第 21 回日本ヒスタミン学会

The 21th Annual Meeting of Japanese Histamine Research Society

December 21-22, 2017
Tokushima, Japan

ABSTRACT BOOK

Preface *written by the chairperson of the annual meeting*

Abstracts *submitted by presenters*

Special Lectures
Beaven Memorial Seminar
Young Investigator Session
Oral Presentations

NEWSLETTER

Mini Reviews *submitted by Wada Prize Winners*

Next Meeting *announced by the President of JHRS*

Recent Events *reported by the Secretary of WHS2018*

Preface

The 21st Annual Meeting of Japanese Histamine Research Society

Eiichi Sakurai, Ph.D., Chief Organizer
Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

The 21st Annual Meeting of Japanese Histamine Research Society was held from December 21 to 22, 2017, in Tokushima, Japan. The theme of this year's meeting was "Taking a new perspective on histamine research endeavor. Our hope was to have 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, the Society has invited basic and clinical researchers: Dr. Akiyoshi Fukamizu (Life Science Center, Tsukuba Advanced Research Alliance, University of Tsukuba) and Dr. Tamotsu Harada (Professor emeritus, Kawasaki Medical School), respectively, for the special lectures.

As an one more special session, we also planed a memorial seminar of Dr. Michael A. Beaven (National Heart Lung & Blood Institute, NIH, USA) demised on April 8th, 2017, which was held by three forefront investigators worked in Mike's lab: Dr. Kazutaka Maeyama (Professor emeritus, Ehime University), Dr. Koichiro Ozawa (Hiroshima University) and Dr. Noriyasu Hirasawa (Tohoku University). Dr. Beaven influenced many Japanese researchers in histamine world. Here, please accept our heartful condolences on the death of Dr. Michael A. Beaven.

In keeping with the Society's tradition, abundant time was allocated to presentations by general members and presentations by candidates for Wada awards to encourage young investigators.

Finally, we deeply appreciate all contributors for their exciting and fruitful discussion in a family atmosphere.

I sincerely hope that we can see all of you at the meeting.

ABSTRACTS

Special Lectures

SL-1 Anti-inflammatory programming of gene expression in cardiorenal pathology

Akiyoshi Fukamizu
Life Science Center, Tsukuba Advanced Research Alliance, University of Tsukuba, Japan

【Abstract】The heart and kidney are two of the most important organs in the regulation of systemic hemodynamics, and abnormalities in either organ can affect the other, leading to accelerated worsening of pathological conditions. The association between these two organs is thought to involve the sympathetic nervous system and the renin–angiotensin system; however, the molecular pathology and mechanism of the onset of this disorders are not yet known, and little progress has been made in developing therapeutic strategies. Therefore, we investigated kidney disease in a mouse model of heart failure in which mice received angiotensin II (A), underwent partial nephrectomy (N), and were loaded with salt (S) (ANS mice), and analyzed changes to factors circulating in the blood and comprehensive gene expression patterns in heart and kidney tissues using RNA-seq. In addition to heart failure, ANS mice developed proteinuria and reduced kidney function, as well as an increase in serum histamine levels. Given the elevated histamine levels, we administered histamine receptor antagonists and agonists and found that cardiorenal function improved significantly after agonist treatment. Moreover, RNA-seq analysis revealed that inflammation-related genes that were elevated in the kidneys decreased when treated with the agonist. I will discuss the effect of histamine-related molecules from a gene expression perspective.

SL-2　Epidemiology and treatment of allergic rhinitis, with a focus on Japanese cedar and Japanese cypress pollinosis

Tamotsu Harada
Honorary Professor, Kawasaki Medical School

【Abstract】The number of patients in Japan with Japanese cedar or Japanese cypress pollinosis, or both, is estimated to exceed 10 million. For this reason it is known as a national disease.

We counted the number of airborne pollen grains of Japanese cedar and Japanese cypress for 24 years (1994–2017) and studied the disease from a variety of perspectives. We used the CAP-radioallergosorbent test (RAST) to demonstrate allergen sensitization rates in 2276 patients with allergic rhinitis and examined the patients' current status and the future likely trends in the disease. In addition, the effectiveness of antihistamines as a treatment was evaluated by using QOL questionnaires. Here we report and discuss the findings.

(1) Pollen shedding by Japanese cedar and Japanese cypress over 24 years

The highest number of airborne pollen grains was 8194/cm^2 (in 1995); the lowest was 280/cm^2 (in 2004), and the average was 2591/cm^2. Although the number of airborne pollen grains increased every 4 years, it has recently been on a downward trend.

(2) Evaluation of sensitization rates in 2276 patients using the CAP-RAST test (8 items)

The highest sensitization rate was 58.1% for Japanese cedar, followed by 47.6% for house dust, 46.0% for *Dermatophagoides farinae*, 38.8% for Japanese cypress, 29.8% for orchard grass, 23.4% for animal dander allergens, 19.5% for mugwort, and 6.1% for pine.

(3) Yearly variation in allergen sensitization rates

The rate of sensitization to Japanese cedar was approximately 20% thirty-five years ago. It peaked at approximately 70% ten years ago and thereafter decreased. The average rate over the 13 years from 2000 to 2012 was approximately 68%. The rate of sensitization to house dust was approximately 60% thirty-five years ago, peaked at approximately 65% twenty-five years ago, and has been decreasing recently. The average rate was approximately 58%. The rate of sensitization to orchard grass was approximately 5% thirty-five years ago and peaked at approximately 34% fifteen years ago. The rate has remained at around 30% in recent years.

(4) Evaluation of effectiveness of antihistamines by using QOL questionnaires

Effectiveness, time to onset of effect, adverse effects, and particularly sleepiness varied according to the type of antihistamine. Use of antihistamines for initial treatment was effective as a whole; however, the effectiveness differed markedly among the types of drugs, and some antihistamines showed sex difference in effectiveness. The degree of sleepiness was consistent with brain histamine H_1 receptor occupancy (Tashiro et al. Taniuchi et al.).

ABSTRACTS

Beaven Memorial Seminar

BMS-1　A memorial seminar for Dr. Michael A Beaven (1936-2017)
Dr. Beaven in histamine world

Kazutaka Maeyama,
Emeritus professor, Ehime University

First, I would like to refer the histamine study in his 55 years research history at NIH. He developed highly sensitive radioenzymatic assay methods for histamine using rat histamine N-methyltransferase, contributing to histamine research in neuronal activity, gastric secretion and allergy (Monogr. Allergy 13. 1-113, 1978).

In 1982-1983, he got a sabbatical leave and brought rat basophilic leukemia (RBL-2H3) cells in his pocket to Cambridge University, UK. He first clarified the relationships between histamine release and the second messenger signals in 2H3 cells, Ca signal and the hydrolysis of inositol phospholipids after antigen stimulation (J. Biol. Chem 259, 7129-7136, 7137-7142, 1984).

When he was back from Cambridge to NIH, I enjoyed the research of Ca signal in mast cells. Many Japanese researchers were encouraged under his supervision and had solved mysteries of mast cell activation. All members heartily thank Dr. Beaven and remember his humor personality and histamine and mast cell world at this seminar.

BMS-2　My Dear Mike as a teacher and a father

Koichiro Ozawa
Department of Pharmacotherapy, Institute of Biomedical & Health Sciences, Hiroshima University

【Abstract】I visited Dr. Michael Beaven's laboratory in National Institute of Health in April 16, 1991.　That day was the first time I met him.　From that day on Mike was a teacher and a father in USA.
In his laboratory we researched roles of protein kinase C (PKC) on antigen-induced release of chemical mediators from rat basophilic RBL-2H3 cells.　At that time roles of PKC on signal transductions of cell activities were the hot topics in life science.　With using permeabilized and reconstituted RBL-2H3 cells, we found that a full secretory response to antigen could be reconstituted by the subsequent addition of nanomolar concentrations of either beta or delta isozymes of PKC but only in the presence of 1 micromolar free Ca^{2+} to indicate distinct roles for Ca^{2+} and PKC in exocytosis.　We found also that either alpha or epsilon isozymes of PKC, exclusively, inhibit antigen-induced hydrolysis of inositol phospholipids in the same permeabilized RBL-2H3 cells. As far as I know, these findings were the first reports that each isozymes of PKC have different functions on signaling for cell activities.　In the process of the study, I learned a lot from Mike and I believe that his instruction made me a scientist. May he rest in peace.

BMS-3　In Memory of Dr. Michael A. Beaven: MAP kinase and glucocorticoids

Noriyasu Hirasawa
Graduate School of Pharmaceutical Sciences, Tohoku University

【Abstract】My first study with Mike was investigating the regulation of arachidonic acid release in RBL-2H3 cells. We first found that MAP kinase regulated arachidonic acid release via activation of cytosolic PLA2, whereas degranulation was regulated primarily by Ca^{2+} and protein kinase C.　Secondly, we demonstrated that the crosslink of FcεRI γ chains was enough to activate MAP kinase and that tyrosine kinase Syk was involved in the antigen-induced MAP kinase activation, but not in the carbachol-induced one. Thus, we showed the existence of FcεRI-Syk-MAP kinase pathway.　Lastly, we examined the effects of the glucocorticoid dexamethasone on the antigen-induced activation of MAP kinase. We found the inhibition of arachidonic acid release by dexamethasone was related to the inhibition of MAP kinase activation, and that the possible target of dexamethasone was the step immediately before the activation of Raf-1.　Mike influenced many Japanese researchers in the mast cells and histamine world. We all miss him greatly.

ABSTRACTS

Young Investigator Session

Y-1　Induction of histamine production and its function in mouse dendritic cells

Kazuyuki Furuta, Eriko Ohno, Satoshi Tanaka
Department of Immunobiology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University

【Abstract】
Dendritic cells (DCs) induce an acquired immune response by presenting antigens to T cells. In this study, we investigated histamine production and the autocrine action in DCs using histidine decarboxylase (HDC) deficient (HDC-KO) mice. Lipopolysaccharide (LPS) stimulation induced HDC mRNA expression and histamine production in bone marrow-derived dendritic cells (BMDCs). LPS stimulation induced increase in the cell surface expression of MHC-II, CD86, CD40, and CCR7 to the same extent in WT and HDC-KO BMDCs. No significant difference was observed in cytokines expression (IL-6, IL-10, IL-12, and TNF-α) induced by LPS stimulation between WT and HDC-KO BMDCs. Furthermore, no significant difference in migration of dermal DCs to lymph nodes was observed between WT and HDC-KO mice in the FITC-induced contact hypersensitivity model. In summary, although histamine production was induced in activated DCs, we could not find the autocrine function of histamine in DCs.

Y-2　Novel anti-allergic mechanism of Tranilast

Sawada Akiho, Mizuguchi Hiroyuki, Kitamura Yoshiaki, Fujino Hiromichi, Fukui Hiroyuki, Takeda Noriaki
Faculty of Pharmaceutical Sciences, Tokushima University

【Abstract】

We have clarified that these two intracellular signalings are responsible for the pathogenesis of pollinosis symptoms, because pollinosis symptoms can be remarkably improved by simultaneously inhibiting histamine H_1 receptor signaling and NFAT signaling. We also found that up-regulation of NFAT signaling-dependent IL-9 gene expression is caused by elevation of intracellular Ca^{2+} in mast cells. As mast cell degranulation is also caused by the increase in intracellular Ca^{2+} concentration, NFAT signaling could participate in this event. However, the relationship between degranulation and NFAT signaling is not clear. Here, we investigated the effect of Tranilast on NFAT signaling. We also examined the effect of NFAT inhibitor on mast cell degranulation. Tranilast inhibited NFAT-mediated IL-9 gene up-regulation in RBL-2H3 cells. Tranilast also suppressed the binding of NFAT to chromatin. NFAT inhibitors inhibited mast cell degranulation. Our data suggest that Tranilast suppressed NFAT signaling in addition to the inhibition of mast cell degranulation. And this findings could help shed light on a novel therapeutic use of Tranilast in Allergic diseases.

Y-3　Roles of charged amino acid residues in the transmembrane domains of human histamine H_1 receptor

Hayato Tsukamoto, Ryosuke Yamamoto, Shigeru Hishinuma, Masaru Shoji
Department of Pharmacodynamics, Meiji Pharmaceutical University

【Abstract】

The transmembrane domains of G protein-coupled receptors (GPCRs) consist of hydrophobic amino acid residues so that the receptor molecules are well incorporated into the lipid bilayer of cell membranes. Accordingly, the $G_{q/11}$ protein-coupled human histamine H_1 receptor (H_1R) possesses only three charged amino acid residues in its transmembrane domains, i.e. Asp73, Asp107 and Lys191 in the second, third and fifth transmembrane domains of H_1R, respectively. Since Asp107 and Lys191 are known to form an orthosteric binding site for histamine, we investigated roles of Asp73 in regulation of H_1R function using a mutant receptor in which Asp73 was changed to asparagine: Asp73 appeared to be involved in not only Na^+-mediated allosteric regulation of the affinity of ligands for H_1R but also the coupling with G proteins. These results suggest that charged amino acid residues in the transmembrane domains of H_1R play crucial roles in regulation of H_1R function.

Y-4 The role of neuromedin-U for mast cells activation.

Yoshimi Matsuo[1], Yuhki Yanase[1], Satoshi Tanaka[2], Kazuyuki Furuta[2], Michihiro Hide[1]

[1] Department of Dermatology, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan.

[2] Department of Immunobiology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Mast cells play critical roles for innate and acquired immune responses by release of chemical mediators, such as histamine and lipid mediators, in response to various stimuli through IgE receptor-dependent or -independent pathways. Recent reports suggest that a neuropeptide, neuromedin-U (NMU), induces an early inflammatory response by directly activating mast cells in mouse. However, detailed mechanism of NMU-induced mast cells activation has not been clarified. In this study, we investigated the effects of NMU on the activation of mouse bone marrow-derived mast cells (BMMCs), connective-tissue type mouse mast cells differentiated from BMMCs (CTMCs), and human skin derived mast cells (hsMCs) *in vitro*.

Y-5 Effect of histamine on tight junctional function in cultured mouse lung microvascular endothelial cells

Chika Inoue[1], Yukari Ueda[1], Kotomi Takeuchi[1], Tadatoshi Tanino[1], Eiko Sakurai[2], Eiichi Sakurai[1]

[1]Dept. Pharmaceutics, Fac. Pharmac. Sci., Tokushima Bunri Univ., and [2]Fac. Pharmacy, Iwaki Meisei Univ.

We aimed to examine paracellular barrier function in cultured mouse lung microvascular endothelial cells (LMECs) and effect of histamine on open of tight junction.The transcellular resistance of LMEC monolayers yielded an electrical resistance of approximately 19 Ω× cm^2 at days 6–7 in culture when the cells reached confluence, and paracellular permeable clearance of sodium fluorescein was the lowest on day 6 in culture, suggesting the formation of tight junction in cultured LMECs. Moreover, the expression of TJ-associated proteins, occludin, claudin-1 and -4 and zonula occludents 1 (ZO-1) was detected in LMECs at day 6 in culture. Although mRNAs of occludin, claudin-1 and -4 and ZO-1 were already expressed on day 1 after culture, the claudin-1 mRNA level gradually increased up to approximately 7-fold on day 7 in culture over the basal level. These results indicate that the drastic increase in the mRNA expression level of claudin-1 leads to the strong formation of tight junction. This tight junction was reversibly opened by an addition of histamine (10 μmol/L). Histamine (10 μmol/L) also caused a significant reduction of claudin-1, occluding and ZO-1 protein and mRNA contents at 1 hr after treatment, mediated H_2 receptor.

ABSTRACTS

Oral Presentations

O-1 Involvement of histamine H_4 receptors in the cisplatin-induced anorexia in mice

Kouichi Yamamoto, Atsushi Yamatodani
Department of Medical Science and Technology, Division of Health Sciences, Graduate School of Medicine, Osaka University

【Abstract】
We investigated the involvement of H_4 receptor in the development of chemotherapy-induced anorexia in mice. Mice received cisplatin (7.5 mg/kg) with or without pretreatment with a histamine H_4 receptor antagonist (JNJ7777120, 10 mg/kg), then their daily food intake was monitored for 3 days. Additionally, we examined the effect of JNJ7777120 on the cisplatin-induced expression of Tumor Necrosis Factor (TNF)-α mRNA in the hypothalamus. Cisplatin-induced anorexia occurred within 24 hours and continued for 3 days. JNJ7777120 abolished delayed phases (day 2 and 3), but not early phase (day 1), of cisplatin-induced anorexia. Cisplatin significantly increased TNF-α mRNA expression in the hypothalamus, and pretreatment with JNJ7777120 inhibited the increased expression. These results suggest the TNF-α mRNA expression via H_4 receptors may contribute to the development of cisplatin-induced anorexia.

O-2 Histamine H3 receptor inverse agonist suppresses microglial functions and improves depression-like behavior in mice

Tomomitsu Iida, Takeo Yoshikawa, Kazuhiko Yanai
Department of Pharmacology, Graduate School of Medicine, Tohoku University

【Abstract】

Microglia play important roles in maintaining brain homeostasis. Recent reports showed that abnormally activated microglia were involved in various neurological disorders such as depression. Our previous *in vitro* study revealed that histamine regulated microglial functions through histamine H3 receptor (H3R). However, the impact of H3R inverse agonist on microglial functions remained to be elucidated.

Intraperitoneal injection of JNJ10181457 (JNJ), an H3R inverse agonist, suppressed LPS-induced increase in cytokine expressions of microglia. Microglial chemotaxis in was also suppressed by JNJ treatment in hippocampal slice assays. We confirmed the inhibitory effect of JNJ on in vivo phagocytosis. Finally, we investigated the effect of JNJ on the mouse model of human depression. JNJ decreased immobility time in tail suspension test coupled with the decreased production of IL-1β in microglia. These results demonstrated that JNJ suppressed microglial activities and improved depression-like behavior.

O-3 The involvement of spinal release of histamine on nociceptive behaviors induced by intrathecally administered spermine

Shinobu Sakurada[1], Takafumi Hayashi[2], Yoko Namioka[1], Chizuko Watanabe[1], Hirokazu Mizoguchi[1]
[1]Department of Physiology and Anatomy, [2]Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University

The involvement of spinal release of histamine on nociceptive behaviors induced by spermine was examined in mice. Intrathecal (i.t.) spermine produced nociceptive behaviors. The nociceptive behaviors induced by spermine at 0.02 amol and 10 pmol were abolished in histidine decarboxylase-deficient mice. In histamine H1 receptor-deficient mice, the nociceptive behaviors induced by spermine were abolished at 0.02 amol, but suppressed at 10 pmol. The i.t. pretreatment with takykinin NK1 receptor antagonists eliminated the nociceptive behaviors induced by 0.02 amol of spermine, but not affect those induced by 10 pmol of spermine. On the other hand, the nociceptive behaviors induced by spermine at both 0.02 amol and 10 pmol were suppressed by i.t. pretreatment with antagonists for the NMDA receptor polyamine-binding site. The present results suggest that the nociceptive behaviors induced by i.t. administered spermine are mediated through the spinal release of histamine and are elicited via activation of NMDA receptors.

O-4 Synthesis of Functional G-Protein-Coupled Receptor in Large scale: The Ligand Binding Assay of Human Histamine H1 Receptor Synthesized by a Wheat Germ Cell-Free Protein Synthesis System Combined with Asolectin Glycerosomes.

Yasuyuki Suzuki, Kazutaka Maeyama
Department of Pharmacology, Ehime University Graduate School of Medicine

G-protein-coupled receptors (GPCRs) have been synthesized by cell-free protein synthesis systems (CF) combined with chemical chaperones that include liposomes and glycerol. Liposomes containing high concentrations of glycerol are known as glycerosomes, which have greater morphological stability than liposomes. In this study, we synthesized human histamine H1 receptor (HRH1), which is one of the most studied GPCRs, by a wheat germ CF combined with asolectin glycerosomes. The amount of synthesized HRH1 in one synthesis reaction was 434 ± 66.6 μg (7.75 ± 1.19 × 10^3 pmol). The specific binding of [^{3}H]pyrilamine to the HRH1 proteoglycerosomes became saturated as the concentration of the radioligand increased. The dissociation constant (*Kd*) and maximum density (*Bmax*) of the synthesized HRH1 were 9.76 ± 1.25 nM and 21.4 ± 0.936 pmol/mg protein, respectively. The findings of this study highlight that HRH1 synthesized using a wheat germ CF combined with glycerosomes has the ability to bind to H1 antagonists.

O-5 Suppression of IFN-γ production in murine splenocytes by histamine receptor antagonists

Miho Kamei[1], Kazuyuki Furuta[2], Tadaho Nakamura[3], Kazuhiko Yanai[4], Satoshi Tanaka[2]
[1]Dept. Immunobiology, Fac. Pharmac. Sci., and [2]Dept. Immunobiology, Grad. Sch. Med. Dent. Pharmac. Sci., Okayama Univ., [3]Dept. Pharmacol., Tohoku Med. Pharmac. Univ., [4]Dept. Pharmacol., Grad. Sch. Med., Tohoku Univ.

Introduction: We found that the splenocytes obtained from the tumor-bearing mice produced histamine and IFN-γ when they were co-cultured with the tumor cells. This IFN-γ production was found to be suppressed by several histamine receptor antagonists. Here, we investigated the mechanism of this suppression. **Results:** IFN-γ production in murine splenocytes was significantly and dose-dependently suppressed in the presence of pyrilamine (H_1 antagonist), diphenhydramine (H_1), thioperamide (H_3/H_4), and JNJ7777120 (H_4) when they were stimulated with Concanavalin A or with the combination of an anti-CD3 antibody and an anti-CD28 antibody whereas it was not changed in the presence of cimetidine (H_2 antagonist). These responses were reproduced in the splenocytes obtained from the $HDC^{-/-}$ mice and the $H_1R^{-/-}$ mice. Murine splenocytes were found not to express H_3 and H_4 receptor mRNAs. **Conclusion:** These findings strongly suggest that these histamine receptor antagonists should have potentials to suppress IFN-γ production in murine spleen T cells and the actions of these antagonists might be independent of the histamine receptor subtypes.

O-6 Involvement Effects of ER stress in antigen-induced histamine release from RBL-2H3 cells.

Koichiro Ozaw, Mai Kakimoto, Toru Hosoi, and Megumi Mino
Department of Pharmacotherapy, Institute of Biomedical & Health Sciences, Hiroshima University

【Abstract】Endoplasmic reticulum (ER) plays important roles in protein maturation and Ca^{2+} homeostasis in cells. Load of various stresses to cells causes dysfunction of ER and then unfolded/misfolded proteins are accumulated in ER, called as ER stress. When cells are suffered from ER stress, cells activate the unfolded protein response (UPR) in order to prevent the damages of cells. Inositol-requiring enzyme-1(IRE1), which is one of UPR and has endoribonuclease activity and kinase activity, catalyze the splicing of XBP1 mRNA to produce spliced XBP1 protein. Spliced XBP1 (sXBP1) functions as a transcriptional factor that regulates UPR-related genes. In this report we investigated whether IRE1-XBP1 pathway may be involved in antigen-induced histamine release from RBL-2H3 cells. We found that sXBP1 was increased after 1 hour by stimulating cells with antigen. By using an inhibitor of endoribonuclease of IRE1, the increase of sXBP1 was reduced but antigen-induced histamine release was not affected. On the other hand, inhibitor of IRE1 kinase suppressed antigen-induced histamine release. These results suggest that the activation of IRE1 kinase might be involved in histamine release from RBL-2H3 cells. (1027 characters)

O-7 L-Asparaginase-induced allergy in mice: in vivo sensitization and in vitro activation of RBL-2H3 cells.

Mitsunobu Mio[1], Ai Nogami-Hara[1], Kazue Yabuki[1], Kawori Suwaki[1], Ai Handa[1], Chie Mitsuhata[1], Koji Kajiyama[1], Akira Shimada[2]
[1]Laboratory of Pharmacology, School of Pharmacy, Shujitsu University and [2]Department of Pediatric Hematology/Oncology, Okayama University Hospital, Okayama, Japan.

【Abstract】
L-Asparaginase (L-ASP) challenge-induced ear edema in L-ASP sensitized mice was inhibited by a pretreatment of mice with anti-IgE antibody (BD Bioscience, clone R35-92). Cyclophosphamide (CY) at 150 mg/kg (i.p.) enhanced L-ASP-induced ear edema. L-ASP-induced CY-enhanced ear edema was also inhibited by anti-IgE Ab. L-ASP sensitization increased total IgE concentration in mice sera and CY also augmented the increase in IgE in the sera. When RBL-2H3 cells were sensitized by anti-L-ASP sera, L-asp challenge induced beta-hexosaminidase release. Anti-L-ASP serum of CY-treated mice induced higher beta-hexosaminidase release than normal anti-L-ASP serum. Anti-IgE Ab was effective in inhibiting L-ASP-induced beta-hexosaminidase release from sensitized RBL-2H3 cells.
From the present results, it became clear that L-ASP sensitization induced IgE production *in vivo*, and the serum was effective to induce beta-hexosaminidase release from RBL-2H3 cells.

O-8 Hepatic Cytochrome P450 and Flavin-Containing Monooxygenase Enzymes Regulated by Type 1 Allergy-Induced Cytokines and Chemical Mediators

Tadatoshi Tanino, Toru Bando, Yukie Nojiri, Yuna Okada, Yukari Ueda and Eiichi Sakurai
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Bouji Nishihama, Yamashiro-cho, Tokushima, Tokushima 770-8514, Japan

【Abstract】This study examined the interaction of cytochrome P450 (CYP) or flavin-containing monooxygenases (FMO) with allergy-produced serotonin, histamine or NO. Seven days after primary or secondary sensitization (PS7 and SS7, respectively), hepatic CYP1A2, CYP2C, CYP2E1 and CYP3A activities were decreased to 45%-75% of the corresponding control. PS7 and SS7 did not change the expression levels of five CYP proteins. Concerning FMO isozymes, at 7 days after primary sensitization or secondary sensitization, benzydamine (BDZ; FMO1 and FMO3 substrate) N-oxygenation was significantly decreased to 70% of individual controls. The expression levels of FMO1 and FMO3 proteins were not significantly changed in the sensitized mice. Hepatic inducible NO synthase (iNOS) mRNA level increased 5-fold and 15-fold in PS7 and SS7 mice, respectively, and hepatic tumor necrosis factor-α levels were greatly enhanced. Serotonin and histamine did not directly interact with hepatic CYP or FMO isoforms. Therefore, NO is highly expected to participate in regulatory mechanisms of the four CYP isoforms. Type 1 allergic mice had differentially suppressed FMO3-dependent BDZ N-oxygenation. The suppression of FMO3 metabolism related to reversible S-nitrosyl modifications of iNOS-derived NO.

NEWSLETTER

Mini Reviews
Next Meeting
Recent Events

Novel anti-allergic mechanism of Tranilast

Sawada Akiho[1], Mizuguchi Hiroyuki[2], Kitamura Yoshiaki[3], Fujino Hiromichi[4], Fukui Hiroyuki[5], Takeda Noriaki[3]

Faculty of Pharmaceutical Sciences[1], Tokushima University; Departments of Otolaryngology[3], Molecular Pharmacology[4], and Molecular Studies for Incurable Diseases[5], Institute of Biomedical Sciences, Tokushima University Graduate School; [2]Laboratory of Pharmacology Faculty of Pharmacy Osaka Ohtani University.

[Introduction]

Since the symptoms of pollinosis can be remarkably improved by suppressing the histamine H_1 receptor signal and the NFAT signal at the same time, it has been clarified that these two intracellular signals are responsible for the pathogenesis of pollinosis symptoms. The mechanism of action of anti-allergic drug tranilast is thought to be the suppression of degranulation reaction from mast cells. However, unlike sodium cromoglycate having the same mechanism of action, it has been reported that the efficacy rate in prophylactic administration before pollen scattering is higher than that after onset in patients with cedar pollinosis (1). This suggests that tranilast has an anti-allergic effect due to an unknown mechanism of action different from sodium cromoglycate. We also found that up-regulation of NFAT signaling-dependent IL-9 gene expression is caused by elevation of intracellular Ca^{2+} in mast cells. As mast cell degranulation is also caused by the increase in intracellular Ca^{2+} concentration, NFAT signaling could participate in this event. However, the relationship between degranulation and NFAT signaling is not clear. In the present study, we investigated the effect of Tranilast on NFAT signaling. We also examined the effect of NFAT inhibitor on mast cell degranulation.

[Methods]

Effect of Tranilast on the inhibition of NFAT signal was evaluated by investigating the effect of Tranilast on ionomycin-induced up-regulation of IL-9 gene expression in RBL-2H3 cells and on PMA + ionomycin (P/I)-induced up-regulation of IL-2 gene expression in Jurkat cells using real-time-RT-PCR. Effect of Tranilast on stimuli-induced NFAT translocation was determined using immunocytochemistry. Effect of Tlanilast on NFAT transcriptional activity was investigated using luciferase assay. Effect of Tranilast on the binding of NFAT to chromatin was investigated by western blot analysis after cells were fractionated using the fractionation kit. The effect of Tranilast on mast cell degranulation was investigated by measuring IgE-antigen stimulated release of beta-hexosaminidase in RBL-2H3 cells.

[Results and Discussion]

Tranilast suppressed ionomycin-induced up-regulation of IL-9 gene expression in RBL-2H3 cells. Tranilast also suppressed P/I-induced up-regulation of IL-2 gene expression in

Jurkat cells. However, sodium cromoglycate did not suppress either gene up-regulations. As NFAT signaling pathway was involved in these gene expression, these data suggest that Tranilast suppresses NFAT signaling. This is confirmed by the NFAT promoter assay.

Western blot analysis revealed that Tranilast inhibited the binding of NFAT to chromatin-bound DNA. Treatment with NFAT signaling inhibitors including cyclosporin A, INCA-6 and pyrogallol inhibited mast cell degranulation reaction. Our data suggest that Tranilast suppressed NFAT signaling in addition to the inhibition of mast cell degranulation. And this finding could shed light on a novel therapeutic use of Tranilast in Allergic diseases.

[References]

1) 木村廣行他　スギ花粉症における予防的投薬の検討－(1) DSCG, Tranilast の予防効果－耳鼻 32: 416-424, 1986.

Roles of charged amino acid residues in the transmembrane domains of human histamine H_1 receptor

Hayato Tsukamoto, Ryosuke Yamamoto, Shigeru Hishinuma, Masaru Shoji

Department of Pharmacodynamics, Meiji Pharmaceutical University

The seven transmembrane domains of G protein-coupled receptors (GPCRs) are composed of hydrophobic amino acid residues so that the receptor molecules are well incorporated into the lipid bilayer of cell membranes. The $G_{q/11}$ protein-coupled human histamine H_1 receptor (H_1R), which consists of 487 amino acid residues, possesses only three charged amino acid residues in its transmembrane domains, i.e. Asp73 in the second transmembrane domain, Asp107 in the third transmembrane domain and Lys191 in the fifth transmembrane domain (Figure 1) [1]. In this review, we briefly summarize roles of these three amino acid resides in the transmembrane domains of H_1R in regulation of the ligand binding and the G protein-coupling, together with our recent findings.

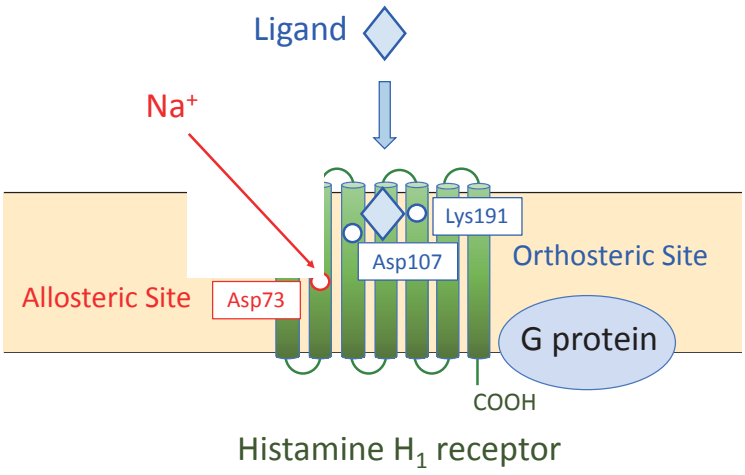

Figure 1. Three charged amino acid residues in the transmembrane domains of human histamine H_1 receptor

Roles of Asp107 and Lys191 as an orthosteric binding site

The aspartate residue in the third transmembrane domain of GPCRs, which corresponds to Asp107 of H_1R, is highly conserved between GPCRs to form an orthosteric binding site for endogenous bioactive amines. Mutational analyses have suggested that Asp107 plays an essential role in both agonist and antagonist binding to H_1R [2]. On the other hand, Lys191 in the fifth transmembrane domain of H_1R is not conserved in other aminergic receptors, but it is also known to form an orthosteric binding site for histamine [3]. Mutational analyses and lligand-receptor docking simulation indicated that Lys191 might play an important role in the binding of second-generation antihistamines containing a carboxyl group via formation of a salt bridge so as to increase their specificity against H_1R [4, 5].

Roles of Asp73 as an allosteric regulatory site

The aspartate residue in the second transmembrane domain of GPCRs, which corresponds to Asp73 of H_1R, is highly conserved between GPCRs to form an allosteric binding site for Na^+ [6, 7]. Our mutational analyses have suggested that Asp73 is involved in Na^+-mediated regulation of the affinity of both agonists and antagonists for H_1R [8]. Since the affinity of ligands for their receptors is known to be determined by their thermodynamic binding forces [9], we evaluated Na^+-mediated changes in thermodynamic binding properties of ligands: Na^+ reduced the binding enthalpy of ligands (electrostatic interaction with H_1R) but concomitantly increased the binding entropy of ligands (hydrophobic interaction with H_1R) in a manner dependent on Asp73 so that the affinity of ligands for H_1R could be diversely regulated by Na^+. We also examined whether Asp73 might be involved in the coupling with G proteins. Since histamine-stimulated accumulation of [^{3}H]inositol phosphates was completely lost by mutation of Asp73 to asparagine, Asp73 appeared to be an essential amino acid residue of H_1R to couple with G proteins. Thus, Asp73 was revealed to regulate not only the affinity of ligands for H_1R but also the coupling of H_1R with G proteins.

Conclusions

H_1R possesses only three charged amino acid residues in its transmembrane domains, but they play crucial roles in regulation of H_1R function.

References

1. Fukui H, Fujimoto K, Mizuguchi H, et al. Biochem. Biophys. Res. Commun. 1994; 201: 894-901.
2. Ohta K, Hayashi H, Mizuguchi H, et al. Biochem. Biophys. Res. Commun. 1994; 203: 1096-1101.
3. Fukui H, Ohta K, Yamamoto D. Folia Pharmacol. Jpn. 1999; 113: 289-297.
4. Gillard M, Van Der Perren C, Moguilevsky N, et al. Mol. Pharmacol. 2002; 61: 391-399.
5. Shimamura T, Shiroishi M, Weyand S, et al. Nature 2011; 475: 65-70.
6. Liu W, Chun E, Thompson AA, et al. Science 2012; 337: 232-236.
7. Katritch V, Fenalti G, Abola EE, et al. Trends. Biochem. Sci. 2014; 39: 233-244.
8. Hishinuma S, Kosaka K, Akatsu C, et al. Biochem. Pharmacol. 2017; 128: 46-54.
9. Hishinuma S, Sugawara K, Uesawa Y, et al. Biochem. Pharmacol. 2014: 91, 231-241.

The role of neuromedin-U for mast cells activation.

Yoshimi Matsuo[1], Yuhki Yanase[1], Satoshi Tanaka[2], Kazuyuki Furuta[2], Michihiro Hide[1]

[1] Department of Dermatology, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan.

[2] Department of Immunobiology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Mast cells play critical roles for innate and acquired immune responses by release of chemical mediators, such as histamine and lipid mediators, in response to various stimuli through IgE receptor-dependent or -independent pathways. Recent reports suggest that a neuropeptide, neuromedin-U (NMU), induces an early inflammatory response by directly activating mast cells in mouse.[1)] However, detailed mechanism of NMU-induced mast cells activation has not been clarified. In this study, we investigated the effects of NMU on the activation of mouse bone marrow-derived mast cells (BMMCs), connective-tissue type mouse mast cells differentiated from BMMCs (CTMCs), and human skin derived mast cells (hsMCs) *in vitro*. NMU induced the degranulation of CTMCs and hsMCs, but not BMMCs, in a concentration-dependent manner. Moreover, the degranulation of CTMCs and hsMCs was clearly inhibited by the treatment with pertussis toxin, suggesting that NMU activates mast cells via Gi /o protein-coupled receptor. Although no or only slight NMU receptors (NMUR1) were expressed in CTMCs and hsMCs, MAS-related G protein coupled receptor-X2 (MRGPRX2) and MrgprB2, mouse analog of MRGPRX2 were highly expressed in hsMCs and in CTMCs, respectively, but not in BMMCs at mRNA level. Moreover, we confirmed that NMU directly bound and activated MRGPRX2 by means of TGFα shedding assay. We also detected expression of NMU in human epidermis and keratinocytes isolated from human skin. These results suggest that NMU, presumably released from keratinocytes, may activate skin mast cells via MRGPRX2. Development of specific antagonist of MRGPRX2 may be a potent therapeutic tool to regulate the activation of mast cells.

1) Moriyama M, Sato T, Inoue H, et al. JEM. 2005; 202: 217–224.

World Histamine Symposium 2018 in Kobe

President of JHRS　Hiroyuki Fukui

Department of Molecular Studies for Incurable Diseases, Tokushima University Graduate School of Biomedical Sciences, Fujii Memorial Institute of Medical Sciences Rm #306, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan

I hope all of you are fine, and everything is going well with you. I would like to announce World Histamine Symposium 2018 in Kobe, WHS2018 Kobe,held from the 7th (Saturday) to the 9th (Monday) in coming July at Kobe Chamber of Commerce and Industry Hall. WHS2018 will be held as a satellite meeting of 18th World Congress of Basic and Clinical Pharmacology, WCP2018 Kyoto, at Kyoto International Conference Center from July 1 to 6.

Two international histamine meetings were held in Okayama in 1981**,** and in Sendai, in 2000, organized by Prof. Kenji Tasaka and Prof. Takehiko Watanabe, respectively. Eighteen years have been passed after the last meeting. WCP2018 Kyoto will be held in this July. Then It is thought a good chance to have the third international histamine meeting in Japan. WHS2018 will also be arranged as the Second Joint Meeting of European and Japanese Histamine Research Societies. Many European Histamine Research Society members will come to Japan for the joint meeting in Kobe.

Histamine has been elucidated to play an important roles in allergy, secretion of gastric juice, neuronal functions, and immunological functions. Histamine is closely related to diseases, and it is expected to elucidate more important pathological mechanisms of histamine. Then the theme of WHS2018 is, “Expanding Histamine Research”, and the sub-theme is, “Towards novel therapeutics for histamine-related diseases”. Hopefully, WHS2018 Kobe will be the base of amplification of histamine research.

The venue of WHS2018 Kobe is Kobe Chamber of Commerce and Industry Hall. The Hall is located south to San-no-miya Station, the center of Kobe. It takes 10 minutes by Port-liner train to reach “Shimin-hiroba” station from “San-no miya” station. The venue is 5 minutes walking distance form “Shimin-hiroba” station. The address of venue is 6-1 Minatojima-Nakamachi, Chuo-ku, Kobe, 650-8543 Japan. Please refer the location using Google map, “https://www.google.co.jp/maps/@34.707751,135.228996,12z?hl=ja”.

Excurtion to Himeji Castle, the most beautiful national treasure castle of Japan is scheduled in the afternoon of July 8, Sunday. The farewell party will be planned in the evening of July 9.

I sincerely and eagerly hope every participant will enjoy WHS2018 Kobe.

The First Joint Meeting of the European and Japanese Histamine Research Societies
May 11–14, 2017, Amsterdam, The Netherlands

就実大学薬学部　見尾光庸

2017 年のヨーロッパヒスタミン学会（EHRS）の年会は、初めての試みとして、日本ヒスタミン学会（JHRS）とのジョイントミーティングとして、アムステルダム自由大学の Rob Leurs 教授のお世話で開催されました。共同開催にあたり、JHRS 側は JHRS 会長である福井裕行先生が代表を務められました。私は日本側事務局として関わらせていただきました。

共同開催に至るまでに、2016 年にフィレンツェで開催された EHRS の年会での打ち合わせに始まり、EHRS 会長の Paul Chazot 先生や Leurs 先生、JHRS の福井先生との度重なるメールの交換、JHRS 会員の先生方への情報提供などを行いました。

学会会場はアムステルダム自由大学の O|2 Lab Building という近代的な建物でした。

5 月 11 日のオープニングセッションでは、Leurs 先生、Henk Timmerman 先生、福井先生、Chazot 先生の挨拶がありました。福井先生はその中で、日本のヒスタミン研究ならびに JHRS の歴史についてもご紹介されました。引き続き、福井先生に対する EHRS 名誉会員授与のセレモニーが行われました。EHRS の名誉会員は福井先生で 15 人目、日本人としては渡邉建彦先生に続いて二人目だそうです。その後、谷内一彦先生による G.B. West Lecture が行われました。ご承知の通り、G.B. West は肥満細胞の中にヒスタミンが存在することを初めて明らかにした人で、EHRS の創立者であり、その名を冠した講演を依頼されるということは、ヒスタミンの世界での第一人者であることの証左と言えましょう。

この後、シンポジウム、一般口演、ポスター発表の口頭説明が３日間にわたって行われ、白熱した議論が展開されました。長くなってもいけませんが、記録のため、日本からの発表者のお名前だけ、プログラムの順番に従って書いておきたいと思います：谷内先生、服部裕一先生、田中智之先生、福井先生、森山芳則先生、梅原隼人先生、井浪義博先生、森山理美先生、中村正帆先生、見尾。

EHRS といえば、学術的なセッションだけでなく、供される食事やエクスカーションも大きな楽しみなわけですが、今回もしっかりと楽しませてもらいました。初日の夕食は、フィンガーフードとオランダのビールを片手にポスターセッション、その後、インドネシア料理。２日目のセッションが終わってからは、船の中でディナーをとりながらアムステルダムの運河めぐり、３日目の夜はベルギー料理のレストランでフェアウェルパーティーが行われました。フェアウェルパーティーでは、YIA の表彰式と優秀ポスターの表彰式があり、YIA のプレゼンター

フェアウェルパーティーの優秀ポスター発表表彰式
左から Paul Chazot, Arianna Rosa, 森山理美, Guadalupe-Elide Morales-Figueroa, Anne Hall, 見尾

を谷内先生がなさいました。

実は、アムステルダムに出発する２週間ぐらい前だったと思いますが、EHRS 会長の Chazot 先生から、ポスター発表の審査員を依頼するという旨のメールが届きました。ポスター発表者の２分間の口頭発表やビール片手のポスタービューイングの時間も、緊張して（？）臨むことができたかもしれません。最終日のお昼過ぎに、審査を担当した Arianna Rosa 先生、Vanina Medina 先生と私が会長の Chazot 先生を交えて、それぞれの審査結果をもとに、各自の評価のポイントや他の審査員の評価に対する疑問点の指摘などの意見交換を、おそらく小１時間はしたと思います。最終的に表彰する３名が決まった時に、Chazot 先生から、プレゼンターをするよう指名され、フェアウェルパーティーの盛り上がりの中、私も３人の表彰をすることになりました（Medina 先生は先に帰られたので、Rosa 先生にも前に来てもらいました）。

ハーグのマウリッツハイス美術館
真珠の耳飾りの少女に会いに行きました。

オランダという国は、レンブラント、フェルメール、ゴッホなど、美術史の上で重要な画家が多数生まれた国だと思います。学会前日の到着から飛行機に乗る日まで、限られた時間ではありましたが、アムステルダム国立美術館やゴッホミュージアム、マウリッツハイス美術館を訪れることができたのも、また楽しい思い出となりました。