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Original article

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

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Abbreviations:
IL, interleukin; HR, histamine receptor; Th2, type 2 T-helper; Th1, type 1 T-helper; FEV_1, forced expiratory volume in 1 s; TNF-α, tumor necrosis factor-alpha; CNS, central nervous system; OVA, ovalbumin; RS, restraint stress; FSS, forced swimming stress; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; PAS, periodic acid–Schiff; PC_{200}, lung resistance to 200% above baseline; SEM, standard error of the mean; HPA, hypothalamic-pituitary-adrenal; CRH, corticotropin-releasing hormone

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 H_1R antagonist), thioperamide (an $H_{3/4}R$ 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 H_1R, but not $H_{3/4}R$, 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

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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 (FEV_1), 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 (H_1R, H_2R, H_3R, and H_4R), 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 H_1Rs in the CNS were required for exacerbation of asthmatic airway responses induced by the stress exposure. In contrast, $H_{3/4}Rs$ 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 H_1R antagonist (Tokyo Chemical Industry, Tokyo, Japan),[25] ranitidine as a selective H_2R antagonist (Sigma–Aldrich, St. Louis, MO, USA),[25] or thioperamide as a selective $H_{3/4}R$ 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, 2×10^5 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.