phosphorylated heterochromatin protein (p-HP1γ), which is involved in gene silencing by methylating histone H3 in mice hippocampus. Notably, increasing p-HP1γ becomes more euchromatic and activates transcription. To determine whether this effect reflected the binding of HP1γ and KLF11 to the enhancer, we designed primers within the KLF11 enhancer region (−700 bp) and performed chromatin immunoprecipitation (ChIP) assays. Notably, HP1γ was recruited to the KLF11 enhancer by CGRP i.c.v. in the mouse hippocampus. We also observed that CGRP (1000 pmol) infusion into the hippocampus significantly increased anxiety-like behavior in the plus-maze test. Therefore, CGRP reduces hippocampal dopamine and exhibits anxiety-like behavior through epigenetic regulation.

Anxiolytic effects of inhibitors of endocannabinoid degrading enzyme on anxiety-like behavior in restraint-stressed mice.

拘束ストレス負荷マウスにおける不安様行動に対する内因性カンナビノイド分解酵素阻害薬の抗不安作用

○福森 良、中島 良佐、上尾 海南、山口 拓

長崎国際大・薬

In this study, we investigated the effects of endocannabinoid (eCB) degrading enzyme inhibitors on anxiety-like behavior and brain eCB levels in restraint-stressed mice.

For restraint stress, mice were forced in 50mL syringe for 30 minutes. Mouse brain was separated into the prefrontal cortex (PFC), hippocampus (HC), striatum (ST), periamygdaloid cortex (AM) and medulla oblongata (MO). Brain tissues were homogenized with acetonitrile, which quantified for 2-arachidonoylglycerol (2-AG) or arachidonylethanolamide (AEA) content by UPLC/MS/MS. In addition, we measured anxiety levels of mice in the elevated plus-maze test.

Restraint stress decreased 2-AG and AEA levels in PFC and HC, but not ST, AM, and MO. In the elevated plus-maze test, restrain-stressed mice showed anxiogenic behavior. This anxiogenic behavior was ameliorated by administration of JZL184 (an inhibitor of monoacylglycerol lipase which hydrolyze 2-AG) or URB597 (an inhibitor of fatty acid amide hydrolase which hydrolyze AEA).

These results suggest that restraint stress induces anxiogenic behavior through the region-specific decrease of eCB in PFC and HC. This anxiogenic behaviors were ameliorated by inhibitors of eCB degrading enzyme, which indicates that anxiogenic behavior induced by restraint stress might be due to the reduction of eCB in PFC and HC.

Protective effects of antidepressants on amyloid β oligomers-induced neurotoxicity in SH-SY5Y cells

アミロイドβオリゴマー誘発性神経毒性に対する抗うつ薬の保護作用

○山本 謙[1,3]、辻 まゆみ[2]、小口 達敬[1,2]、門馬 佑太郎[1,3]、大橋 英朗[1,3]、野原 哲人[1,3]、井藤 尚仁[1,3]、永田 未希[4]、村上 秀友[3]、木内 祐二[1,2]

[1]昭和大・院医・薬理学部門 医科薬理学講座、[2]昭和大・薬理科学研究センター、[3]昭和大・医学部内科学講座・脳神経内科学部門、[4]昭和大・薬・病院薬剤学講座

[Introduction] It is widely known that patients with Alzheimer's disease (AD) often present depression, which is one of the risk factors for developing AD. Although antidepressants have been used in many patients with AD, the evidence of their cognitive benefit remains inconsistent. AD is characterized pathologically by amyloid-β peptide (Aβ) deposition and neurofibrillary tangles in brain. In this study, we evaluated if treatment with antidepressants could modulate neuronal damage induced by the highly toxic Aβ oligomers (Aβo).

[Methods] $A\beta_{1-42}$ aggregation was measured using thioflavin T. We prepared 5 μM Aβo solution using $A\beta_{1-42}$peptides and induced neuronal toxicity in SH-SY5Y cells (human neuroblastoma) using Aβo. SH-SY5Y cells were treated with duloxetine (Dlx), venlafaxine (Ven), mirtazapine(Mir) and fluoxetine(Flx) and Aβo. Cell viability and oxidative stress were measured.

[Results] Dlx and Flx inhibited $A\beta_{1-42}$ aggregation in concentration-dependent manner. Dlx and Flx significantly reduced Aβo-induced cytotoxicity compared to Aβo-alone group after 24 hr of treatment. All four antidepressants reduced ROS production significantly in concentration-dependent manner compared to Aβ-o alone group after 30 min of treatment.

[Conclusion] These results suggest that antidepressants, especially Dlx and Flx, may be effective in preventing and suppressing the progression of AD through multiple mechanisms such as inhibition of Aβ aggregation and antioxidative effect.

Involvement of the agranular insular cortex in antidepressant actions of arketamine

アールケタミンの抗うつ作用発現には島皮質が関与する

○横山 玲[1]、吾郷 由希夫[2]、笠井 淳司[1]、田沼 将人[1]、林田 美鈴[1]、島﨑 雄人[1]、樋口 桃子[1]、五十嵐 久人[1]、勢力 薫[1]、山口 瞬[3]、中澤 敬信[4]、橋本 謙二[5]、橋本 均[1,6,7,8,9]

[1]大阪大・院薬・神経薬理、[2]広島大・院医・細胞分子薬理、[3]岐阜大・院医・高次神経形態学、[4]東農大・生命科学、[5]千葉大・社会精神保健教育研究センター、[6]大阪大・院連合小児発達・子どものこころセンター、[7]大阪大・データビリティフロンティア機構、[8]大阪大・先導的学際研究機構、[9]大阪大・院医・分子医薬

(*S*)-ketamine (esketamine), one of ketamine enantiomers, has been approved for treatment-resistant depression. Although several preclinical studies have shown that another enantiomer (*R*)-ketamine (arketamine) has greater potency antidepressant-like effects than esketamine, the antidepressant mechanism of ketamine enantiomers is not fully understood. Here, we aimed to identify brain regions that contribute to the difference in antidepressant action between ketamine enantiomers using isolation-reared mouse model of depression. We found that lower doses of arketamine than esketamine had antidepressant-like effects in isolation-reared mice in the forced swim test. Then, the machine learning classifiers with brain-wide activation mapping in isolation-reared Arc-dVenus reporter mice revealed that the agranular insular cortex (aIC) may contribute to the antidepressant-like effect of arketamine and discrimination between the effects of ketamine enantiomers. Furthermore, a temporary suppression of neuronal activity in the aIC blocked the antidepressant-like effect of arketamine, but not of esketamine, and conversely activation of neurons in the aIC induced antidepressant-like effects in isolation-reared mice. These findings suggest that activation of the aIC is involved at least partly in the antidepressant-like effects of arketamine.

Evaluation of the effects of Kir4.1 channel inhibitors on lipopolysaccharide-induced depressive behavior

Lipopolysaccharideによるうつ症状に対するKir4.1チャネル阻害薬の作用評価

○清水 佐紀、中野 諒子、廣瀬 由佳、堀名 宏紀、大野 行弘

大阪医薬大・薬・薬品作用解析

Astroglial potassium channels (Kir4.1) are involved in the onset of depression. Previous study showed that Kir4.1 channel is upregulated in the lateral habenula in rat models of depression (Nature, 554(7692), 323-327, 2018). In addition, it is reported that serotonin reuptake inhibitors (e.g., SSRIs) inhibit Kir4.1 channel currents, suggesting that astroglial Kir4.1 channels might be involved in the pharmacological action of antidepressants. In this study, to explore the role of Kir4.1 channels in the treatment of depression, we evaluated the effects of the Kir4.1 channel inhibitors, VU0134992 and quinacrine, on lipopolysaccharide (LPS)-induced depression in mice using forced swim test. First, treatments of mice with VU0134992 (10–30 mg/kg, s.c.) and quinacrine (10-100 mg/kg, s.c.) reduced immobility time in despaired model by forced swimming. Next, we observed that LPS (0.8 mg/kg, i.p.) increased immobility time in the forced swim test, and the immobility time was dose-dependently reversed by quinacrine (30-100 mg/kg, s.c.). Especially, quinacrine (100 mg/kg, s.c.) significantly attenuated prolonged immobility time by LPS in forced swim test. Furthermore, quinacrine (100 mg/kg, s.c.) also significantly improved LPS-induced anxiety like behavior in the elevated plus maze test. These results demonstrated that Kir4.1 channel inhibitors have antidepressant effects, suggesting that the Kir4.1 channel inhibitors are useful as new antidepressants.

Chronic administration of oxytocin exerts anxiolytic and antidepressant effects in a dose-independent manner in corticosterone-induced depression model of female mice

コルチコステロン誘発性うつ病モデル雌性マウスに対して、オキシトシン慢性投与は濃度特異的な治療効果を示す

○森 征慶、田村 美咲、隅 憲廣、川邉 隼輔、村田 雄介、大江 賢治、遠城寺 宗近

福岡大・薬・臨床薬物治療学

Background

Oxytocin (OT) is thought to have potential as a new therapeutic strategy for depression. In spite of the fact that women are at a far greater risk for depression than are men, many experimental studies use male subjects. Thus, we investigated the anxiolytic and antidepressant effects of OT on steroid-induced depression model of female mice.

Methods

Adult female C57BL/6J mice were injected with OT (0.01, 0.1, or 1.0 mg/kg, i.p.) and corticosterone (CORT; 40 mg/kg, s.c.) for 4 weeks. Mice were randomly assigned to the following groups: (1) vehicle; (2) CORT; (3) OT 0.01 + CORT; (4) OT 0.1 + CORT; (5) OT 1.0 + CORT. To assess the anxiolytic and antidepressant effects of OT, mice were subjected to the open field test (OFT) and forced swimming test (FST).

Results

OT 0.1 + CORT group showed a significantly higher number of entries into the center zone in OFT than the CORT group. In the FST, the immobility time of the OT (0.01, 0.1, and 1.0) + CORT groups were significantly lower than that of the CORT group. The immobility time of OT (0.01 and 0.1) + CORT groups were comparable to the vehicle group.

Discussion

Under CORT exposure, the anxiolytic effects of OT were seen only in the middle dose of OT (0.1 mg/kg), whereas OT treatment (0.01 and 0.1 mg/kg) ameliorated the depressive behavior. Previous studies showed that OT (1.0 mg/kg) improved both the CORT-induced anxiety and depressive behaviors in males. These results suggest that the effective dose of OT may be different between sexes.

Regulation of proliferation by glutathione in neural/stem progenitor cells generated after neuronal degeneration in the adult hippocampal dentate gyrus

成体脳海馬歯状回神経変性後に生成された神経系幹・前駆細胞のグルタチオンによる増殖制御

○米山 雅紀、山口 太郎、尾中 勇祐

摂南大・薬

Various neurological injuries are widely recognized as promoting endogenous neurogenesis in adult mammalian hippocampal dentate gyrus. Our previous studies demonstrated that the systemic treatment with trimethyltin chloride (TMT) causes the granule cell loss in the dentate gyrus of adult mice, with being regenerated in the dentate granule cell after the neuronal loss. The goal of the present study was to elucidate the roles of glutathione in proliferation of neural/stem progenitor cells (NPCs) after neuronal degeneration. Using the NPCs isolated from the dentate gyrus of mice on day 3 post-TMT treatment, the exposure to buthionine sulfoximine (BSO, inhibitor of glutathione synthesis) significantly attenuated the cell proliferation without cell damage. Next, BSO-induced attenuation of proliferative activity was completely abolished by singly tested N-acethyl cysteine (NAC), which is precursor of glutathione. However, NAC did not affect the proliferative activity of the NPCs. Our results suggest that glutathione has a critical role in proliferative activity in the NPCs generated following neuronal degeneration in the hippocampal dentate gyrus.

The pivotal role of PACAP/PAC1 signaling in the locus coeruleus noradrenergic system

PACAP/PAC1シグナルによる青斑核ノルアドレナリン神経制御機序の解明

○NGUYEN THI THU、栗原 崇、宮田 篤郎、神戸 悠輝

鹿児島大・院医歯・生体情報薬理学

Both pituitary adenylate cyclase-activating polypeptides (PACAP) and noradrenaline are known to be involved in anxiety and fear memory. However, the crosstalk of these substances has not been clarified previously. Here we investigated the PACAP action in the locus coeruleus (LC) noradrenergic system based on the mice. To evaluate the significance of PACAP on the mice behavior, we used the PACAP-knockout (-/-) mice.
PACAP (-/-) mice less stayed in the open arm of the elevated plus maze than wild-type (WT) mice, suggesting PACAP has an anxiogenic effect. When a fear conditioning test was conducted using PACAP (-/-) mice, the freezing time was significantly decreased compared to WT mice suggesting that PACAP is important for fear memory formation.
PAC1 is PACAP-specific receptor. To examine whether PAC1 was expressed in LC noradrenergic neurons, we observed coexistence of tyrosine hydroxylase immunoreactivity and PAC1 mRNA, revealing expression of PAC1 in the LC noradrenergic neurons. To examine whether PAC1 functional expressed in the LC, we measured extracellular noradrenaline in the hippocampus after PACAP treatment. Intracerebroventricular injection of PACAP increased hippocampal noradrenaline, suggesting PACAP/PAC1 signaling in the LC is active.
From the results show above, PACAP/PAC1 signaling might activate LC noradrenergic system. In the future, we will clarify the significance of LC PACAP/PAC1 signaling on mice behavior.

Potential effects of Semaphorin3A-PlexinA signaling on the function and metabolism of amyloid-beta precursor protein

Semaphorin3A–PlexinAシグナルがアミロイドβ前駆タンパク質の機能や代謝に与える影響

○関口 拓己[1]、櫻井 隆[1]、山下 直也[1,2]

[1]順天堂大・医・薬理、[2]神奈川工大・応用バイオ

Amyloid-beta (Aβ) aggregation has been believed to be the fundamental trigger of the development of Alzheimer's disease (AD). Therefore, elucidating the mechanisms that induce Aβ overproduction from its type I transmembrane precursor protein (APP) is one of the important issues in providing potential therapeutic targets for AD. Semaphorin3A (Sema3A), a secreted type of repulsive axon guidance molecule, is implicated in the development of various neurodegenerative diseases. It was previously reported that Sema3A and its signaling molecules accumulate and aggregate in AD patients' brain. However, the molecular link between Sema3A signaling and AD pathogenesis remains unknown. Here we show evidence that APP interacts with PlexinA, a Sema3A receptor component. APP and PlexinA interacted through the extracellular regions and we were able to narrow down these regions to less than 100 amino acids. Based on these findings, we are now investigating whether the APP-PlexinA interaction affects APP function and metabolism, which might provide new perspective that aberrant Sema3A signaling induces Aβ overproduction.

In vivo molecular analysis of a novel gene involved in the maintenance of wakefulness

覚醒維持に関与する新規遺伝子のIn vivo分子解析

○王 乙萌[1,2]、戸根 大輔[1,2]、山田 陸裕[2]、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ンター・合成生物学研究チーム

The molecular basis of mammalian sleep-wake regulation remains largely unexplored. Several studies have identified sleep-regulating kinases that strongly suggest that protein phosphorylation plays a key role in promoting sleep (Tatsuki, 2016; Funato, 2016; Mikhail, 2017), but the regulation of protein phosphorylations involved in the induction and/or maintenance of wakefulness remains unknown. In this study, we identified a novel gene involved in the maintenance of wakefulness. This geneX controls protein phosphorylation in various cellular signaling pathways. AAV-mediated neuronal expression of gene X inhibits the transition from wakefulness to sleep, leading to a marked increase in wake duration in mice. We also found that this change in wake duration was accompanied by a significant decrease in delta power during NREM sleep. Conversely, inhibition of ProteinX (encoded by geneX) function in neurons resulted in a decrease in wake duration. These results imply that ProteinX is a key regulator of signaling involved in the maintenance of wakefulness. It has been suggested that kinases such as CaMKII are activated during wakefulness and induce sleep, but in contrast, ProteinX might exert its wake-promoting function through interaction with the sleep-promoting kinases.

Acute effects of guanfacine on neuronal activities in the prefrontal cortex of mice

マウス前頭前皮質における神経活動に対するグアンファシンの急性作用

○齋藤 文仁、鈴木 秀典、荒川 亮介

日本医科大・薬理学

Neurodevelopmental disorder, attention deficit hyperactivity disorder (ADHD) is considered to affect the whole-brain, and one of candidate responsible brain region is the prefrontal cortex (PFC). The PFC regulates high-order cognitive functions, including attention, behavior and planning through working memory. As a medication for ADHD, α_{2A}-adrenergic receptor agonist, guanfacine (GFC) has been shown to improve PFC cognitive function, including working memory. However, it is poorly understood how GFC is effective in ADHD pathology in the PFC. In the present study, we first investigated the acute effects of GFC on ion channels that were proposed to be modulated by cAMP-PKA pathway in brain slice preparation of C57BL/6 mice. We used the somatic recording of patch-clamp techniques to record HCN channel- and KCNQ channel-mediated currents from layer 5 neurons of the PFC (2~3 month-aged). However, GFC did not show obvious modulatory effect on these current components. Next, we examined the modulatory effect of GFC on both EPSCs and IPSCs onto pyramidal neurons. For the recording of synaptic currents, recorded neurons were classified to two types (Callosal/Commissural (COM) and Corticopontine-projecting (CPn)). Although IPSCs were comparably inhibited in both types of neurons, EPSCs were suppressed in only COM-type neurons. These results may be able to explain a part of therapeutic mechanisms of GFC. In the future, it is necessary to clarify whether this target cell-dependent modulatory action is involved in the clinical effect of chronic administration of GFC.

Extracellular ATP-induced hyperexcitability: its molecular mechanism and its role in synapse and behavior

細胞外ATPを介した神経過興奮：分子メカニズムとシナプス及び行動への影響

○繁冨 英治[1,2]、鈴木 秀明[1,2]、平山 幸歩[1]、佐野 史和[1,2,3]、田中 謙二[4]、尾藤 晴彦[5]、小泉 修一[1,2]

[1]山梨大院医・薬理、[2]山梨大院医・GLIAセンター、[3]山梨大院医・小児科、[4]慶應義塾大・医・先端医科研・脳科学、[5]東京大院医・神経生化学

Astrocytes show dramatic changes at the molecular level in response to brain insult and disease, becoming reactive astrocytes. Among the changes found in reactive astrocytes, we have focused on P2Y1 receptor (P2Y1R), which is activated by extracellular ATP and is upregulated in reactive astrocytes in some neurological disorders including epilepsy. To reveal the pathophysiological significance of P2Y1R upregulation in astrocytes, we have used transgenic mice in which astrocytes specifically overexpress P2Y1R using Tet-Off system (P2Y1OE). P2Y1OE mice were more susceptible to drug-induced seizures and showed more abnormal spikes in EEG recordings, suggesting P2Y1OE triggered neuronal hyperexcitability. We analyzed the cellular mechanism underlying the hyper-excitability by imaging techniques and electrophysiology in the hippocampal slices. We found evidence showing that excitatory synaptic transmission was enhanced in P2Y1OE with the increase in P2Y1R-mediated Ca^{2+} signals in astrocytes. Interestingly, the enhancement of excitatory synaptic transmission was due to a novel excitatory molecule X derived from astrocytes rather than glutamate release from astrocytes. Overall, our data show a novel mechanism of astrocytic regulation of excitatory synapses which could contribute to hyperexcitability in neurological diseases.

Neocortical slow oscillations gate the transfer of hippocampal activity to the visual cortex

海馬情報の視覚野への伝達の皮質徐波によるゲーティング

○鮫島 華[1]、池谷 裕二[1,2]

[1]東京大・薬・薬品作用学教室、[2]東京大・Beyond AI 研究推進機構

Recent evidence shows that the primary visual cortex (V1) is plastic and associated with storage of visual long-term memory. Although the hippocampal formation is not monosynaptically connected with the V1, neuronal activity of the V1 is modulated during hippocampal ripples, a form of 150-250 Hz oscillations that plays a role in memory consolidation, and the modulation may contribute to memory formation and retention; however, little is known about subthreshold activity in individual V1 neurons during hippocampal ripples. We recorded membrane potentials (Vms) from V1 layer Ⅱ/Ⅲ pyramidal cells of urethane-anesthetized mice using the whole-cell current-clamp technique, together with recording local field potentials (LFPs) from the hippocampal CA1 region, to explore the Vm fluctuations in V1 neurons in response to hippocampal ripples. Time series analyses of hippocampal LFPs and V1 neuronal Vm revealed a significant portion of V1 neurons are briefly depolarized after the ripple onsets when they are at DOWN states of the Vm slow oscillations, whereas V1 neurons exhibited a variety of responses (or no responses) after hippocampal ripples at the UP states. The latencies of the depolarizations at DOWN states relative to the ripple onsets ranged from 20 to 40 ms, suggesting multisynaptic transmission from the hippocampus to the V1. Our findings advance our understanding about the neuronal mechanisms underlying neocortical consolidation of visually relevant memories.

Oxytocin administration to head gamma-irradiated mice results in increased KCC2 mRNA expression through decreased phosphorylated CREB

頭部ガンマ線照射マウスに対するオキシトシン投与はリン酸化CREBの減少を介してKCC2 mRNA発現の増加をもたらす

○五十嵐 健人[1,2]、田中 康一[1,2,3]、北中 純一[2]、北中 順惠[3]、西山 信好[2]、富田 和男[1,2]、佐藤 友昭[1]

[1]鹿児島大・院医歯、[2]兵庫医科大・薬、[3]兵庫医科大・医

BACKGROUNDS: Cranial gamma-ray irradiation is one of the effective treatments for brain tumors. So far, we have found that nasal administration of oxytocin prevents cognitive dysfunction in gamma-irradiated mice, and restores reduction in mRNA expression of KCC2, a chloride ion transporter (Igarashi et al., BBRC, 2022). However, it remains unclear how oxytocin is involved in the increased expression of KCC2.

METHODS: 3-week-old male C57BL6/J mice were exposed to a 60Co source for 4 minutes and irradiated with 1.5 Gy gamma rays. Irradiation was performed once a day and repeated for 3 days. Mice in the oxytocin-administered group were nasally administered 0.1 mM oxytocin 5 μL after each irradiation. Hippocampal tissue was excised from gamma-irradiated mice, mice treated with oxytocin after gamma-irradiation, and non-gamma-ray irradiated mice, and the amounts of phosphorylated CREB and phosphorylated ERK1/2 were examined by Western blot.

RESULTS: In mouse hippocampus administered nasally with oxytocin after gamma-irradiation, phosphorylated CREB was reduced by approximately 24% compared with non-gamma-irradiated mice ($p < 0.05$). In addition, mice nasally administered oxytocin after gamma irradiation had a 51% decrease in phosphorylated CREB ($p<0.001$) compared to non-irradiated mice, and a 35% decrease compared to gamma-irradiated mice ($p<0.05$). In the mouse hippocampus to which oxytocin was nasally administered after gamma-irradiation, the phosphorylated ERK1/2 was increased by about 24% as compared with the non-gamma-ray-irradiated mouse, but it was not significant.

DISCUSSION: In this study, we found that phosphorylated CREB decreased in mice treated with oxytocin after gamma irradiation. Previous studies have suggested that phosphorylated CREB is involved in the reduction of KCC2 expression (Rivera et al., J. Neurosci. 2004). From these facts, it is considered that oxytocin administration after gamma-ray irradiation may lead to an increase in the expression level of KCC2 through a decrease in phosphorylated CREB.

ATP-sensitive K^+ channel activation in the brain is involved in elevation of plasma levels of adrenaline

脳内ATP感受性カリウムチャネル活性化は血中アドレナリン増加に関与する

○岡田 尚志郎、山口 奈緒子

愛知医科大・医

Recently, we reported that restraint stress-induced elevation of plasma levels of adrenaline, but not of noradrenaline, was suppressed by intravenous and intracerebroventricular (i.c.v.) administration of glucose via brain thromboxane A_2-dependent mechanisms. (Yamaguchi et al., 2022). Since glucose can be converted to ATP in the brain cells, in the present study, we investigate whether ATP-sensitive K^+ channel (K_{ATP} channel) activity in the brain is involved in changes of plasma levels of catecholamines in urethane anesthetized rats. I.c.v. administered diazoxide (20 μg/rat), a K_{ATP} channel opener, and A-769662 (25 μg/rat), an AMPK activator, elevated plasma levels of adrenaline, but not noradrenaline. In contrast, i.c.v administered glibenclamide (25 μg/rat), a K_{ATP} channel antagonist, did not alter plasma levels of adrenaline. I.c.v. pretreatment with SQ29548 (10 μg/rat), a thromboxane A_2 receptor antagonist, attenuated diazoxide- and A-7969662-induced elevation of plasma levels of adrenaline. These results suggest that K_{ATP} channel activation in the brain might trigger the activation of thromboxane A_2 receptor, resulting in elevation of plasma levels of adrenaline.

Roles of sorting nexin protein 33 on amyloid precursor protein processing and the level of amyloid β

アミロイド前駆体タンパク質プロセシングやアミロイドβ産生におけるSorting Nexin 33の役割

○高鳥 悠記[1]、水川 裕美子[1]、漆谷 徹郎[1]、泉 安彦[2]、赤池 昭紀[3]、土田 勝晴[1]、尾﨑 惠一[1]、久米 利明[4]
[1]同志社女子大・薬学部(京田辺)、[2]神戸薬科大・薬、[3]和歌山県立医科大・薬、[4]富山大・院医薬

Alzheimer disease (AD), one of the most common diseases presenting dementia, is a progressive neurodegenerative disorder characterized by the depletion of high-affinity nicotinic acetylcholine receptors (nAChRs) and a marked loss of cholinergic neurons. Selective depletion of cholinergic neurons in the basalis of Meynert, decreased activities of choline acetyltransferase, or down-regulation of nAChRs suggest the roles of cholinergic deficits in AD. Donepezil is a therapeutic acetylcholinesterase inhibitor currently being used for the treatment of AD. However, recent studies have also reported off-target effects of donepezil that likely contribute to its therapeutic effects.We investigated the role of donepezil on the processing of amyloid precursor protein (APP) and the involvement of sorting nexin (SNX) 33, a member of the SNX protein family in this study. Donepezil induced an increase in SNX33 expression. The expression of full-length APP in the cell lysate remained unchanged, but the secretion of sAPPα in culture media increased. Donepezil led to a decrease in amyloid β (Aβ) protein levels in a concentration- and time-dependent manner. SNX33 knockdown by target-specific morpholino oligos inhibited the effects of donepezil. Donepezil treatment increased cell membrane surface expression of APP in SNX33 expression-dependent manner. This study shows that donepezil increases SNX33 expression and APP cleavage by α-secretase and reduces the level of Aβ by in primary cortical neurons.

Neuroprotective effects of Raloxifene on Aβ oligomer-induced neuronal injury

Aβ oligomer誘発性神経細胞傷害に対するRaloxifeneの神経保護作用

○野原 哲人[1,2]、辻 まゆみ[3]、小口 達敬[1,3]、門馬 佑太郎[1,2]、大橋 英朗[1,2]、永田 未希[4]、井藤 尚仁[1,2]、山本 謙[1,2]、村上 秀友[2]、木内 祐二[1,3]

[1]昭和大・医・薬理学講座医科薬理学部門、[2]昭和大・医・内科学講座脳神経内科学部門、[3]昭和大・薬理学研究センター、[4]昭和大・薬・病院薬剤学講座

[Introduction] Increasing Alzheimer's disease (AD) is a social problem in an aging society. In addition, the prevalence of osteoporosis increases with aging and various interventions are being performed. The hypothesis that aggregates and accumulation of Amyloid beta(Aβ) induces nerve cell death has been proposed. Raloxifene (Ral), a therapeutic agent for postmenopausal osteoporosis, is a selective estrogen receptor modifier, but it is controversial about slowing the progression of AD. Therefore, we investigated the protective effects of Ral on Aβ-induced cytotoxicity.

[Method] The effect of Ral on the aggregation of Aβ was measured by the thioflavin T fluorescence method. In addition, highly toxic Aβ42 oligomer (Aβo) was exposed to human neuroblastoma cells (SH-SY5Y) to induce cytotoxicity. The effects of Ral on Aβo-induced cell damage were assessed by cell viability (MTT), oxidative stress (ROS production, cell membrane phospholipid peroxide) and intracellular Ca^{2+}.

[Results] Ral inhibited Aβ42 and Aβ40 aggregation in concentraiton-dependent manner. The effect of Ral on the cytotoxicity of cells induced by Aβo exposure showed an increase in cell viability and a decrease in oxidative stress, and repair of membrane damage. Ral reduced the sustained increase in intracellular Ca^{2+} due to Aβo exposure.

[Conclusion] Ral, a therapeutic agent for osteoporosis, has showed a protective effect against Aβo-induced nerve damage by inhibiting Aβ aggregation and antioxidant effects. Ral is expected to have beneficial effects on the prevention and progression of AD.

Airway inflammation induced by LPS exposure in a mouse model of papain-induced asthma

Papain誘導喘息モデルマウスにおけるLPS曝露による気道炎症

○木村 元気、安藤 大稀、入江 孝祐、片山 侑紀、佐藤 しおり、鈴木 智大、原田 真衣、吉田 翼、西本 裕樹、木澤 靖夫

日本大・薬

In severe asthma patients, it is difficult to treat because of resistance to corticosteroid therapies. We have previously demonstrated that LPS induced steroid insensitive airway inflammation in mice. In this study, we investigated the effects of LPS exposure on airway inflammation in papain-induced asthma mice model. Papain-sensitized A/J mice were challenged to papain every other day, then were exposed with LPS intranasally twice daily for 3 days. Fluticasone propionate (FP) were administered intranasally at 2 h before each LPS exposure. BALF was collected at 24 h after the last LPS exposure and eosinophils and neutrophils were quantified by FACS analysis. The level of inflammatory cytokine and chemokine in BALF were measured by ELISA, and mRNA expression in lung was measured by RT-qPCR. The number of neutrophils and eosinophils in mice co-exposed to papain and LPS in BALF was significantly increased compared to that in the papain alone-exposed group, especially the number of neutrophils was significantly increased. Chemokine and cytokine levels and mRNA expression were also significantly increased by co-exposure to papain and LPS, especially CXCL1. However, these indicators of airway inflammation were resistant to FP. These profiles provide new insights into steroid insensitive airway inflammation in severe asthma.

Drug evaluation of LPS-induced acute lung injury (ARI) mice models (2nd Report)

LPS誘発急性肺炎症モデルに対する薬物評価の追加研究

○牛島 壮太、和田 肇、岡本 公英、守住 孝輔、片山 誠一、廣中 直行、西 勝英

(株)LSIM 安全科学研究所 熊本研究所・薬理研究部

In the 94th Annual Meeting of the Japanese Pharmacological Society, we reported two kinds of pulmonary inflammatory models using lipopolysaccharide (LPS), LPS inhalation model and ☒-GalCer-LPS instillation model.

In the previous report, we concluded that these LPS-induced ARI models could be useful to evaluate therapeutic efficacy of drugs used for treatment of pneumonia.

In the present study we carried out further research on LPS instillation model with a pretreatment of α-garactosyl ceramid (α-GalCer), and we also examined the effect of exosome from human adipose cell.

We evaluated inflammatory cell infiltration in BALF (Broncho Alveolar Lavage Fluid), BALF supernatant cytokine level and SpO2.

Exosome was found to be slightly effective to improve symptomatic parameters in this model. The action of exosome changed along with the time of administration. Exosome administrated 4hr after LPS instillation decreased body weight loss, inflammatory cell infiltration, IL-6 and IL-10 levels. On the other hand, administration of exosome 24hr after LPS instillation showed advanced histopathological appearance image of the lung.

The results indicate that characteristic pathophysiological change of this model was identified.

Inhibition of androgen receptor signaling exacerbates pathological changes in a mouse model of porcine pancreatic elastase-induced pulmonary emphysema.

アンドロゲン受容体シグナルの阻害はエラスターゼ誘発肺気腫形成を増悪させる

○青野 健太郎[1]、松本 純一[2]、松本 太一[3]、池田 弥恵[2]、河村 莉奈[2]、山内 淳史[1]

[1]福岡大・院薬・生物薬剤学、[2]福岡大・薬・生物薬剤学、[3]福岡大・薬・医薬品情報学

Chronic obstructive pulmonary disease (COPD) is characterized progressive airflow obstruction due to the chronic inflammation in bronchitis and the alveolar destruction. We previously showed that testosterone deficiency exacerbated porcine pancreatic elastase (PPE)-induced destruction of the alveolar structure in orchiectomy (ORX) mice, suggesting testosterone may have a suppressive effect on the progression of pulmonary emphysema. However, it remains unclear the mechanism by which testosterone suppresses the progression of pulmonary emphysema. In this study, we investigated whether testosterone supplementation attenuates pathological change (body weight loss, the infiltration of T lymphocytes into the lung, and alveolar destruction) in ORX/PPE mice. Furthermore, we examined effect of flutamide, androgen receptor (AR) inhibitor, on the enlargement of alveolar space in PPE-inhaled mice. Testosterone supplementation significantly attenuated the loss of body weight, the infiltration of T cells in bronchoalveolar lavage fluid and alveolar destruction in ORX/PPE mice. Furthermore, flutamide-treated mice show more severe emphysematous change than vehicle-treated mice. These results indicate that testosterone-androgen receptor signaling have a pivotal role in suppressing the progression of pulmonary emphysema through modulating T cell-mediated immune response.

Roles of the senescent alveolar epithelial cells in lung fibrosis using alveolar epithelial cell-specific p16 knockout mice

p16コンディショナルノックアウトマウスを用いた肺線維化における老化肺胞上皮細胞の役割の解析

○竹之内 康広、北風 圭介、杉本 理栄、石丸 浩靖、坪井 一人、岡本 安雄

川崎医科大・医

Senescent cells are strongly implicated in various diseases including idiopathic pulmonary fibrosis. In our previous study, we confirmed expression of p16, a key marker of cellular senescence, in the alveolar epithelial cells (AECs) of lungs from bleomycin-induced pulmonary fibrosis model mice. Although AECs have been reported to contribute to lung fibrosis through epithelial-mesenchymal transition (EMT), it remains unclear whether the induction of EMT is influenced by AEC senescence. In the present study, we investigated the roles of AEC senescence in lung fibrosis using AEC-specific p16 knockout (cKO) mice. Intraperitoneal administration of bleomycin caused pulmonary fibrosis in both control and cKO mice. However, the amount of soluble collagen in bronchoalveolar lavage fluid and collagen deposition in lung tissue, indicators of lung fibrosis, were lower in cKO compared with control group. Furthermore, the mRNA expression of *Col1a1*, which promotes lung fibrosis, tended to be decreased in the lungs of cKO mice compared with controls. In addition, the expression of EMT marker genes (*Cdh2* and *Vim*) also tended to be lower in the lungs of cKO mice. These results suggest that cellular senescence mediated by p16 expression in AEC contributes to progression of lung fibrosis.

Evaluation of insulin secretion by organ bath of isolated pancreases

摘出膵臓とオルガンバス実験系を用いたインスリン分泌評価

○大内 基司[1]、森田 亜州華[1]、佐藤 慶太郎[2]、安西 尚彦[1,3]、藤田 朋恵[1]

[1]獨協医大・医・薬理、[2]明海大・歯・薬理、[3]千葉大・院医・薬理

In order to investigate molecules related to insulin secretion, we conducted a practical method to imitate human insulin secretion through rats via organ baths of pancreatic preparations. Our previous study showed that insulin secretion from rat pancreas tissue was stimulated by glucagon-like peptide-1 and glimepiride. 1,5-anhydro-D-glucitol (1,5-AG) is a glucose analog and exists in humans. This study aims to assess the effects of short-term 1,5-AG stimulation of insulin secretion in rat pancreases to better understand the effects in humans. Rat pancreases were assigned to eight groups: two glucose concentrations (100 and 400 mg/dl) and pairs of varying 1,5-AG concentrations (0, 0.1, 1, and 10 mM). There was a significant increase in insulin outflow from low to high glucose concentrations. However, there was no significant enhancement of insulin secretion between the four groups with low and high 1,5-AG concentrations. This suggests that short-term exposure to 1,5-AG has no effect on insulin secretion in rat pancreas tissues.

To justify whether the methods and techniques were useful as an experimental system, we isolated pancreases from another rodent species, the mouse, and similarly measured insulin outflow. In mouse pancreas preparations, stimulating with 400 mg/dl glucose significantly increased insulin outflow. Therefore, organ baths of isolated mouse pancreases are also considerably effective for assessing effects of novel molecules and/or therapeutics on insulin secretion.

Mechanisms of the Antioxidant and Anti-inflammatory of SMTP-44D through Its Soluble Epoxide Hydrolase Inhibitory Action

SMTP-44D の可用性エポキシドヒドロラーゼ阻害作用を介した抗酸化作用および抗炎症作用メカニズムの検討

○篠内 良介[1,2]、柴田 佳太[1,2]、蓮見 惠司[3]、野部 浩司[1,2]

[1]昭和大・薬・薬理、[2]昭和大・薬理科学研究セ、[3]東京農工大学・院農・応用生命化学専攻

We have previously reported the efficacy of SMTP-44D for diabetic neuropathy (DN) through its potential antioxidant and anti-inflammatory activities. However, the mechanisms underlying the antioxidant and anti-inflammatory activities of SMTP-44D remain unclear. The present study aimed to reveal the mechanism of these effects of soluble epoxide hydrolase (sEH) inhibition by SMTP-44D. In the *in vivo* assay, SMTP-44D (30 mg/kg) was administered to 200 mg/kg streptozotocin (STZ)-induced diabetic mice from the 8 to the 28 days after the injection of STZ. In the *in vitro* assay, IMS32 cells were incubated in a high glucose medium for 48 h and then treated with SMTP-44D (30 μM) for 48 h. The effects of the ratio of epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs), oxidative stress markers, and inflammatory factors by administration of SMTP-44D were assessed by LC-MS/MS, TBARS, and ELISA assay, respectively. Furthermore, apoptosis was evaluated by TUNEL assay. SMTP-44D treatment considerably increased the ratio of EETs to DHETs and mitigated oxidative stress, inflammation, and apoptosis. These results suggested that SMTP-44D can suppress the induction of apoptosis by exerting antioxidant and anti-inflammatory effects, possibly through sEH inhibition. SMTP-44D can be a potential therapeutic agent against DN.

The influence of testes on diazepam-induced recognition memory impairment in rats

雄ラットのジアゼパム誘導性再認記憶障害における精巣の関与についての検討

○畑 実野里[1]、石井 敦大[2]、川口 真以子[1,2]、畠山 太一[3]

[1]明治大・農・動物環境学、[2]明治大・院農・動物環境学、[3]明治大・研究知財戦略機構

Benzodiazepines, including diazepam (DZP), induce recognition memory impairment as a side effect, and in this class of drug, there are sex differences in pharmacokinetics and pharmacodynamics. Therefore, we focus on whether the effect of DZP on recognition memory depends on sex difference. Our previous study showed the improvement effects of ovaries on recognition memory deficit induced by DZP in female rats. Here we examined the influence of testes on DZP-induced recognition memory impairment in male rats. Recognition memory was assessed by a spontaneous object recognition (SOR) and a spontaneous place recognition (SPR) tests that utilize the natural tendency of rats to explore a novel stimulus more than the familiar one. Thus, if rats could retain the object identity or place information, they explored a novel object or place more than the familiar one. First, using intact male rats, we examined the effect of DZP on SOR and SPR tests. Rats treated with DZP (2.0 mg/kg, i.p.) or vehicle, explored a novel object more than the familiar one in the SOR test, but in the SPR test, they with DZP, but not vehicle, investigated equally two objects. Second, we had castrated intact male rats, and then, the SOR and SPR tests were conducted. Results showed that in the SOR test, they with DZP or vehicle explored a new object more than the familiar one, but in the SPR test, they with DZP, but not vehicle, explored equally two objects. These results suggest that male rats even with DZP could retain the object identity, but not place information, regardless of whether or not castration was conducted.

Investigation of the function of DGKζ depending on its localization in pancreatic β-cells

ジアシルグリセロールキナーゼζの膵β細胞内局在と機能の関連解析

○渡邉 直也、金子 雪子、石川 智久

静岡県立大・院薬・薬理学講座

Increasing β-cell mass is a crucial therapeutic target for diabetes mellitus. Our previous study showed that diacylglycerol kinase δ（DGKδ）is localized in the nucleus and acts as a suppressor of β-cell proliferation. DGKζ, a type IV DGK isoform, has been shown to localize in the nucleus of rat β-cells and be a negative regulator of cell cycle in the fibroblasts. We therefore examined the possibility that DGKζ, similarly to DGKδ, regulates β-cell mass. The intracellular localization of DGKζ was examined in MIN6 cells, a mouse β-cell line, and in isolated mouse islets by subcellular fractionation. Insulin secretion was measured by batch incubation and cell cycle was analyzed by flow cytometry. In MIN6 cells, DGKζ was localized in the cytoplasm and knockdown of DGKζ caused defective insulin secretion but did not affect cell cycle. In contrast, DGKζ in isolated islets was detected in the nucleus fraction as well as in the cytoplasm fraction. EGFP-tagged DGKζ overexpressed in MIN6 cells was also distributed in the nucleus. Moreover, overexpression of DGKζ induced cell cycle arrest in MIN6 cells. These results suggest that cytoplasmic DGKζ regulates insulin secretion, whereas DGKζ expressed in the nucleus regulates cell cycle. Thus, DGKζ is likely to have different function depending on its localization in pancreatic β-cells.

Intracellular serpin A1 regulates inflammatory cytokines expression via toll-like receptor signaling pathway in endometrial stromal cells

子宮内膜間質細胞のSerpin A1によるToll様受容体シグナル経路を介した炎症性サイトカイン発現の調節

○草間 和哉[1]、里吉 彩華[1]、安曇 麻奈[1]、吉江 幹浩[1]、梶原 健[2]、田村 和広[1]

[1]東京薬科大・薬・内分泌薬理学、[2]埼玉医科大・医・産科婦人科学

Endometriosis is characterized by the presence of inflamed and fibrotic endometrial tissue outside the uterine cavity. We have previously found that serpin A1 (SERPINA1) was decreased in the endometriosis-like lesion of the mouse model, which makes us hypothesize that a decrease in SERPINA1 may exacerbate inflammation in the lesion. However, the molecular mechanisms by which SERPINA1 affects the expression of inflammatory cytokines and the development of endometriosis remains unclear. To investigate the role of SERPINA1 in endometrial stromal cells (ESCs), RNA-seq analysis was performed using RNA extracted from ESCs knocking down SERPINA1. The analysis has identified several toll-like receptor (TLR)-related factors as the upregulated genes. Silencing SERPINA1 expression increased the expression of TLR3, TLR4, their downstream factor MYD88, and inflammatory cytokines in cultured ESCs. Treatment with TLR3 or TLR4 agonists enhanced the expression of inflammatory cytokines, whereas inhibitors of TLR3 or TLR4 decreased the expression of these cytokines in SERPINA1-silenced ESCs. Immunohistochemical analysis showed that TLR3, TLR4, and MYD88 were localized in the endometriotic lesion. Thus, our data indicate that the decrease in SERPINA1 induces the expression of inflammatory cytokines in ESCs, accompanying with TLR3/4 signaling. The regulation of intracellular SERPINA1 could be a potential strategy to inhibit inflammatory responses in endometriotic lesions.

Evaluation of anti-diabetic effects of the *Cyclolepis genistoides* D. Don (Palo azul), an herb native to Paraguay.

パラグアイ原産ハーブ*Cyclolepis genistoides*D. Don（パロアッスル）の抗糖尿病生物活性成分含有画分の活性評価

○三竿 顕也[1]、北島 満里子[2]、村木 拓斗[2]、林 隼太郎[2]、高橋 晃輝[1]、福島 圭穣[1]、北井 淳一郎[3]、奥村 明子[3]、吉田 博也[3]、石川 勇人[2]、藤野 裕道[1]

[1]徳島大・院薬・生命薬理学、[2]千葉大・院薬・中分子化学、[3]株式会社 IHM

Cyclolepis genistoides D. Don ("Palo azul" meaning "blue twig"; Palo) is an herb widely used for folk remedies against diabetes and renal diseases in Paraguay.

The EtOH extract of Palo has been previously reported that it induced the mRNA expressions of peroxisome proliferator-activated receptor γ (PPARγ) and adiponectin in mouse 3T3-L1 preadipocytes.

Here, to identify bioactive substances in Palo, a newly EtOH extracted crude sample (Palo-E) was separated by liquid-liquid extractions, and the AcOEt fraction (Palo-A), $CHCl_3$ fraction (Palo-C), *n*-BuOH fraction (Palo-B), and water-soluble fraction (Palo-W) were obtained. 3T3-L1 cells were treated with each fraction 3 times in 7 days and total RNA was collected followed by mRNA expression levels of PPARγ and adiponectin were examined by RT-PCR.

As the results, treatment with the Palo-B and Palo-W fractions significantly increased mRNA expression of both PPARγ and adiponectin in 3T3-L1 cells, but Palo-A fraction did not show these effects. On the other hand, cell death was observed in the Palo-C fraction treated cells.

These results suggest that the Palo-B and Palo-W fractions are likely to contain bioactive substances, which need to be identified by further separations in near future.

Impact of chronic social stress on physiological and behavioral functions in cynomolgus monkeys

カニクイザルの生理学的及び行動学的機能に対する慢性社会的ストレスの影響

○林田 健一郎[1]、堀本 泰弘[1,2]、稲留 大輔[2]、宮本 真二[2]、中村 祐里[1]、西方 龍太郎[1,2]、清水 洋志[1]、澤田 和俊[1]、沼田 洋輔[1]、角崎 英志[3]

[1]新日本科学・薬効薬理研究部、[2]新日本科学・実験動物管理部、[3]新日本科学・前臨床カンパニー

Chronic social stress (CSS) stemming from a low social position can result in depressive and anxiety disorders in animals and humans. However, the pathophysiology of the CSS-associated disorders is still unclear. The present study examined the effects of CSS on physiological and behavioral parameters in female cynomolgus monkeys, by comparing those parameters in subordinates and dominants housed in pairs. In the blood, subordinates had more white blood cells, fewer reticulocytes, and higher levels of low density lipoprotein than dominants. Subordinates showed not only anxiety/depression-like behaviors but also impaired negative feedback control of the hypothalamic-pituitary-adrenal axis and higher body temperature compared to dominants, consistent with symptoms in patients with depression. In addition, magnetic resonance spectroscopy revealed that subordinates showed less myo-inositol (an astroglial marker) in the amygdala compared to dominants, consistent with previous observations in patients with depression. These results indicate that cynomolgus monkeys with CSS present physiological and behavioral disorders that resemble the pathophysiology of depression in humans, and may be a useful translational animal model for further research into the mechanisms and treatments of depression.

Developing an experimental autoimmune encephalomyelitis (EAE) model in cynomolgus monkeys: A feasibility study

カニクイザルにおけるEAE作製の試み

○澤田 和俊[1]、中村 祐里[1]、西方 龍太郎[1]、深草 翔太[1]、清水 洋志[1]、林田 健一郎[1]、沼田 洋輔[1]、角﨑 英志[2]
[1]新日本科学・薬効薬理研究部、[2]新日本科学・前臨床カンパニー

EAE is an animal model of multiple sclerosis (MS), which is most commonly used in rodents. Due to the recent diversification of therapeutic modalities, including gene modification, and given the structural similarities in the central nervous system (CNS) to humans, non-human primates become to be considered a better animal model for MS research. In the present study, we evaluated neurological signs, magnetic resonance images (MRI), and histopathology in the CNS to examine whether immunization with myelin oligodendrocyte glycolipid (MOG) could induce EAE in cynomolgus monkeys. After MOG treatment, the animals showed motor dysfunction and visual impairment, indicating neurological deficits. However, there were individual differences in onset and severity of the neurological deficits. MRI and histopathological examination revealed disseminated inflammation in brain and optic nerve, similar to the pathology of MS in humans. Although further research is required to reduce the individual differences of the neurological deficits, these results indicate that MOG treatment can induce EAE in cynomolgus monkeys, and this can be used as a translational animal model for the development of new therapeutic modalities for MS.

Examination of cognitive functions in MK-801 induced schizophrenia-like cynomolgus monkey model using Cambridge neuropsychological test automated battery

ケンブリッジ神経心理テスト自動バッテリーを用いたMK-801惹起統合失調症様カニクイザルモデルおける認知機能評価

○清水 洋志[1]、濱口 弘嗣[1]、大高 優[1]、西方 龍太郎[1]、中村 祐里[1]、澤田 和俊[1]、林田 健一郎[1]、沼田 洋輔[1]、角﨑 英志[2]
[1]新日本科学・安全性研究所・薬効薬理研究室、[2]新日本科学・前臨床カンパニー

Cambridge neuropsychological test automated battery (CANTAB) is a cognitive function test device that examines memory, learning, attention, motivation, and reaction time in animals and humans. Schizophrenia is a serious mental disorder including positive and negative symptoms as well as cognitive dysfunction. In the present study, we utilized a delayed matching to sample task (DMST), one of the CANTAB programs, to examine cognitive functions in a drug-induced schizophrenia-like cynomolgus monkey model. MK-801, an N-methyl-D-aspartate (NMDA) receptor antagonist, that is known to induce schizophrenia-like symptoms in various species, reduced accuracy rates in the DMST in the monkeys, demonstrating the cognitive impairment from MK-801(20 μg/kg im, n=4). This effect was reversed by D-cycloserine, a partial NMDA agonist, and A776363, a dopamine 1 (D1) receptor agonist(0.1 and 1 μg/kg im, n=3). On the other hand, donepezil(100 μg/kg im, n=4), a cholinesterase inhibitor approved as an Alzheimer's disease therapeutic agent, did not affect MK-801-induced cognitive impairment, similarly to the lack of donepezil efficacy on cognitive functions in schizophrenia patients. These results demonstrate the roles of NMDA and D1 but not cholinergic receptors in cognitive functions in MK-801 induced schizophrenia-like symptoms in cynomolgus monkeys.

An early and versatile detection scheme of adverse drug reactions using large-scale administrative claims data

大規模レセプトデータを用いた医薬品副作用の早期網羅的検出

○山本 浩貴[1]、栢沼 玄[1]、長島 卓也[2]、戸田 千尋[1]、永安 一樹[1]、金子 周司[1]

[1]京都大・院薬、[2]日本大・医

Adverse drug reactions (ADRs) are one of the leading causes of mortality and thus should be detected as early as possible to reduce health risks of patients. Data mining approach using large-scale medical record might be a useful method for early detection of ADRs. There have been many researches analyzing medical record to detect ADRs; however most of the researches focused only on a narrow range of late-onset ADRs, limiting its usefulness. In this study, we developed an early and simple detection scheme of ADRs based on association rule mining (ARM) of JMDC insurance claims data. The assessment of its ability in detecting a broader range of ADRs was performed using a global gold standard of ADRs consisting of 92 positive control ADR-drug pairs and 88 negative control pairs rationally selected from the statistical analyses of large-scale ADR self-reports. In this assessment of ARM, the areas under the receiver operating characteristic curve and the precision-recall curve were 0.80 and 0.83, respectively. Moreover, when capability of ARM to detect ADRs with varying short periods of accumulating data was tested, our method detected much more positive controls (65 pairs) than conventional sequence symmetry analysis (9 pairs) frequently-used for ADR detection. These results suggest that ARM is effective in the early and versatile detection of ADRs, complementarily to the existing pharmacovigilance strategies.

A exploratory study on pharmacological actions of acetaminophen on ductus arteriosus constriction based on a systematic review and database analysis using Japanese Adverse Drug Event Report database

システマティックレビューおよび医薬品副作用データベース解析に基づく解熱鎮痛薬アセトアミノフェンの動脈管におよぼす薬理作用に関する探索的検討

○石塚 洋一[1]、近藤 悠希[1]、難波 七海[1]、門脇 大介[2]

[1]熊本大・大学院生命科学研究部(薬学教育)・臨床薬理学、[2]崇城大・薬・医療薬剤学

Background: Acetaminophen (APAP) is widely used as an antipyretic analgesic with a higher safety profile than non-steroidal anti-inflammatory drugs (NSAIDs), however its pharmacological mechanisms are still fully unclear. In this study, we conducted a meta-analysis and database analysis focusing on the effect of APAP on the "ductus arteriosus," i.e., the effect on treatment of patent ductus arteriosus (PDA) in low birthweight infants and the occurrence of adverse reactions of fetal ductus arteriosus occlusion when administered to pregnant women. Methods: Articles on the efficacy of treatment for PDA were collected, and a meta-analysis was performed. The database analysis was conducted by collecting records about the fetal ductus arteriosus contraction from the "JADER" database. Results and Discussion: The combined results of the randomized controlled trials suggest that APAP has some therapeutic effect on PDA. The results of meta-analysis should be interpreted with caution, given the insufficient number and quality of studies analyzed. In addition, an analysis using JADER detected an adverse event signal of fetal ductal arteriosus occlusion was detected. At present, these findings do not support the APAP therapy for the disease, nor rule out the use of APAP in pregnant women. However, these data suggest that APAP shows the effects on ductus arteriosus.

Development of a functional antibody against Ca^{2+}permeable channel TRPV2 for treatment of human muscle degenerative diseases

ヒト筋変性疾患治療を目指したCa^{2+}透過チャネルTRPV2の機能性抗体の開発

○岩田 裕子、ピアソン ジェームズ

国立循環器病研究セ・心臓生理機能部

Abnormal Ca^{2+} handling is essential in the pathophysiology of muscular dysgenesis such as muscular dystrophy (MD) or/and dilated cardiomyopathy (DCM). One of Ca^{2+} permeable channels, transient receptor potential cation channel, subfamily V, member 2 (TRPV2) has been suggested as a principal candidate for Ca^{2+} entry pathways and a potential therapeutic target for muscular dysgenesis. Recently, we produced functional antibodies against TRPV2 with remarkable protective effects against DCM as well as MD animal models. However, the antibodies were rodent TRPV2 specific. Here, we produced selective antibodies recognizing humanTRPV2 from the outside of cells using phage display. One of antibodies inhibited the Ca^{2+} influx via humanTRPV2 in cultured cells and caused TRPV2 to disappear from the plasma membrane via cellular internalization. We measured the change in $[Ca^{2+}]_i$ induced by TRPV2 activator. Although cannabidiol induced massively increased $[Ca^{2+}]_i$ in the Duchenne muscular dystrophy (DMD) cell line, this increase was almost completely abolished by treatment with the functional antibody. These results suggest that the functional antibody we developed has therapeutic potential for treating MD or/and DCM.

Microglia engulf myelin debris in mouse cortical slice cultures

マウス皮質培養スライスにおけるマイクログリアによるミエリン片の貪食

○三上 弘記[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

Proper myelin formation by oligodendrocytes on neuronal axons is essential for brain function. Demyelination causes various neurodegenerative diseases, but the cellular and molecular mechanisms underlying demyelination remain unclear. Here, we developed a slice culture system to examine neuron-glia interactions during demyelination.

To prepare slice cultures, postnatal-6-day mouse brains were sectioned, and then the cortical slices containing the corpus callosum were cultured. By immunostaining cultured slices for the myelin marker Myelin basic protein and the axonal marker Neurofilament, we confirmed that axons are myelinated in the corpus callosum until days in vitro 15. Next, we attempted to induce demyelination in the slice culture system by applying lysophosphatidylcholine (LPC), which is an endogenous lysophospholipid, from days in vitro 14 to days in vitro 15. LPC application induced a decrease in colocalization of Myelin basic protein and Neurofilament, which we defined as demyelination, compared to the control group. Additionally, we found that microglia engulf myelin debris around the demyelinated axons.

Thus, we developed a slice culture system in which demyelination and surrounding microglial response to demyelination can be investigated.

The standardization study to apply microphysiological systems (MPS) in drug development as humanized pharmacological evaluation systems

新薬開発において生体模倣システム（MPS）をヒト型*in vitro*薬理評価法として実用するための規格化研究

最上（重本） 由香里[1]、北村（中山） 貴美子[1]、山崎 大樹[1]、石田 誠一[1,2]、佐藤 薫[1]

[1]国衛研・薬理、[2]崇城大・院・工

Microphysiological systems (MPS)s with human cells are attracting attentions all over the world as novel pharmacological evaluation systems to improve human predictability of safety, PK/PD, and efficiency of new drugs at the preclinical stage. In 2017, we started AMED-MPS project, the collaborative project among industry, academia, and regulatory, in which we established the MPS prototypes of liver, small intestine, kidney, and blood brain barrier (BBB). However, the technical requirements for respective concepts of use (COU) and regulatory acceptance have not been studied sufficiently. We therefore have started the AMED MPS regulatory science (RS) since 2Q of 2022 as the MPS standardization study. This is the first domestic national project aiming at the development of commercially available MPSs 'made in Japan'. For the reference, we will show the ongoing standardization research for BBB MPS. We have established the standard operation procedures (SOP)s to acquire the minimal essential benchmark data that show 'BBB likeliness'. Lastly we summarized the global trend and the domestic trend in MPS development based on our studies and previous reports.