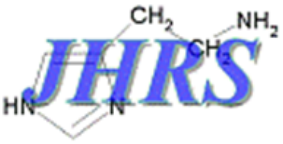


# 第 19 回日本ヒスタミン学会

# *The 19th Annual Meeting of Japanese Histamine Research Society*

*November 13-14, 2015*
*Sendai, Japan*

## *ABSTRACT BOOK*

*Preface written by the chairman*
*Abstracts submitted by presenters*

## *NEWSLETTER*

*Topics submitted by members*

# Preface

The 19th Annual Meeting of Japanese Histamine Research Society was held from November 13 to 14, 2015, in Sendai, Japan. This meeting was a very good opportunity for all participants to exchange of ideas between basic science and clinics, and between allergy/inflammation and central nerve system. In this meeting, we invited two distinguished researchers: Dr. Robin L. Thurmond (Janssen Research & Development) and Dr. Takatoshi Mochizuki (Kyushu University) for the special lectures. In addition, we planed a symposium “New progress in histamine research in inflammation and allergy”, which was held by three forefront investigators: Dr. Yasuo Endo (Tohoku University), Dr. Yoshihiro Inami (Hoyu Co., Temple University School of Medicine) and Dr. Yukihiko Sugimoto (Kumamoto University).

To encourage young investigators, we have the young investigator session and awarded “Young Investigator Award” to three excellent presenters. We also held “Meet the Professor” session to make an opportunity for young investigators to discuss casually with Dr. Robin L. Thurmond.

As an additional event, we held an exhibition “The World of Scientific Illustrations”. We believe that these illustrations stimulate the scientific imagination of many investigators.

We hope that this meeting was a fruitful for all the participants and for histamine research. Finally, we thank all contributors for their exciting talks and kind participation. We also appreciate the members of Japanese Histamine Research Society and the local organizing committee.

Noriyasu Hirasawa, Ph.D.

The Representative Organizer

The 19th Annual Meeting of Japanese Histamine Research Society

Graduate School of Pharmaceutical Sciences,

Tohoku University

# *ABSTRACT BOOK*

## *Abstracts*

## *Special Lectures*

## SL-1 Roles of histamine neurons in narcolepsy

Takatoshi Mochizuki
Dept of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA. Current Affiliation: Academic Research and Industrial Collaboration Management Office, Kyushu University, Fukuoka.

【Abstract】

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, abnormal REM sleep-associated symptoms, and cataplexy, brief episodes of muscle weakness triggered by strong emotions. Since the discovery of neuropeptide orexin/hypocretin, many human and animal studies have demonstrated that orexin neurons are important for wake maintenance and luck of orexin causes narcolepsy symptoms. Role of histamine neurons in narcolepsy has also been investigated but the results were inconclusive; histamine concentration in the CSF was relatively low in people with narcolepsy (Nishino et al., 2009), in contrast, the number of HDC-immunoreactive neurons was increased in the postmortem brains (Valko et al, 2013). To study whether histamine neurotransmission is attenuated or enhanced in narcolepsy, we tested H1 blockers and alpha-fluoromethylhistidine in orexin KO mice and measured sleep/wake behavior. Our results demonstrated larger wake defects in orexin KO mice than WT mice, suggesting that histaminergic neurotransmission is critical for maintaining wake in these mice. Compensatory enhancement of histaminergic neurotransmission would occur in narcolepsy.

## SL-2 Clinical Experience with Histamine $H_4$ Receptor Antagonists

Robin L. Thurmond
Janssen Research & Development, LLC San Diego, CA USA

【Abstract】

Ligands for the most recently discovered histamine receptor, the histamine $H_4$ receptor, have just started to be tested in the clinic. Preclinically the $H_4$ receptor antagonist, JNJ-39758979, has been shown to block scratching in mice and is anti-inflammatory in models of dermatitis, asthma and arthritis. Some of these effects have now been shown to translate into humans. One study evaluated the effect and safety of JNJ-39758979 on histamine-induced pruritus in healthy subjects. Treatment with JNJ-39758979 demonstrated a significant decrease in pruritus when compared with placebo. Another study assessed the safety and efficacy of JNJ-39758979 in patients with moderate atopic dermatitis. Numerical improvements were observed in the EASI score at week 6 compared to baseline, but these changes did not reach statistical significance. Nominally statistically significant improvements were seen in patient-reported itch severity and duration. Studies in asthma, rheumatoid arthritis and psoriasis have also been conducted.

# *Symposium*

## *-New developments in Histamine Research on Immunity and Allergy-*

### S-1 Roles played by histamine/HDC and IL-1 in allergies

Yasuo Endo

Division of Molecular Regulation, Graduate School of Dentistry, Tohoku University, Sendai 980-8575, Japan

【Abstract】

Mast cells and basophils store and release histamine. However, other cell-types can provide *newly formed* histamine via induction of the histamine-forming enzyme histidine decarboxylase (HDC). Interestingly, lipopolysaccharide (LPS, a constituent of Gram-negative bacteria and a typical TLR4 agonist) is a potent exogenous HDC inducer, and LPS is a potent inducer of the inflammatory cytokine IL-1. IL-1 is a potent endogenous inducer of HDC. Recently, we found in mice that LPS and histamine can each promote metal allergies by acting as a potent adjuvant at both the sensitization and elicitation steps. IL-1 also reportedly promotes sensitization to allergies induced by organic chemical haptens. Here, we suggest that (a) histamine and IL-1 exert adjuvant effects at both the sensitization and elicitation steps, (b) these adjuvant effects depend on histamine and on IL-1 , and (c) this histamine is supplied either via release from mast cells or basophils or via HDC-induction in other cell-types.

## S-2 Involvement of epidermal histamine in surfactant-induced itch in mice

Yoshihiro Inami
Hoyu Co., Ltd., Fundamental Research Laboratory
Temple University School of Medicine, Department of Dermatology and Temple Itch Center

【Abstract】
Washing with soap is important to keep skin healthy, but may cause itch. We examined whether topical application of anionic surfactants, which are the main ingredients of detergents, would cause itch in mice. 10% sodium laurate (SL), but not 10% sodium dodecyl sulfate (SDS), increased acute hind-paw scratching between 2 and 3 h after application. Repeated application of 10% SDS increased chronic scratching in a day-dependent manner. Both acute and chronic scratching were inhibited by an H1 histamine receptor antagonist, but were not affected by mast cell deficiency. Histamine and 53-kDa histidine decarboxylase (HDC) levels were increased in the epidermis 2 h after SL application and on day 4 of SDS treatment. Repeated SDS application also increased epidermal HDC mRNA levels and nerve elongation into the epidermis, which were inhibited by HDC deficiency. These results suggest some anionic surfactant-induced itch is mainly due to increased histamine production in the epidermal keratinocytes.

## S-3 Mechanisms underlying prostaglandin $E_2$-induced inflammation in the skin

Yukihiko Sugimoto[1, 2], Shuh Narumiya[3]
[1]Dept. of Pharmaceut. Biochem., Gard. Sch. of Pharmaceut. Sci., Kumamoto University, [2]AMED-CREST, [3]Medical Innovation Center, Kyoto University

【Abstract】
Prostaglandins (PGs) are involved in various types of inflammatory diseases by exerting their pro-inflammatory actions. Particularly, $PGE_2$ plays a key role as one of the pro-inflammatory mediators during the pathological processes. $PGE_2$ actions are mediated by four PGE receptor subtypes, EP1, EP2, EP3, and EP4, which differ in their signal transduction pathways. Both pharmacological and genetic analyses have clarified which EP subtypes are involved in each of $PGE_2$ actions; EP3 receptor is involved in inflammation-associated fever generation, and EP1 is involved in thermal hyperalgesia. However, until recently, it was unknown which EP subtypes mediate $PGE_2$-induced inflammatory response, such as enhancement of vasopermeability, edema formation and leukocyte infiltration. To clarify these points, we employed arachidonate-induced dermatitis models and examined the effect of each EP gene disruption on this model. Finally, we found that $PGE_2$-EP3 signaling triggers acute inflammatory responses by mast cell activation in the skin (Morimoto K, et al. J. Immunol. 192: 1130, 2014). We would like to discuss on potential roles of EP3-mediated mast cell activation in other inflammatory diseases in human.

# *Young Investigator Session*

## Y-1 Roles of Histamine in Ni wire-induced Inflammation

Yu Kishimoto[1], Sanki Asakawa[1], Taiki Sato[1], Shiho Takakuwa[1], Takahisa Nakajo[1], Takafumi Shimizu[1], Takeo Yoshikawa[2], Kazuhiko Yanai[2], Hiroshi Ohtsu[3], Noriyasu Hirasawa[1]

[1]Laboratory of Pharmacotherapy of Life-style Related Diseases, Graduate School of Pharmaceutical Sciences; [2]Department of Pharmacology, Graduate School of Medicine; [3]Department of Quantum Science and Energy Engineering, Graduate School of Engineering; Tohoku University, Sendai, Japan

【Abstract】

Antihistamines improve symptoms such as redness and itch in nickel (Ni) allergy. However the roles of histamine in Ni-induced acute inflammation remain unclear. We used a Ni wire-implantation model in mice to elucidate the functions of histamine. The implantation of a Ni wire induced the increase in the concentration of $Ni^{2+}$, and levels of mRNA for HDC and neutrophil chemoattractants (KC, MIP-2). Ni wire-induced expression of HDC mRNA was also observed in mast cell deficient WBB6F1-W/$W^{V}$ mice. In $HDC^{-/-}$ mice, the $Ni^{2+}$-induced elution of $Ni^{2+}$, and increase in the levels of mRNA for KC and MIP-2 were higher than those in wild-type mice. Histamine inhibited the $Ni^{2+}$-induced expression of MIP-2 mRNA in mouse bone marrow-derived macrophages and IL-8 in human monocyte cell line U937. The elution of $Ni^{2+}$ was reduced in neutrophil-depleted mice. These results suggested that Ni wire-induced the expression of HDC in non-mast cells, and histamine reduced the elution of $Ni^{2+}$ probably through regulating neutrophils infiltration.

## Y-2 Interleukin-33 induces histidine decarboxylase activity in mice

Kanan Bando[1,2], Yukinori Tanaka[1], Toshinobu Kuroishi[1],
Teruko-Takano-Yamamoto[2], Shunji Sugawara[1], Yasuo Endo[1]
Divisions of [1]Oral Immunology and [2]Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Tohoku University

【Abstract】

[Objective] Interleukin-33 (IL-33), a member of the IL-1 family, stimulates mast cells and basophils to produce Th2 cytokines and has been shown to be involved in allergic inflammations. We previously reported that other IL-1 family members (such as IL-1α, IL-1β, and IL-18) induce histidine decarboxylase (HDC) in mice. Histamine is supplied in two ways: by its release from intracellular stores and by its synthesis via the induction of HDC. Several studies have reported that IL-33 induces degranulation of mast cells, but there is no report of it being able to stimulate histamine synthesis. Here, we examined whether IL-33 can induce HDC. [Methods] IL-33 was injected intravenously into mice, and organs were collected and subjected to measurement of HDC activity. [Results] IL-33 induced HDC activity, especially in the spleen and bone marrow, peaking around 4 h after its injection. [Discussion] IL-33 can provide histamine not only via mast cell degranulation, but also via HDC induction.

## Y-3 Changes of Histamine-related molecule expression in murine organ injury with experimental septic shock

Mizuki Hattori[1,2], Mitinori Takashina[1], Toshio Fujimori[2], Takahiro Imaizumi[1],
Kengo Tomita[1], Tokiko Suzuki[1], Yuta Aoki[2], Satoshi Tanaka[3], Wakana Ohashi[1],
Mituaki Yamazaki[2], Yuichi Hattori[1]
[1]Dept. Mol. Med. Pharmacol., [2] Dept. Anesthesiol., Grad. Sch. Med. Pharm. Sci., Toyama Univ. [3]Dept. Immunobiol., Grad. Sch. Med. Dentist. Pharmac. Sci., Okayama Univ.

【Abstract】

Systemic histamine levels are elevated in animal model of sepsis. However, the role of histamine on septic organ injury remains unclear. We investigated whether histamine contributes to organ injury in mice with cecal ligation and puncture (CLP)-induced sepsis. We found that plasma and tissue histamine levels were elevated and tissue gene expression levels of histidine decarboxylase (HDC) were increased in mice after CLP. Furthermore, H1 receptor gene expression was up-regulated in the heart, spleen, and intestine, and H2 receptor gene expression was up-regulated in the liver. Treatment with combination of H1 and H2 receptor antagonists significantly declined increases in serum aminotransferase activity, creatinine levels, and pro-inflammatory cytokines in CLP mice. Histopathological examinations showed that the blockade of H1 and H2 receptors significantly reduced acute lung injury after CLP. These results suggest an aggravating role of histamine in the development of organ injury associated with sepsis.

## Y-4 Effect of narrow-band UVB on PMA-induced up-regulation of Histamine $H_1$ receptor gene expression in HeLa cells

Kentaro Okamoto[1], Hiroyuki Mizuguchi[1], Tatsuya Fujii[2], Mika Kitamura[1], Yoshiaki Kitamura[2], Noriaki Takeda[2], Hiroyuki Fukui[3]

Department of [1]Molecular Pharmacology, [2]Otolaryngology, and [3]Molecular Studies for Incurable Diseases, Institute of Medical Biosciences, Tokushima University Graduate School

【Abstract】

The phototherapy with narrowband-UVB (NB-UVB, wavelength from 308 to 313 nm) has been used to treat skin allergic diseases such as psoriasis. Here, we investigated the effects of irradiation with NB-UVB at a wavelength of 310 nm on phorbol-12-myristate-13-acetate (PMA)-induced up-regulation of histamine $H_1$ receptor (H1R) mRNA in HeLa cells. Pre-irradiation with UVB at 305 nm and NB-UVB at 310 nm, but not UVB at 315 nm at a dose of 200 and 300 mJ/cm$^2$ significantly suppressed PMA-induced up-regulation of H1R mRNA. At a dose of 200 mJ/cm$^2$, irradiation with UVB at 305 nm, but not NB-UVB at 310 nm induced apoptosis in HeLa cells, although exposure of the cells to UVB of both 305 and 310 nm induced apoptosis at a dose of 300 mJ/cm$^2$ after PMA treatment. These findings suggest that low dose irradiation with NB-UVB at 310 nm specifically suppressed up-regulation of H1R gene expression without inducing apoptosis. Intranasal NB-UVB irradiation with LED could be used as phototherapy for allergic rhinitis.

## Y-5 Histamine induces glutamate release from cultured astrocytes

Anikó Kárpáti, Takeo Yoshikawa, Tadaho Nakamura, Fumito Naganuma, Tomomitsu Iida, Yamato Miura, Kazuhiko Yanai

Department of Pharmacology, Tohoku University Graduate School of Medicine

【Abstract】

Astrocytes regulate brain homeostasis and neuronal activity through various mechanisms such as sensing neurotransmitters from neighboring neurons and releasing their own gliotransmitters such as glutamate. Neurotransmitters bind to astrocyte membrane receptors, hereby activating intracellular signaling, which induces an increase of intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$). Increased $[Ca^{2+}]_i$ is essential to trigger gliotransmitter release, contributing to synaptic plasticity, and in some cases exacerbates neurodegeneration.

Until now, little work has examined the impact of histamine on astrocyte signaling and gliotransmitter release, and the molecular mechanisms of these processes still remain unknown.

Our data revealed the expression of $H_1R$ and $H_2R$ in human 1321N1 cells. Histamine can increase $[Ca^{2+}]_i$ in a dose-dependent manner. Pharmacological assays showed $H_1R$ plays a crucial role in $[Ca^{2+}]_i$ elevation and glutamate release from astrocytes.

## Y-6 MAS-related G protein-coupled receptor X2 (MRGPRX2), a new target for IgE-independent allergic inflammatory and anaphylactoid reactions

Muhammad Novrizal Abdi Sahid[1], Shuang Liu[1], Takeshi Kiyoi[2], Kazutaka Maeyama[1]

[1]Dept. of Pharmacology, Ehime University Graduate School of Medicine, and [2]Academic Research Support Center, Ehime University, Ehime 791-0295, Japan

【Abstract】 Mast cells are divided into two types, connective tissue type of mast cells (CTMCs) and mucosal type of mast cells (MMCs). Both types of mast cells are triggered by immunological stimuli, that is, the cross-linking of IgE receptors with antigens. Compound 48/80 and some basic peptides such as substance P stimulate only CTMCs by IgE-independent process that has been considered not via certain receptors but via direct interaction with G proteins. Since last decade, receptors involved in sensory nerve stimulation, such as pain and itching, were clarified and classified as the family of MAS-related G protein-coupled receptors(MRGPR). In human, MRGPRX2 are expressed in primary sensory neurons and mast cells and compound 48/80 and basic peptides cause histamine secretion via MRGPRX2 from mast cells.

We transfected human MRGPRX2 gene into rat basophilic leukemia cells (RBL-2H3), which are characterized as MMCs type and not stimulated with compound 48/80. In transfected 2H3 cells (2H3X2), histamine release occurred dose-dependently by stimulation with compound 48/80. 2H3X2 cells will be a good screening tool for detection of drugs causing anaphylactoid reactions.

## Y-7 LPA-LPA$_1$ signaling promotes mast cell maturation and function.

Kuniyuki Kano[1,2], Yoshitaka Taketomi[3], Kahori Namiki[1], Tomoe Kondo[1], Tatsuji Nishioka[1], Makoto Murakami[3] and Junken Aoki[1,2]

[1] Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University. [2] AMED-CREST, [3] Lipid Metabolism Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

【Abstract】

Lysophosphatidic acid (LPA) is bioactive lipid, which elicits various responses through defined six LPA receptors (LPA$_1$-LPA$_6$). Here, we show LPA-LPA$_1$ signaling promotes mast cell (MC) maturation *in vivo* and *in vitro*. We found that the number of cells, histamine level and MC differentiation markers including Mcpt4 and Hdc were decreased in peritoneal MCs derived from LPA$_1$ KO mice. LPA$_1$ KO mice were highly sensitive to caecal ligation and puncture (CLP)-induced sepsis, in which MCs contribute to antibacterial defense. To examine the role of LPA$_1$ in differentiation of MCs, we cocultured immature bone marrow–derived mast cells (BMMCs) with Swiss 3T3 fibroblasts. LPA$_1$ antagonist and ATX (LPA-producing enzyme) inhibitor significantly attenuated the maturation of BMMCs. In addition, both of LPA$_1$-KO BMMCs and LPA$_1$-KO fibroblasts showed impaired ability to maturate BMMCs. Taken together, these data suggest that LPA-LPA$_1$ signaling is novel factor to facilitate fibroblasts-driven maturation of MCs.

## Y-8 Effect of neuromedin U on the activation of connective tissue-type mast cell

Matsuo Yoshimi[1], Yuhki Yanase[1], Satoshi Tanaka[2], Michihiro Hide[1]
[1]Department of Dermatology, Graduate School of Biomedical and Health Sciences, Hiroshima University, [2]Division of Pharmaceutical Sciences, Graduate School of Medicine, Dentisty, and Pharmaceutical Sciences, Okayama University

【Abstract】

Neuromedin U (NMU), known as a neuropeptide, plays important roles in various physiological functions, such as contraction of smooth muscle, reduction of food intake and body weight, and regulation of circadian rhythm. NMU also induces degranulation of mouse mast cells *in vivo*, which leads to early phase inflammation, such as vasodilation, extravasation, and neutrophil infiltration in inflamed sites, suggesting the critical role of NMU in immunoregulation. However, mast cells model which reacts to NMU *in vitro* has not been established. Therefore, detailed mechanisms and functions of NMU in mast cell activation are unclear.

In this study, we established connective tissue-type mast cells (CTMC) which can react to NMU by co-culture of BMMCs with Swiss 3T3 fibroblasts in the presence of stem cell factor and investigated the effect of NMU on activations of CTMC, such as histamine and leukotriene release.

## Y-9 Elucidation of the role of human basophils in skin allergic diseases

Yuhki Yanase, Tomoko Kawaguchi, Kaori Ishii, Takaaki Hiragun, Michihiro Hide
Department of Dermatology, Graduate School of Biomedical and Health Sciences, Hiroshima University

【Abstract】

Basophils are resident in blood and represent less than 1% of peripheral blood leukocytes. Basophils express high affinity IgE receptor (FcεRI) which binds to IgE antibody in blood. The activation of basophils by the interaction of specific antigen with IgE antibodies results in the release of various chemical mediators, such as histamine, and induction of allergic reactions. Although numerous researches performed using animal model imply basophils play specific and critical roles in skin allergic disorders, detailed roles and functions of human basophils in skin allergic disorders have been unclear. In this study, we investigated the characters of peripheral blood basophils in the patients with atopic dermatitis (AD) and chronic urticarial (CU) by means of flow cytometric analysis and ELISA. Moreover, we examined the effects of high concentration of monomeric IgE antibody on the release of histamine and morphological changes of human basophils.

# *Oral Presentations*

## O-1 An Analysis of the Making Process of Scientific Illustrations

Ariga Kana, Tashiro Manabu
Division of Cyclotron Nuclear Medicine, Tohoku University Cyclotron and Radioisotope Center

【Abstract】
Scientific illustration is a kind of visual representation in science. Although the scientific illustrations are often created by illustrators as well as scientists, little is known in terms of epistemology about how the collaborative works between scientists and illustrators proceeds.

The purpose of this study is to show how scientists and illustrators create images of scientific illustrations collaboratively. Through a case study of making scientific illustrations by a scientist and an illustrator, the following 2 image creating processes were identified: 1) to create a new image based on scientific knowledge and the illustrator's creativity, and 2) to modify and develop existing images through their communication. These processes were not clearly distinguishable, but the levels of creativity were different. The present result suggests that scientific illustrations can reflect ideas from both scientists and illustrators, and that thus illustrators' ideas may affect visual thinking of scientists.

## O-2 Car-driving performance after administration of antihistamine in young and elderly adults.

Akie Inami[1], Rin Matsuda[1], Thomas Grobosch[3], Hiroshi Komamura[1], Yusuke Yamada[1], Kazuko Takeda[1], Masayasu Miyake[1], Kotaro Hiraoka[1], Kazuhiko Yanai[1,2], Marcus Maurer[3], Manabu Tashiro[1]

[1]Division of Cyclotron Nuclear Medicine, Cyclotron and Radioisotope Centre, Tohoku University, [2]Department of Pharmacology, Tohoku University. [3]Department of Dermatology and Allergy, Allergie-Centrum-Charite/ECARF, Charité - Universitätsmedizin, Berlin, Germany

【Abstract】 We assessed car-driving performances of elderly and young adults using a driving simulator to investigate sedative effects of antihistamines. Subjects were tested for 4 drug conditions including second-generation antihistamines such as levocetirizine (LEV) and fexofenadine (FEX), a sedative antihistamine such as diphenhydramine (DIP), as well as placebo (PLA). Driving tests were performed before oral administration and 90 and 180 min post-administration. Additionally, subjective sleepiness was evaluated before and after each driving test. The results of statistical examination using two-way ANOVA have indicated that DIP significantly impaired driving performance compared with other drugs at 90 min post-administration in young adults. In contrast, there was no significant impairment in elderly adults. As for subjective sleepiness, the results of analysis demonstrated significantly stronger sleepiness due to DIP than other agents in young adults. No significant differences between drugs in subjective sleepiness of elderly adults. The present study has demonstrated that sedative effects of LEV is similar to FEX and PLA. In addition, it has been suggested that elderly adults are less susceptible to the sedative effect of antihistamines than young adults.

## O-3 The effects of isoflurane on histaminergic neuronal system

Tadaho Nakamura, Takeo Yoshikawa, Fumito Naganuma, Yamato Miura, Tomomitsu Iida, Takuro Matsuzawa, Ai Horigome, Aniko Karpati, Kazuhiko Yanai

Department of Pharmacology, Tohoku University Graduate School of Medicine

【Abstract】

Volatile anesthetics may exert loss of consciousness (LOC) effect via the modulation of specific neural pathways which relate to sleep/arousal. Histamine neurons, originated from tuberomamillary nucleus (TMN) in posterior hypothalamus, play a pivotal role in evoking and maintaining arousal status. However, the effects of volatile anesthetics on histaminergic neuronal system still remain to be elucidated. We found that low-dose isoflurane, a widely used volatile anesthetic, increased extracellular histamine, whereas high-dose isoflurane decreased extracellular histamine in mouse hypothalamus. Histamine $H_1$ receptor ($H_1$) KO mouse had lower $EC_{50}$ of isoflurane for inducing LOC. $H_1$KO also had prolonged emergence time which indicated $H_1$KO had high sensitivity to isoflurane anesthesia. These results indicated that isoflurane modulated histamine dynamics in brain and histaminergic neuronal system was involved in LOC by isoflurane.

## O-4 Histamine *N*-methyltransferase deficiency causes abnormal sleep-wake cycles.

Fumito Naganuma, Takeo Yoshikawa, Tadaho Nakamura, Ai Horigome, Yamato Miura, Atsushi Miura, Takatoshi Mochizuki, Kazuhiko Yanai.
Department of Pharmacology, Tohoku University Graduate School of Medicine.
Department of Neurology, Harvard Medical School.

【Abstract】

Histamine *N*-methyltransferase (HNMT) plays roles as histamine-metabolizing enzyme in the central nervous system. We investigated HNMT deficient mice and cleared that the brain histamine content of HNMT deficient mouse (KO) was 6 times as abundant as that of wild type (WT). We also found that Hnmt deficiency significantly decreased locomotor activities with prolonged immobility times in dark period. These results indicated that the sleep-wake cycles disrupted in KO. Thus, we performed the sleep analysis. The sleep analysis revealed that prolonged waking time of KO in light period with extended sleep time in dark period, implying that high histamine concentration caused by HNMT deficiency prolonged the wakefulness in light period. In addition, KO showed an increase in EEG in wakefulness and a decrease in theta-wave (5.0 - 10.0 Hz) in NREM, suggested that KO might feel sleepy in the wakefulness and Hnmt deficiency changed the EEG pattern of the NREM sleep. Our study suggested that HNMT was important for the normal sleep-wake cycles through the regulation of brain histamine concentration.

## O-5 Distribution of histaminergic neuronal cluster in the rat and mouse hypothalamus

Seiichi Chiba, Chinatsu Moriwaki, Keisuke Ina, Hirotaka Shibata, Yoshihisa Fujikura.
Oita University, Faculty of Medicine, Molecular Anatomy.

【Abstract】

Histaminergic neurons of rats and mice are found in five subgroups in posterior regions (E1, E2, and E3); adjacent to periventricular regions (E4); and diffusely scattered throughout the more dorsal regions (E5) of the hypothalamus. These histaminergic subgroups seemed to contribute the physiological regulations in different manners, respectively.

## O-6 L-Asparaginase-induced allergy in mice: effects of concomitant drugs and anti-IgE antibody

Mitsunobu Mio[1], Ai Nogami-Hara[1], Kazue Yabuki[1], Kaori Suwaki[1], Ai Handa[1] and Akira Shimada[2]

[1]Lab. Pharmacol., Shujitsu Univ., Sch. Pharm., [2]Dept. Pediatrics, Okayama Univ. Hospital

【Abstract】

L-Asparaginase (L-Asp) derived from *E. coli* is one of the essential drugs for acute lymphoblastic leukemia. *E. coli* L-Asp often causes allergies, including anaphylaxis. At present, there is neither suitable therapy for L-Asp allergy nor alternative L-Asp in Japan. In this study, we induced type I allergy to *E. coli* L-Asp in mice.

Male BALB/c mice sensitized with L-Asp (100 μg with alum i.p. and 10 μg i.d. in the auricle) were challenged with 10 μg L-Asp in the auricle and the changes in auricle thickness were measured. Auricle sections were stained by H&E or toluidine blue.

L-Asp induced significant swelling of the auricles. Anti-IgE significantly inhibited the swelling. Microscopy revealed eosinophil infiltration and mast cell degranulation in challenged auricles; both of which were inhibited by anti-IgE. The swelling was augmented by cyclophosphamide (CY) but not methotrexate.

It was indicated that anti-IgE can inhibit L-Asp allergy and that CY, which reportedly impair Treg cells, may exacerbate L-Asp allergy.

## O-7 New, easy, and rapid method using *in vivo* imaging for evaluating anaphylaxis

Kouya Yamaki, Shin Yoshino

Department of Pharmacology, Kobe Pharmaceutical University

【Abstract】

In this study, we established a new, easy and rapid method using *in vivo* imaging for evaluating anaphylaxis and demonstrated the potential of the method to survey the anti-allergic compounds applied to the mice systemically or topically on skin. In contrast to the requirement of 2 days for the induction of the conventional passive systemic anaphylaxis, only 15 min after the single intravenous injection of the monoclonal anti-ovalbumin IgE (OE-1) premixed with FITC-ovalbumin was sufficient to develop anaphylactic symptoms including scratching and hypothermia, as well as distinctive Anaphylaxis-dependent Spotted Distribution of a fluorescent-labeled Immune complex in Skin, named "ASDIS", in HR-1 hairless mice. Moreover, ASDIS was inhibited by the systemic injection of histamine H1 receptor antagonists or mast cell-depleting antibody MAR-1, or topical application of the antagonists on skin. This new method inducing ASDIS may accelerate the screening of anti-allergic compounds as well as researches in anaphylaxis.

*NEWSLETTER*

*Topics*

# プロのイラストレーターへの依頼のポイント

東北大学サイクロトロン・ＲＩセンター　有賀雅奈・田代学

論文、ポスター、プレゼンテーションから、広報や一般向けの講演まで、科学では多くのイラストレーションが活用されています。このようなイラストは研究者が制作することが多いのですが、研究のプレゼンスを高める作品を作るのは容易ではありません。こういう時、もしも予算があればプロのイラストレーターに依頼するのもいいかもしれません。この記事では、科学者がイラストレーターに依頼する際のポイントをご紹介いたします。

まずはイラストレーターの探し方です。科学者個人がイラストレーターを探す最も安心な方法は、広報や知人の科学者にイラストレーターを紹介してもらうことです。心当たりがない場合は、検索エンジンを駆使して探すほか、イラストレーター紹介ページを参照します。例えば日本サイエンス・ビジュアリゼーション研究会（http://www.geijutsu.tsukuba.ac.jp/~jssv/）、筆者が管理する雅楽堂（http://www.kana-science.sakura.ne.jp/）などに、科学系のイラストレーターが紹介されています。科学系の書籍をめくって誰がイラスト制作を担当したのか探す方法もあります。

イラストレーターを選ぶ際には、ウェブサイトなどを確認し、希望する絵を描ける技量があるか見分けることが重要です。イラストレーターのスキルは様々です。技法、画力、画風、得意な科学分野を確認し、わかればコミュニケーション力も考慮して選びます。

依頼する際には、メール等で次のことを伝えます。1）誰に対する何の目的の絵か（研究発表／学生向けの広報等）、2）掲載する媒体（ウェブ／冊子等）、3）何回利用するのか、4）どんな内容の図か、5）枚数とサイズ、6）希望の表現方法（3DCG／水彩／ペン画等）、7）希望の金額、8）納期、です。未確定の項目があれば、要相談などと伝えます。

価格の決め方は複雑です。制作時間、納期、技法、数、打ち合わせ交通費、資料収集量、著作権の条件、イラストレーターのキャリアなど複合的な要因で変わるためです。目安として日本イラストレーター協会（http://jpn-illust.com/illust.html）には一般的なイラストで10cm 角くらいならば「モノクロイラストで１点あたり 3,000 円～5,000 円、カラーで 5,000 円～10,000 円」とあります。条件によりますが、一般的に科学の場合は描きこみが細かく背景知識・資料調査が要求されるため、これよりも高額になる可能性が大きいです。価格を決める際は、依頼側が予算を示すかイラストレーターが見積もりを出し、その価格をもとに交渉をします。

依頼決定後は、科学者がイラストレーターに資料を提供し、打ち合わせを行い、ラフスケッチや制作過程でチェックと修正を重ね、完成に至ります。イラストは完成に近いほど修正が困難になるので、制作初期の段階でできるだけ要望を伝えることが重要です。

以上が依頼のポイントでした。今すぐ依頼する予定のない方も、いつか機会があるかもしれません。ご参照いただけたら幸いです。

# MAS-related G protein-coupled receptor X2 (MRGPRX2), a new target for IgE-independent allergic inflammatory and anaphylactoid reactions

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Mast cells play important roles in allergy and inflammation by synthesizing and releasing inflammatory mediators such as histamine, proteases, lipid mediators and cytokines. Mast cells are divided into two types, connective tissue type of mast cells (CTMCs) and mucosal type of mast cells (MMCs). Both types of mast cells are triggered by immunological stimuli, that is, the cross-linking of IgE receptors with antigens, resulting in exocytotic release of histamine and secretion of other mediators. Some basic peptides such as substance P and bee venoms stimulate only CTMCs by IgE-independent process. Compound 48/80 is a common drug to stimulate rat peritoneal mast cells for the pharmacological researchers. It has been considered that the stimulation of compound 48/80 or basic peptides occurs not via certain receptors but via direct interaction with G proteins. Since last decade, receptors involved in sensory nerve stimulation, such as pain and itching, were clarified and classified as the family of MAS-related G protein-coupled receptors (MRGPR). In human, MRGPRX2 are expressed in primary sensory neurons and mast cells and compound 48/80 and basic peptides cause histamine secretion via MRGPRX2 from mast cells.

We transfected human MRGPRX2 gene into rat basophilic leukemia cells (RBL-2H3), which are characterized as MMCs type and not stimulated with compound 48/80. These transfected cells still have a normal response toward DNP-BSA (antigen) stimulation (Fig. 1A). Unlike in wild type RBL-2H3 cells, in transfected 2H3 cells (2H3X2) histamine release occurred dose-dependently by stimulation with compound 48/80 and mastoparan, a bee venom basic peptide (Fig. 1B). These results are in accordance with the increase in calcium influx measured using fura-2, calcium probe, after stimulation with these compounds (Fig. 2). Our investigation also showed that MRGPRB3 (MRGPR family that responsible on mast cells activation in rat) expression is much lower in mast cells deficient (-/-) rat compare to wild type rat (+/+) (Fig. 3).

Taken together, our results showed a promising potency of MRGPRX2 as a target for IgE-independent allergic inflammation and anaphylactoid reactions. Specific expression of MRGPR family in sensory neurons and mast cells is a good property in developing drug with a specific target of action. The 2H3X2 cells will be helpful as a screening tool for detection of drugs causing anaphylactoid reactions. Furthermore, we need to elucidate the relation

between mast cells and primary sensory neurons functions, where MRGPR families mainly expressed in.

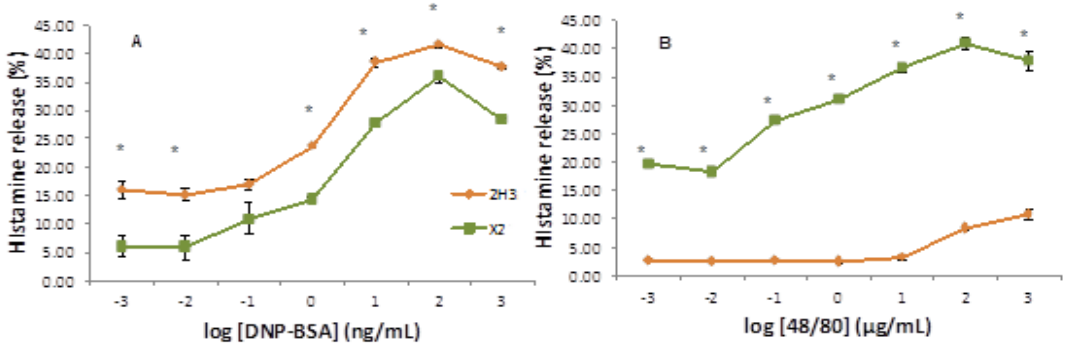


Fig 1. Histamine release from RBL-2H3 cells and 2H3X2 cells after stimulation with 20 ng/mL of DNP-BSA (A) and 50 µg/mL of compound 48/80 (B).

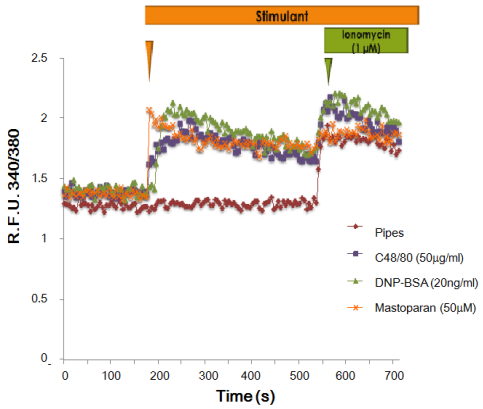


Fig 2. Calcium signal in 2H3X2 cells after secretagogues administration.

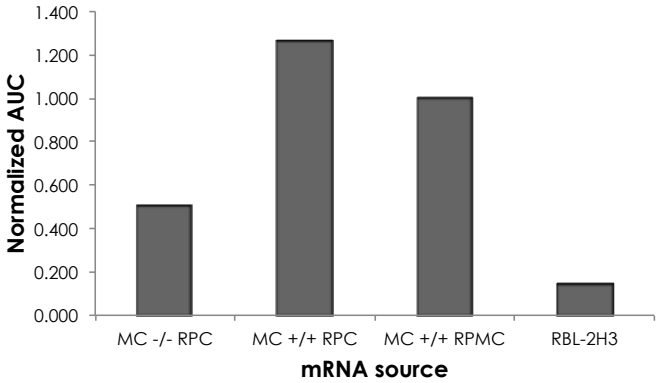


Fig 3. MRGPRB3 mRNA expressions in rat peritoneal cells (RPCs), rat peritoneal mast cells (RPMCs) and RBL-2H3 cells.