The involvement of central nervous system histamine receptors in psychological stress-induced exacerbation of allergic airway inflammation in mice

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Abstract

Background: Psychological stress is one of the major risk factors for asthma exacerbation. Although histamine in the brain acts as an excitatory and inhibitory neurotransmitter associated with psychological stress, the contribution of brain histamine to psychological stress-induced exacerbation of asthma remains unclear. The objective of this study was to investigate the role of histamine receptors in the CNS on stress induced asthma aggravation.

Methods: We monitored the numbers of inflammatory cells and interleukin (IL)-13 levels in bronchoalveolar lavage fluid, airway responsiveness to inhaled methacholine, mucus secretion in airway epithelial cells, and antigen-specific IgE contents in sera in a murine model of stress-induced asthma treated with epinastine (an H1R antagonist), thioperamide (an H3/4R antagonist), or solvent.

Results: All indicators of stress-induced asthma exacerbation were significantly reduced in stressed mice treated with epinastine compared with those treated with solvent, whereas treatment with thioperamide did not reduce the numbers of inflammatory cells in the stressed mice.

Conclusions: These results suggest that H1R, but not H3/4R, may be involved in stress-induced asthma exacerbations in the central nervous system.

Introduction

Asthma is characterized by chronic airway inflammation in response to various types of inherited and environmental factors, which leads to wheezing, coughing, tightness in the chest, and shortness of breath. Increasing evidence has indicated that asthma is not a disease but a syndrome with heterogeneous pathobiology, clinical course, and therapeutic response to medicines. Therefore, asthma phenotypes based on pathological and clinical features...
such as types of airway inflammation and risk factors for exacerbations have been proposed to identify more effective asthma therapies specific for each phenotype.1

In asthma pathophysiology, type 2 T-helper (Th2) cytokines, such as interleukin (IL)-4, IL-5, and IL-13, play pivotal roles in regulating the behavior of inflammatory cells; inducing B cell isotype switching to produce IgE; and promoting the accumulation, activation, and prolonged survival of eosinophils. These events initiate and maintain the cardinal features of asthma, such as asthmatic airway inflammation, thereby accelerating airway responsiveness and epithelial mucus secretion.2

Psychological and psychosocial stressors have been long recognized as important universal risk factors for asthma exacerbations,3–9 which are accompanied by aggravation of airway inflammation attributable to further skewing towards a Th2-dominant cytokine profile.10–14 The cognitive processes accompanying psychological stress are deeply connected to asthma exacerbations. Activation of the anterior cingulate cortex and insula by psychological stimuli exhibited a greater decrease in forced expiratory volume in 1 s (FEV1), increased recruitment of eosinophils, and diminished glucocorticoid inhibition of TNF-α production.15 The intensity of activation in the anterior insula in response to asthma-relevant psychological stimuli is correlated with the severity of airway inflammation evoked by allergen inhalation.16 Stress-induced brain activation results in the production and release of stress neuropeptides, such as opioid peptides, substance P, and histamine, which bind to membrane receptors in the central nervous system (CNS).17,18 However, the molecular mechanism linking psychological stress perceived in the CNS to exacerbations of asthma pathophysiology in airways is not yet fully understood.

Histamine receptors (HRs) are a group of seven-transmembrane G protein-coupled receptors classified to four major subtypes (H1R, H2R, H3R, and H4R), which are expressed throughout the body including in immune cells, gastric mucosal cells, and neurons.19 Histamine in the brain is produced by histaminergic neurons comprising the tuberomammillary nucleus in the posterior hypothalamus. Projections from the tuberomammillary nucleus extend to almost all areas in the brain and are implicated in basic homeostatic and higher brain functions such as arousal, cognition, nociception, and responses to perceived stress.19–21

In the present study, we aimed to determine the role of HRs in the CNS in psychological stress-induced asthma exacerbations using a murine model of stress asthma. We found that H3Rs in the CNS were required for exacerbation of asthmatic airway responses induced by the stress exposure. In contrast, H4Rs might be irrelevant in this process or function in the negative feedback control of stressor signals in asthma exacerbation.

Methods

Mice and ethical statement

Specific pathogen-free female C57BL/6 mice were purchased from CLEA Japan (Osaka, Japan). All mice were kept under specific pathogen-free conditions at the Institute for Animal Experimentation, Tohoku Pharmaceutical University, Sendai, Japan. Mice were housed under a 12 h/12 h light/dark cycle at a constant temperature (22 ± 2 °C). Sterilized food and water were available ad libitum. All experimental procedures involving animals were approved by the Committee of Animal Experiments at Tohoku Pharmaceutical University (approval numbers: 13001-cn-a, 14002-cn, and 15001-cn). We took the utmost care to alleviate any pain and suffering on the part of the mice.

Sensitization, antigen challenge, and stress exposure

A schematic of the experimental protocol is shown in Figure 1. Six- to eight-week-old mice were sensitized and made to inhale an aerosolized antigen as previously described.22 Briefly, mice were sensitized by intraperitoneal injections of 8 μg ovalbumin (OVA; Grade V, Sigma–Aldrich, St. Louis, MO, USA) adsorbed with aluminum hydroxide (Wako Pure Chemical Industries, Osaka, Japan) on days 0 and 5. On days 17 and 24, the mice were challenged with aerosolized OVA (0.5% in saline) for 1 h. Psychological stressors were applied on the following schedule to avoid habituation to psychological stress: 6 h restraint per day from day 17 to day 19, and 3 min forced swim per day from day 20 to day 23. For restraint stress (RS), each mouse was placed in a 50-mL conical centrifuge tube with multiple ventilation holes. For forced swimming stress (FSS), the mice were placed in a plastic tank (19 cm in diameter, 27 cm in height) containing 15 cm water at 32 °C. Non-stressed mice were deprived of food and water, which has been used as a non-stress condition in other rodent experiments that investigated the effect of stress.23,24 RS and FSS are category D procedures, as are other types of psychological stress, such as electric foot shock stress, sound stress, and communication box-induced stress, that have been used to investigate the exacerbation of stress-induced allergic airway inflammation by many groups including us. Category D procedures are used in these studies because exposure to stress is required to induce asthma exacerbation, and alternative methods for reducing stress fail to exacerbate the condition.

To test the effects of HR antagonists, 2 μL epinastine as a selective H1R antagonist (Tokyo Chemical Industry, Tokyo, Japan),25 ranitidine as a selective H2R antagonist (Sigma–Aldrich, St. Louis, MO, USA),25 or thioperamide as a selective H3R antagonist (Sigma-Aldrich)26,27 in calcium- and magnesium-free phosphate-buffered saline (PBS) was intracerebroventricularly administered to selectively block HRs in the CNS 1 h before each stress exposure at the dose indicated. Solvent was used as a negative control. Mice were placed in a stereotactic device under anesthesia induced by inhalation of 2% isoflurane in oxygen (DS Pharma Animal Health, Tokyo, Japan). A 25G microinjection needle tip was aligned with the bregma and then inserted into the ventricle using the following coordinates from bregma: 1.0 mm anterior and 1.0 mm lateral. Intracerebroventricular injection has been used generally to selectively administer agents to the CNS.22 In our preliminary experiments, the intracerebroventricularly injected solution was not detected outside the CNS within 1 h for volumes below 5 μL. The doses of HR antagonists used herein were based on those used in previous studies.28,29 At the end of the experiments, the mice were euthanized by an overdose of pentobarbital (Somnopentyl; Kyoritsu Seiyaku, Tokyo, Japan).

Preparation of bronchoalveolar lavage (BAL) fluids

BAL samples were collected with 2 × 0.25 mL chilled PBS through a cannula inserted in the trachea. Total cell numbers recovered from BAL fluid were counted with a hemocytometer. After centrifugation of the BAL fluids, supernatants were stored at −80 °C for cytokine assay. For each sample, 2 × 10⁶ cells were centrifuged onto a glass slide using a Cytospin 4 (Thermo Scientific, Waltham, MA, USA) and stained with Diff-Quick solution (International Reagents, Kobe, Japan). The fractions of leukocytes were analyzed by counting a minimum of 200 cells under a light microscope.
Measurement of airway responsiveness

Lung resistance at the baseline and in response to aerosolized saline and acetyl-β-methylcholine (methacholine; Sigma–Aldrich) were measured for 3 min under each condition. To measure airway sensitivity, the concentration of acetyl-β-methylcholine needed to increase lung resistance to 200% above baseline (PC200) was calculated by interpolating the log concentration-lung resistance curve from individual mice.

Lung histology

Lungs were isolated from mice 3 days after the last OVA challenge, fixed in 10% buffer formalin, dehydrated, and embedded in paraffin. Sections were cut at 4-μm-thick and stained with periodic acid–Schiff (PAS). Mucin production was estimated as the proportion of PAS-positive cells in the total airway epithelium of bronchioles in the PAS-stained section. The percentage of PAS-positive cells was calculated in each of the five random bronchioles in three lung sections from each mouse.

Measurement of OVA-specific IgE and cytokine

Serum samples were collected 3 days after the last OVA challenge. The quantities of OVA-specific IgE in serum were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates (Nunc A/S, Roskilde, Denmark) were coated with 10 μg/mL OVA in bicarbonate buffer for 1 h at 37 °C and then blocked with 1% bovine serum albumin (BSA) in PBS at 4 °C overnight. Prior to testing, serum samples were diluted with 1% BSA/PBS. Horseradish peroxidase-conjugated goat anti-mouse IgE antibodies (Bethyl Laboratories, Montgomery, TX, USA) diluted 1:2500 were used for detection. IgE concentrations were determined by interpolating the log concentration-OD absorbance at 450 nm using that at 650 nm for reference and calculated based on a reference standard of pooled sera from sensitized female mice. The concentrations of IL-13 in BAL fluid were measured using an ELISA kit (eBioscience, San Diego, CA, USA) according to the manufacturer’s instructions. The limit of detection was 1.5 pg/mL.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). Differences between the two groups were tested using two-tailed analysis in an unpaired Student’s t-test. Differences among four or more groups were tested using ANOVA with post hoc analysis (Tukey’s multiple comparison test). A p-value less than 0.05 was considered significant. Data are expressed as the mean ± standard error of the mean (SEM).

Results

Effects of HR antagonists on stress-induced exacerbations of airway inflammation

The numbers of total cells, eosinophils, and lymphocytes in BAL fluid were significantly increased by the stress exposure but showed a dose-dependent reduction with epinastine treatment (Fig. 2). Treatment with 3.0 × 10⁻⁸ mol epinastine significantly reduced the numbers of inflammatory cells in stressed mice to the levels observed in non-stressed mice treated with the same dose of epinastine and tended to reduce the numbers of total cells, eosinophils, and lymphocytes in BAL fluids of non-stressed mice compared with solvent treatment, although the difference was not statistically significant (Fig. 2). Intracerebroventricular administration of 3.0 × 10⁻⁸ mol ranitidine significantly reduced the increased numbers of total cells, eosinophils, and lymphocytes in BAL fluids of stressed mice, whereas treatment with a higher dose (3.0 × 10⁻⁸ mol) of ranitidine did not reduce the stress-induced increases (Supplementary Fig. 1). The effects of ranitidine in stressed mice were not dose-dependent but reverse bell-shaped. The maximal suppressive effect was observed at 3.0 × 10⁻⁸ mol. In contrast, the effects of ranitidine in non-stressed mice seemed to be dose-dependent but were not statistically significant (Supplementary Fig. 1). Treatment with thioperamide, an H2/3 antagonist, did not significantly reduce the stress-induced increased numbers of inflammatory cells in BAL fluid (Fig. 3), even at a higher dosage (4.9 × 10⁻⁸ mol; data not shown).

Effects of an H₄R antagonist, epinastine, on stress-induced exacerbations of asthmatic airway responses

Lung resistance increased dose-dependently in response to inhaled methacholine in all groups of mice. The lung resistance to 200% above baseline (PC200) with solvent treatment tended to be lower in stressed mice than in non-stressed mice, although the difference was not significant (Fig. 4). In the stressed mice, epinastine treatment significantly increased the PC200 to a level similar to that in the non-stressed mice treated with epinastine. However, in the non-stressed mice, no significant difference in PC200 was observed between solvent and epinastine treatments.

Effects of epinastine on stress-induced exacerbations of airway mucus secretion

The number of PAS-positive cells under solvent treatment was significantly higher in the stressed mice than in the non-stressed mice (Fig. 5). In the stressed mice, treatment with epinastine significantly reduced the number of PAS-positive cells to the level...
observed for non-stressed mice treated with epinastine. However, in the non-stressed mice, no significant difference in cell number observed between solvent and epinastine treatments.

**Effects of epinastine on the stress-induced increase of IL-13 in BAL fluid**

The IL-13 contents in BAL fluids under solvent treatment were significantly higher in the stressed mice than in the non-stressed mice (Fig. 6). In the stressed mice, treatment with epinastine significantly reduced the IL-13 contents to the levels measured for non-stressed mice treated with epinastine. In the non-stressed mice, there was no significant difference in IL-13 contents between solvent and epinastine treatments.

**Effects of epinastine on the stress-induced increase of OVA-specific IgE in sera**

OVA-specific IgE concentrations in sera under solvent treatment tended to be higher in the stressed mice than in the non-stressed mice, although the difference was not statistically significant (Fig. 7). In the stressed mice, treatment with epinastine significantly decreased IgE concentrations to the levels of those in the non-stressed mice treated with epinastine. In the non-stressed
mice, epinastine treatment yielded no significant difference in IgE concentrations compared with solvent treatment.

Discussion

To our knowledge, this is the first report demonstrating that HRs in the CNS are involved in both the development and psychological stress-induced exacerbations of allergic asthma. Histamine is a neurotransmitter whose release is upregulated in response to perceived stress. Ito et al. showed that chronic RS increased histamine turnover in the nucleus accumbens and striatum in Fisher rats. In addition, Gadek-Michalska et al. showed that intra-cerebroventricular administration of an H1R agonist in rats increased the levels of corticosterone, one of the stress hormones released in the neuroendocrine response to psychological stress. The increase of serum corticosterone after stress exposure was previously observed in this model of stress asthma. Therefore, it is likely that histamine was released upon stress exposure in the present study.

Epinastine seemed to increase the PC200 (Fig. 4), and reduce the eosinophil counts (Fig. 2), the IL-13 contents in BAL fluid (Fig. 6) and the OVA-specific IgE levels in sera (Fig. 7) of non-stressed mice compared with solvent treatment, although the differences were

![Fig. 4. Effects of epinastine on methacholine-induced airway hyperresponsiveness. Mice were sensitized and challenged with ovalbumin (OVA) in the absence (open bars) or presence (closed bars) of psychological stress. Solvent or 3.0 × 10⁻⁹ mol epinastine was administered intracerebroventriculatly before each stress exposure. Airway sensitivity was measured 1 day after the last OVA challenge. Each group consisted of six to eight mice from two independent experiments. Data are shown as the mean ± SEM. #p < 0.05 compared with the stressed mice treated with solvent using an unpaired Student's t-test.](image)

![Fig. 5. Effects of epinastine on airway mucus secretion. Mice were sensitized and challenged with ovalbumin (OVA) in the absence (open bars) or presence (closed bars) of psychological stress. Solvent or 3.0 × 10⁻⁹ mol epinastine was administered intracerebroventriculatly before each stress exposure. Lungs were isolated from mice 3 days after the last OVA challenge. Lung sections were prepared and stained with periodic acid Schiff (PAS). (A) Representative images of lung sections from the non-stressed and stressed mice treated with solvent or epinastine (scale bar, 20 μm). (B) The percentage of PAS-positive cells was calculated in each of five random bronchioles in three lung sections from each mouse. Each group consisted of 8–12 mice from two independent experiments. Data are shown as the mean ± SEM. *p < 0.05 compared with the non-stressed mice treated with solvent; #p < 0.05 compared with the stressed mice treated with solvent using an unpaired Student’s t-test.](image)

![Fig. 6. Effects of epinastine on interleukin (IL)-13 contents in bronchoalveolar lavage (BAL) fluid. Mice were sensitized and challenged with ovalbumin (OVA) in the absence (open bars) or presence (closed bars) of psychological stress. Solvent or 3.0 × 10⁻⁹ mol epinastine was administered intracerebroventriculatly before each stress exposure. BAL samples were collected on day 3 after the last OVA challenge. The concentration of IL-13 in BAL fluids was measured by enzyme-linked immunosorbent assay. Each group consisted of 4–10 mice from two independent experiments. Data are shown as the mean ± SEM. *p < 0.05 compared with the non-stressed mice treated with solvent; #p < 0.05 compared with the stressed mice treated with solvent using an unpaired Student’s t-test.](image)

![Fig. 7. Effects of epinastine on ovalbumin (OVA)-specific IgE production in serum. Mice were sensitized and challenged with OVA in the absence (open bars) or presence (closed bars) of psychological stress. Solvent or 3.0 × 10⁻⁹ mol epinastine was administered intracerebroventriculatly before each stress exposure. Serum samples were collected on day 3 after the last OVA challenge. The concentrations of OVA-specific IgE in sera were measured by enzyme-linked immunosorbent assay. Each group consisted of 8–12 mice from two independent experiments. Data are shown as the mean ± SEM. #p < 0.05 compared with the stressed mice treated with solvent using an unpaired Student’s t-test.](image)
not statistically significant. In addition, ranitidine tended to dose-dependently reduce the eosinophil counts in non-stressed mice compared with solvent treatment. Previous studies demonstrated that H$_2$Rs mediated the excitatory CNS functions of brain histamine such as maintenance of wakefulness, acceleration of locomotor activity, enhancement of learning and memory processes, and increased pain perception, and H$_2$Rs are thought to play similar roles. Therefore, histamine physiologically released in the CNS might be partially involved in the development of allergic airway responses through the activation of H$_1$Rs and H$_2$Rs, which could explain the (non-significant) epinastine- and ranitidine-mediated decreases in the development of allergic asthma under stress-free conditions. However, we cannot rule out the possibility that the procedure for intracerebroventricular administration of solvent could induce stress via HR activation in the non-stressed mice. In our previous study investigating the effect of a glucocorticoid receptor antagonist using the same stress model, subcutaneous administration of the solvent tended to aggravate allergic airway responses in non-stressed mice compared to untreated non-stressed mice (unpublished data). Thus, epinastine and ranitidine might attenuate the aggravation of asthma-related features caused by stress from handling in non-stressed mice.

Epinastine (3.0 × 10$^{-9}$ mol) and ranitidine (3.0 × 10$^{-9}$ mol) significantly attenuated the stress-enhanced enhancement of asthmatic airway responses and Th2 immune responses to the levels of non-stressed mice treated with the antagonists (Supplementary Fig. 1, Fig. 2A, 4, 5, 6, 7). These results suggest that the stress exposure exaggerated asthmatic airway responses by increasing histamine release, leading to the further activation of H$_1$Rs and H$_2$Rs in the CNS.

On the other hand, the intracerebroventricular administration of an H$_3$R antagonist, thioperamide, exhibited no significant effect on the airway inflammation at the same dose (Fig. 3) or at a higher dose (4.9 × 10$^{-8}$ mol; data not shown). However, within the group receiving thioperamide administration, a bell-shaped enhancement effect was observed, and the maximal effect was observed at 0.3 × 10$^{-9}$ mol. H$_3$Rs act as inhibitory auto- and hetero-receptors in the CNS, which inhibit the synthesis and release of histamine, and a number of neurotransmitters, including acetylcholine, dopamine, noradrenaline, and serotonin, in a pathway-dependent manner, leading to suppression of convulsion, stress-induced excitation, and feeding behavior. The structure–activity relationships of H$_3$R and H$_4$R overlap as G$	ext{pp}_i$ protein-coupled receptors. Therefore, the activation of H$_3$Rs and H$_4$Rs by the stress exposure might play a suppressive role in stress-induced asthma exacerbations.

Taken together, our findings and the physiological properties of each HR subtype suggest that the interaction of histamine with HR subtypes playing distinctive roles in the CNS is associated with the severity of allergic airway responses, particularly in the case of psychological stress-induced exacerbations. Further studies will be required to identify the exact roles of each HR subtype in the CNS and to elucidate how the stressor signal via HRs in the CNS modulates Th2 immune responses in asthmatic pathophysiology. The present study presents a new insight into the global role of the histamine-regulated CNS in allergic asthma, which might lead to the development of more effective strategies for the management of asthmatic patients with the stress-related asthma phenotype.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alit.2016.05.015.

Conflict of interest

The authors have no conflict of interest to declare.

Authors’ contributions

Conceived and designed the experiments: IO, TM; performed the experiments: TM, KO; analyzed the data: TM, IO, TK; KSC; contributed reagents/materials/analysis tools: IO, YO, HM, SS, MT; contributed to the writing of the manuscript: TM, IO, HM.

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