1-LBS-01

Oral L-histidine supplementation improves working memory through the activation of brain histamine system in male mice

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Brain histamine is produced from L-histidine in histamine neurons and controls various CNS functions. Although histidine was known as a precursor of histamine, the impact of oral histidine intake on brain histamine concentration and brain function has not been fully elucidated. Here, we aimed to elucidate the importance of histidine intake in the histaminergic nervous system and working memory in stressful conditions. First, we confirmed that sleep deprivation by water-floor (WF) stress in male mice increased histamine consumption and resulted in histamine depletion and impaired working memory. This memory impairment was rescued by intracerebroventricular injection of histidine, indicating that oral histidine intake could also improve memory function. Histidine intake increased extracellular histamine concentration around the prefrontal cortex (PFC) and the basal forebrain (BF), leading to a robust increase in the number of c-fos-positive cells around these areas. We also revealed that histidine supplementation alleviated impaired memory function induced by WF stress through histaminergic activation.

These results demonstrate that oral histidine intake replenishes brain histamine and leads to the recovery of impaired working memory induced by sleep deprivation through histaminergic activation.

1-LBS-02

NCC-3902, a novel late sodium current blocker, exhibits potent antiarrhythmic effect in isolated guinea pig pulmonary vein and canine rapid atrial pacing model

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Atrial fibrillation (AF) is caused by an interaction between an "initiating trigger" such as ectopic activity in the pulmonary vein (PV) and a "vulnerable status" such as an atrial effective refractory period (ERP) shortening. Controlling both causal factors is an ideal therapeutic strategy for AF, but it is difficult to be achieved by existing drugs. We hypothesized that the blockade of persistent component of the sodium current (late I_{Na}; I_{NaL}) could be an innovative approach for AF treatment. Using NCC-3902; a selective I_{NaL} blocker, we examined whether inhibition of I_{NaL} has an effect on ectopic activity, atrial ERP, and AF induction.

NCC-3902 blocked I_{NaL}, but had no effect on other major cardiac ion channel currents stably expressed in cell lines. In isolated guinea pig PV, NCC-3902 decreased the automatic firing frequency of the myocardium. In canine rapid atrial pacing models, NCC-3902 prolonged the ERP and intra atrial conduction time in a dose-dependent manner. In addition, NCC-3902 suppressed AF induction without proarrhythmic potentials. Our results suggest that the blockade of I_{NaL} could achieve an ideal AF management with controlling both an "initiating trigger" and a "vulnerable status" without proarrhythmic potentials. A novel I_{NaL} blocker, NCC-3902, could bring more effective and safer approach for AF treatment.

1-LBS-03

Effects of cellular senescence on secretion of extracellular vesicles in neonatal rat cardiac fibroblasts

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Extracellular vesicles (EV) are lipid-bilayer-capsuled particles released by stress from various cells. It has been recently revealed that EV secretion is altered by a status of cellular senescence. In the present study, we examined the effects of cellular senescence on EV secretion. Neonatal rat cardiac fibroblasts (NRCF) were treated with doxorubicin (DOX, 24 h) for the induction of cellular senescence. EV were isolated from NRCF culture media using a total exosome isolation kit. The mRNA expression of cell was measured by a real-time quantitative PCR. Protein expression of EV was measured by Western blotting. DOX changed NRCF morphology to swelling one, and increased mRNA expression of p16 and p21, a senescence-associated gene. In EV isolated from NRCF culture media, DOX stimulation increased protein expression of an exosomal maker. Further study is required to elucidate whether senescence-associated EV could affect cellular function as well as protein profiles of their cargo.

1-LBS-04

Search for novel antifibrotic agents using nonalcoholic steatohepatitis (NASH) organoid model

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Nonalcoholic steatohepatitis (NASH) is a disease in which fatty liver develops independently of alcohol intake and progresses to cirrhosis and liver cancer, and there are approximately 5 million affected people in Japan. Currently, no effective therapeutic agents have been found to improve the fibrosis of NASH, and a new approach to elucidate the pathogenesis of the disease is required. It has been suggested that this disease is associated with abnormal mitochondrial function. Therefore, we measured the expression of mitochondria-related factors and the production of mitochondria-derived reactive oxygen species (ROS) using liver organoids that can reproduce the pathology of NASH established in our laboratory. The results showed that the expression of the mitochondrial fission protein DRP1 and the production of ROS were increased compared to normal liver organoids. Furthermore, the mitochondrial fission inhibitor Mdivi-1 treatment suppressed the dendritic-like morphology of NASH organoids, suggesting that it may have anti-fibrotic effects.

1-LBS-05

Ramelteon enhances memory acquisition of object recognition in mice

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Ramelteon, a synthetic and selective melatonin MT_1/MT_2 receptor agonist, tunes sleep-wake rhythms and is used for the treatment of insomnia. We previously reported that ramelteon modulated neocortical extracellular oscillations, suggesting the contribution of ramelteon on information processing in the brain. We supposed that ramelteon affects cognitive functions such as memory and learning and investigated whether ramelteon modulates the acquisition, consolidation, and/or retrieval of memory. To assess the effect of ramelteon on memory, we intraperitoneally injected saline or ramelteon (3mg/kg/day) into mice and conducted a novel object recognition test, in which two same objects were presented to freely moving mice in an open field on Day 1 (training session), and one object of the two was replaced with a novel object that had distinct color and shape on Day 2 (test session). To specify which phase (*i.e.*, acquisition, consolidation, and/or retrieval) of memory was affected by ramelteon, we injected the drug 20 min before the training (acquisition group), immediately after the training (consolidation group), or 20 min prior to the probe test (retrieval group). We found that the discrimination performance for two distinct objects in the acquisition group was significantly higher in ramelteon-treated mice than saline-treated mice, but neither index for the consolidation nor retrieval group differed between ramelteon- and saline-treated mice. These results suggest that ramelteon specifically facilitates the acquisition phase of object recognition memory that possibly requires the perirhinal cortex and hippocampal CA1 area.

2-LBS-06

SUMO1 Modification of Tau in Progressive Supranuclear Palsy

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Small ubiquitin-like modifiers (SUMO) have been implicated in several neurodegenerative diseases. SUMO1 conjugation has been shown to promote aggregation and regulate phosphorylation of the tau protein linked to Alzheimer's disease and related tauopathies. The current study has demonstrated that SUMO1 colocalizes with intraneuronal tau inclusions in progressive supranuclear palsy (PSP). To examine the effects of SUMOylation on the tau based pathology of PSP, several tau-SUMO fusion proteins were prepared. As the results, truncated tau especially showed a higher propensity for tau oligomerization and accumulation on microtubules as compared to the full-length protein. In addition, tau-SUMO1 fusion protein
showed the greater effect than SUMO2 fusion protein. PSP may represent a detrimental event that promotes aggregation and impedes the ability of cells to remove the resulting protein deposits. This combination of tau truncation and SUMO1 modification may be a contributing factor in PSP pathogenesis.

2-LBS-07

Nalmefene, a reducing alcohol consumption drug, enhanced oxLDL uptake in macrophages and aggravates atherosclerotic plaque formation in apolipoprotein E knockout mice.

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Backgrounds: Nalmefene, an opioid receptor modulator, is one of the reducing alcohol consumption drugs for patients with alcohol dependence. Opioid receptors have been reported to involve in peripheral inflammatory diseases such as colitis and arthritis in animal models. The uptake of oxidized low-density lipoprotein (oxLDL) in macrophages in atherosclerotic plaques develops atherosclerosis, one of the inflammatory diseases. In this study, we determined if nalmefene increases risk of atherosclerosis formation using apolipoprotein E knockout (ApoE KO) mice and peritoneal macrophages.

Methods and results: ApoE KO mice (8-week-old) were fed a high-fat diet and intraperitoneally administrated with vehicle (saline) or nalmefene (1 mg and 3 mg kg^{-1} day^{-1}) for 21 days. Atherosclerotic lesions were histologically analyzed by oil red O-staining and immunohistochemistry using anti-MOMA2 (monocytes / macrophages) antibody. The atherosclerotic plaque formation in ApoE KO mice was dose-dependently progressed by nalmefene treatment. In addition, nalmefene significantly increased the MOMA2-positive area in aorta in ApoE KO mice. Next, to examine the effects of nalmefene on oxLDL uptake in peritoneal macrophage, peritoneal macrophages were treated with nalmefene (300 μg / mL) for 24 h and then DiI-labeled oxLDL (DiI-oxLDL) was added to the cells for 4 h. Nalmefene significantly increased DiI-oxLDL uptake in cells compared with vehicle ($P<0.01$).

Conclusion: Nalmefene may progress the atherosclerotic plaque formation via enhancing oxLDL uptake in macrophages.

2-LBS-08

Metabolic alterations associated with cellular senescence induced by sustained NFκB activation

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Nuclear Factor-kappa B (NFκB) is an important transcription factor involved in many biological phenomena such as cancer, inflammation, aging, and immune response. The canonical pathway of NFκB has multiple feedbacks, and the NFκB activation display oscillation behavior. NFκB inhibitor alpha (IκBα) is a target gene of NFκB, but is also a negative feedback factor and cause oscillation of NFκB activity. Knockout of the IκBα gene induces sustained NFκB activation in cells stimulated with tumor necrosis factor α (TNFα), resulting in gene expression different from oscillatory NFκB activation (Cheng et al., Science, 2021). However, how this change in the dynamics of NFκB activity link to cell properties or fate is not fully understood.

In breast cancer MCF7 cells, the IκBα knockdown-induced sustained NFκB activation promoted cellular senescence phenotypes such as increased cell size, cell cycle arrest, elevated SA-βgal activity, and senescence-associated secretory phenotype (SASP) gene expression. Furthermore, combined metabolomic and transcriptomic analysis revealed that sustained activation of NFκB leads to metabolic hallmarks associated with cellular senescence. In this presentation, we would like to discuss the molecular mechanisms by which dynamics change in NFκB activity induce cellular senescence from the viewpoint of metabolism.

3-LBS-09

PDGFRβ signal inhibition in brain pericytes reverses the decreased expression of astrocytic glutamate transporter EAAT2 in the hippocampus of traumatic brain injury model mice

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Impaired glutamate uptake and lowered expression of glutamate transporter (excitatory amino acid transporter: EAAT) in activated astrocytes are involved in the development of neuronal hyperexcitability in traumatic brain injury (TBI). Our previous study showed that astrocytic activation, characterized by increased GFAP expression, is preceded by increased expression of platelet-derived growth factor receptor (PDGFR) β in brain pericytes of TBI mice. However, little is known about the role of pericyte in dysregulation of glutamate uptake via EAAT in astrocytes under TBI pathology. Here, we investigated whether reactive pericytes in the early phase after TBI modulate EAAT2 expression in astrocytes. EAAT2 expression in astrocytes was significantly lower in the ipsilateral hippocampus 28 days after TBI than after sham operation. The decreased EAAT2 levels in TBI mice on postoperative day 28 were reversed by treatment with imatinib, a PDGFRβ inhibitor, during a period of postoperative day 0–4. In this period, PDGFRβ expression was increased in pericytes. These findings suggest that increased PDGFRβ expression in pericytes in the early phase causes the downregulation of EAAT2 expression in astrocytes in the late phase after TBI and drives the development of impaired glutamate uptake in astrocytes after TBI.

3-LBS-10

HDL mimetics promotes mitochondrial function in mouse myoblast cells.

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High-density lipoprotein (HDL) has been reported to have pleiotropic effects for antiatherosclerosis, and large clinical trial has been shown that an induction of HDL by torcetrapib improves glucose metabolism. Recently, HDL and apolipoprotein A-I (ApoA-I), the major constituting protein of HDL had been demonstrated to enhance mitochondrial function in skeletal muscle in vitro.

One of the HDL mimetics, Fukuoka University ApoA-I Mimetic Peptide (FAMP) was developed as a low-amino acid residues peptide preserving human ApoA-I activity without phospholipids and has been reported to enhance HDL functions. In this study, we evaluated whether FAMP with HDL would affect mitochondrial functions in C2C12 mouse myoblast cells using with the extracellular flux analyzer. As the results, HDL induced oxygen consumption rate changes that was the significant elevation of basal respiration (+35%), maximal respiration (+54%), ATP production (+35%) and spare respiratory capacity (+68%). Furthermore, an incubation of HDL with FAMP at 37°C significantly increased maximal respiration and spare respiratory capacity in a dose dependent manner.

In conclusion, HDL mimetics improved mitochondrial function in skeletal muscle cells through enhancement of HDL function.

3-LBS-11

Induction of metallothionein expression by Cyclosporin A

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[Purpose] It is widely known that drug-induced gingival overgrowth is caused by the administration of an antihypertensive drug Amlodipine, the antiepileptic drug Phenytoin, or the immunosuppressant Cyclosporin A (CyA). The mechanism of gingival overgrowth is thought to be fibroblast overgrowth and collagen metabolism disorders due to the drug's direct action, but the details are unknown. Metallothionein (MT) is attracting attention as a multifunctional protein that has various roles such as detoxification of heavy metals as cadmium (Cd), and its relationship with proliferation has been suggested. In this study, we experimented to investigate the relationship between MT and drug-induced gingival overgrowth. [M & M] CyA or Cd was added to mouse gingival epithelial cells, and the changes in gene expression of MT or type I collagen (Col Ia) were analyzed 1, 3, 6 hours after CyA or Cd treatment by using real-time RT-PCR. In addition, the cytotoxicity was observed using lactate dehydrogenase assay, and cell viability assay. [Results] Cell viability and cytotoxicity in CyA treatment were not significant to control. The gene expression of MT increased after Cd or CyA treatment. The CyA treatment increased Col Ia gene expression after 1 hour. There is a difference in the reactivity of MT gene expression by the type of stimulation to gingival cells. [Conclusions] The MT of gingival cells could work as a multifunctional protein induced during proliferation as well as a biological defense reaction to heavy metal, it indicates the pharmacological significate of MT in gums.