

Nasal Allergic Response Mediated by Histamine H₃ Receptors in Murine Allergic Rhinitis

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Background: Histamine is one of the most important chemical mediators causing nasal allergic symptoms, and H₁ receptor antagonist have been used as the treatment first choice in nasal allergy. The presence of H₃ receptors has also been determined in the human nasal mucosa, but few studies have investigated the involvement of H₃ receptors in nasal allergy. **Objective:** We used a murine allergic model to investigate the presence of nasal mucosa H₃ receptor mRNA and any H₃ receptor agonist or antagonist influences on clinical nasal allergic symptoms. **Methods:** H₃ receptor mRNA in nasal mucosa was investigated by reverse-transcription polymerase chain reaction. OVA-sensitized mice were given an intraperitoneal injection of H₃ receptor agonist or antagonist, and clinical nasal allergic symptoms were scored over 10 minutes after nasal provocation of OVA. Inhibition of nasal allergic symptoms was also examined using an H₁ receptor antagonist alone and using a both an H₃ receptor agonist and an H₁ receptor antagonist. **Results:** H₃ receptor mRNA was identified in the murine nasal mucosa. The H₃ receptor agonist (R)- α -methylhistamine significantly inhibited clinical nasal allergic symptoms of OVA-sensitized mice. The H₃ receptor agonist and H₁ receptor antagonist inhibited clinical nasal allergic symptoms in the murine allergic model more strongly than the single drug. **Conclusion:** The foregoing results indicate that H₃ receptors are involved in modulation of nasal allergy. H₃ receptor agonists can also be useful as a novel therapeutic approach in nasal allergy. Both H₃ receptor agonist and H₁ receptor antagonist may be more effective than a single drug. **Key Words:** Histamine H₃ receptor, murine model, allergic rhinitis, H₃ agonist, H₃ antagonist, (R)- α -methylhistamine, thioperamide, PCR.

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INTRODUCTION

Histamine functions as a chemical messenger and an aminergic neurotransmitter and is one of the most important mediators of allergy and inflammation. It plays an important role in a great number of physiologic processes in central and peripheral tissues. Histamine plays an important role in eliciting nasal symptoms of allergic rhinitis such as sneezing, rhinorrhea, and nasal obstruction.¹ Nasal provocation by histamine induces sneezing, rhinorrhea, and nasal congestion within minutes.¹ Histamine H₁ receptor antagonists are used widely in the treatment of allergic nasal disease. Such agents alleviate sneezing and hypersecretion but have little effect on nasal obstruction.

There are three recognized subtypes of histamine receptors (H₁, H₂, H₃),^{2,3} and recently, a novel histamine receptor gene (H₄) has been identified.⁴ The Histamine H₃ receptor has been identified as a presynaptic autoreceptor on histamine-containing neurons in the brain, regulating the synthesis and release of histamine by a feedback mechanism.² Arrang et al.³ also studied the in vivo effects of the H₃ receptor agonist (R)- α -methylhistamine and H₃ receptors antagonist thioperamide in the rat lung, abdominal skin, and spleen, demonstrating that (R)- α -methylhistamine modified the synthesis and release of histamine.

Recently, H₃ receptors have been found to have a widespread distribution in animal and human peripheral tissues.^{5–8} We therefore used an immunohistochemical method to study the localization of histamine receptor subtypes in human nasal mucosa and reported that H₃ receptors were localized on the epithelium and H₁ receptor-positive nerves in human nasal mucosa.⁹ This result indicates that H₁ receptors and H₃ receptors adjoin and regulate each other in the same nerves. But there is no study regarding the presence of H₃ receptor mRNA in the nasal mucosa.

Sugimoto¹⁰ reported that H₃ receptors were involved in modulation of dermal pruritus, but as yet, there is very little information on the role of H₃ receptors in peripheral tissue. There are also no reports concerning the role of H₃ receptor in nasal allergy.

In the present study, we used a murine allergic model to investigate the presence of H₃ receptor mRNA in murine nasal mucosa and any H₃ receptor agonist or antag-

onist influences on clinical nasal allergic symptoms. We also studied the influence of an H₃ receptor agonist and an H₁ receptor antagonist.

MATERIALS AND METHODS

Animals and OVA Sensitization

Male 6-week-old BALB/c mice were obtained from Japan CLEA Corporation (Tokyo, Japan) and housed in an air-conditioned room maintained at 22 to 24°C and 50% to 60% humidity. Mice were immunized by intraperitoneal injection of 2 µg OVA (Worthington Biochemical Corp., Lakewood, NJ) per 2 mg of aluminum hydroxide gel on days 0 and 5, followed by 4 days daily intranasal challenge with OVA diluted in sterile normal saline (20 µL 33 mg/mL OVA solution, twice per mouse) beginning on day 11. All animal experiments conducted in this study were approved by the animal research ethics board of the University of Tokyo.

Reverse-Transcription Polymerase Chain Reaction Detection of Mouse H₃ Receptor mRNA in the Nasal Mucosa

Normal BALB/c mice and OVA-sensitized mice were killed by intraperitoneal overdose of sodium ketamine hydrochloride, and the nasal mucosa were removed. Specimens were preserved in RNA later RNA Stabilization Reagent (Qiagen, Valencia, CA) for 24 hours. Total RNA (1 µg) was extracted from the nasal mucosa using an Isogen RNA isolation kit (Nippon Gene, Tokyo, Japan). Total RNA (1 µg) was used for synthesis of a first strand of cDNA. Two primers, 5'-TGTCATGCTCGCCTTCGT-3' (coding region 161-179) and 5'-CGTCTTGTTGCCCCCTGCT-3' (coding region 434-452), were used to amplify the entire coding region of the mouse H₃ receptor cDNA. Some cDNAs from normal and OVA-sensitized BALB/c nasal mucosa were used as templates for polymerase chain reaction (PCR) amplifications. PCR reaction conditions were 34 cycles of 94°C, 30 seconds; 62°C, 30 seconds; and 72°C, 30 seconds in a Perkin-Elmer GeneAmp System 9600 apparatus (Norwalk, CT). PCR products were run in a 2% agarose gel stained with ethidium bromide (10 µg/mL) and then photographed under ultraviolet irradiation. The heart cDNAs from the both normal and OVA-sensitized mice were used as negative control. A mouse β-actin probe was amplified as positive control.

Drugs

(R)-α-methylhistamine and thioperamide were purchased from Biomol International LP (Plymouth Meeting, PA). d-chlorpheniramine maleate was purchased from Schering-Plough Corp. (Osaka, Japan). These drugs investigated were given 30 minutes before OVA treatment.

Clinical Symptoms

Mice were given an intraperitoneal injection of 200 µL saline, (R)-α-methylhistamine, d-chlorpheniramine maleate, both (R)-α-methylhistamine and d-chlorpheniramine maleate, or thioperamide. Both H₃ receptor agonist (R)-α-methylhistamine and H₁ receptor antagonist d-chlorpheniramine maleate were not mixture of them and means that injection of 200 µL d-chlorpheniramine maleate soon after injection of 200 µL (R)-α-methylhistamine. Nasal symptoms were evaluated by counting the number of sneezes and nasal itching motions (nasal rubbing) that occurred in the 10 minute interval after OVA intranasal provocation by the same dosage of OVA given during daily challenge in the 30 minutes after injection of drugs. Observations were performed with the mice in a cage (60 × 40 × 30 cm).

Eosinophil in Murine Allergic Nasal Mucosa after Drug Stimulation and Nasal Challenge with OVA

To investigate the effect of drugs ([R]-α-methylhistamine, d-chlorpheniramine maleate, or thioperamide) objectively, murine nasal mucosa were obtained 24 hours after observation of clinical symptoms. Mice were killed by intraperitoneal injection of an overdose of sodium ketamine hydrochloride. Tissue samples for histochemistry were fixed in 10% formaldehyde for 48 hours at 20°C and embedded in paraffin. The paraffin-embedded specimens were cut continuously at 4 µm thickness on a microtome and deparaffinized. Specimens were then stained with hematoxylin-eosin. Eosinophils were counted by light microscopy. The number of eosinophil was quantified per unit (1 mm²) of epithelial basement membrane to a uniform depth of submucosa by using an eyepiece reticule. The average of cells counted in at least six sections was used for data analysis.

Immunocytochemistry

Normal BALB/c mice and OVA-sensitized mice were killed by means of an intraperitoneal overdose of sodium ketamine hydrochloride and subjected to perfusion fixation with saline and 10% formaldehyde. The decapitated heads were fixed in 10% formaldehyde for 48 hours at 20°C. Specimens were then decalcified 7 days in 20% EDTA-Na and embedded in paraffin. The paraffin-embedded nasal cavity was transversely sectioned at the level of the maxillary sinus by continuous microtoming at a 4 µm thickness, and specimens were then deparaffinized.

To activate the antigenicity, all specimens were heated by autoclave for 10 minutes at 120°C in sodium citrate (0.1 mol). To suppress the endogenous peroxidase activity of erythrocytes and other inflammatory cells, all serial sections were pretreated with 100% methanol containing 3% H₂O₂. Serial sections were incubated 24 hours at 4°C with rabbit polyclonal antibodies for H₁ receptor (dilution 1:60) and H₃ receptor (dilution 1:60) (Alpha Diagnostic International, San Antonio, TX). A control was provided by omitting the primary antibody in the staining procedure and incubating serial sections with rabbit immunoglobulin G (Vector Labs, Burlingame, CA) diluted to 1:500. Sections were treated with avidin-biotinylated horseradish peroxidase complex (ABC) reagents (Vector Labs) and then stained with 0.001% 3,3'-diaminobenzidine including 0.03% H₂O₂. All sections were then mounted with Kaiser's glycerin gelatin, evaluated, and documented by light microscopy.

Statistical Analysis

All data are presented as mean ± SE. Statistical analysis was performed using Student's t test, with a critical value less than .05 considered significant.

RESULTS

Reverse-Transcription PCR Analysis of Mouse H₃ Receptor mRNA in the Nasal Mucosa

The histologic distribution of mouse H₃ receptor mRNA was analyzed by reverse-transcription PCR amplifications. As shown in Figure 1, H₃ receptor mRNA was expressed in the nasal mucosa of both normal and OVA-sensitized mice.

Inhibition of Nasal Symptoms in Murine Allergic Mode by H₃ Receptor Agonist

As shown in Figure 2, intraperitoneal injection of (R)-α-methylhistamine inhibited nasal allergic symptoms, such as sneezing and nasal rubbing, in OVA-sensitized

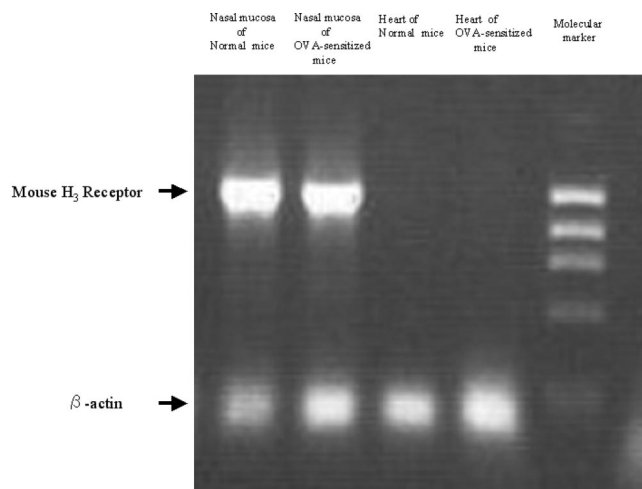


Fig. 1. PCR detection of mouse H₁ receptor mRNA and β -actin mRNA expression profile. Two primers: 5'-TGGTCATGCTCGCCTTCGT-3' and 5'-CGTCTTGTGTCCCCCTGCT-3' were used for RT-PCR. β -actin probe was amplified as positive control. H₁ receptor mRNA expressed in both normal and OVA sensitized mice nasal mucosa.

mice. After 200 μ L saline intraperitoneal injection and intranasal provocation, mean sneezing count was 25.75 ± 8.99 , and mean rubbing count was 43.50 ± 17.75 ($n = 8$). After intraperitoneal injection of 50 mg/kg (R)- α -methylhistamine and intranasal provocation, mean sneezing count was 11.25 ± 4.92 , and mean rubbing count was 24.75 ± 6.84 ($n = 8$). Mean sneezing count was significantly lower ($P < .005$) in the (R)- α -methylhistamine group (50 mg/kg) versus the saline group. Mean rubbing count was also significantly lower ($P < .05$) in the (R)- α -methylhistamine group (50 mg/kg) versus the saline group. Mean sneezing and rubbing counts in the (R)- α -methylhistamine group (25 mg/kg) were lower than the saline group, although there were not significant differences between groups.

Inhibition of Nasal Symptoms in Murine Allergic Model by H₁ Receptor Antagonist

As shown in Figure 3, intraperitoneal injection of d-chlorpheniramine maleate inhibited nasal allergic symptoms such as sneezing and nasal rubbing in OVA-sensitized mice. After intraperitoneal injection of 40 mg/kg d-chlorpheniramine maleate and intranasal provocation, mean sneezing count was 10.63 ± 5.24 , and mean rubbing count was 10.00 ± 8.94 ($n = 8$). Mean sneezing and rubbing counts were significantly lower ($P < .001$) in the d-chlorpheniramine maleate group (40 mg/kg) versus the saline group. Mean sneezing and rubbing counts in the d-chlorpheniramine maleate group (20 mg/kg) were lower than in the saline group, although there were not significant differences between groups.

Inhibition of Nasal Symptoms in Murine Allergic Model by H₃ Receptor Agonist and H₁ Receptor Antagonist

As shown in Figure 4, intraperitoneal injection of (R)- α -methylhistamine and d-chlorpheniramine maleate

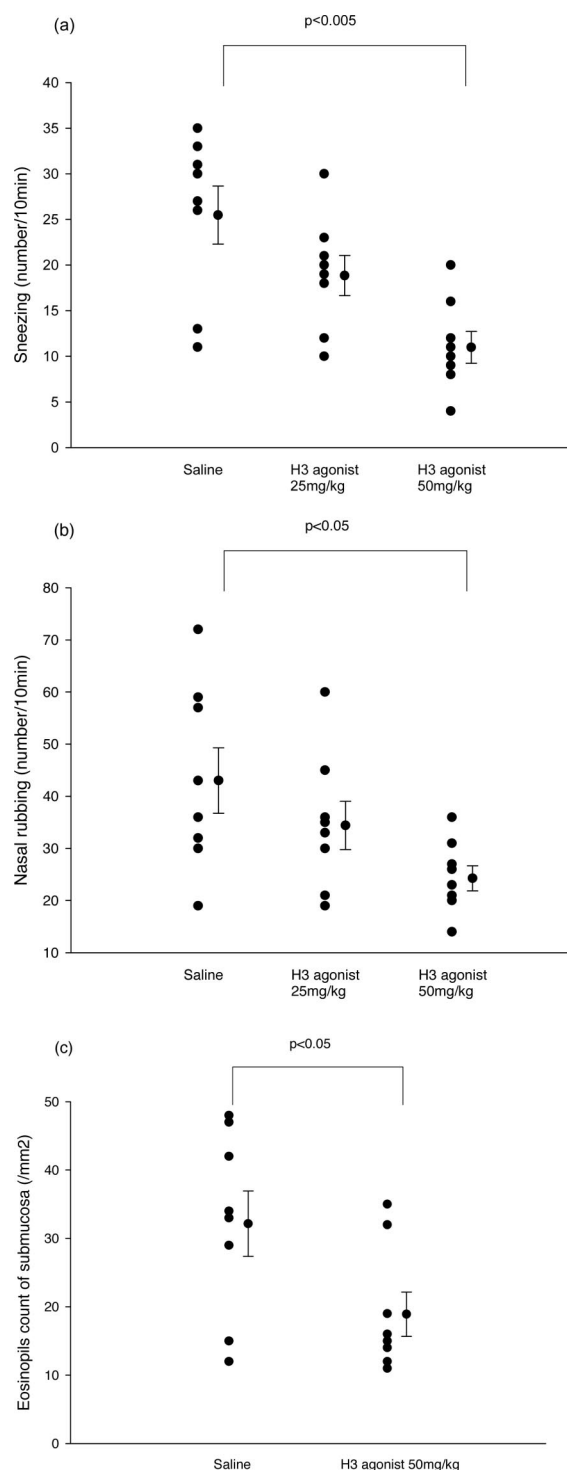


Fig. 2. Inhibition of nasal symptoms in murine allergic model by H₃ agonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or (R)-methylhistamine (25 and 50 mg/kg). The nasal symptoms of (A) sneezing and (B) nasal rubbing were counted over 10 minutes after intranasal drip of OVA. (C) Decrease of eosinophils in the submucosa of murine allergic model by H₃ agonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or (R)-methylhistamine (50 mg/kg). Eosinophils were counted after intranasal drip of OVA. Each bar represents the mean $\pm$ SE.

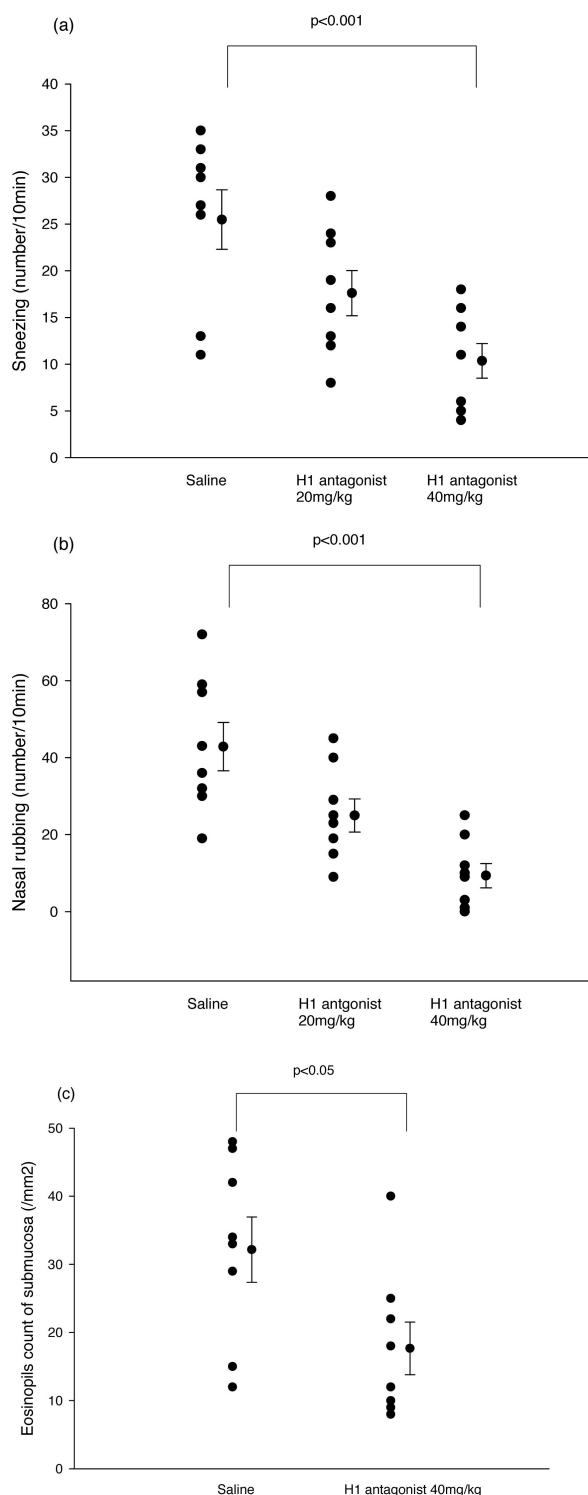


Fig. 3. Inhibition of nasal symptoms in murine allergic model by H₁ antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or d-chlorpheniramine maleate (20 and 40 mg/kg). The nasal symptoms of (A) sneezing and (B) nasal rubbing were counted over 10 minutes after intranasal drip of OVA. (C) Decrease of eosinophils in the submucosa of murine allergic model by H₁ antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or d-chlorpheniramine maleate (40 mg/kg). Eosinophils were counted after intranasal drip of OVA. Each bar represents the mean ± SE.

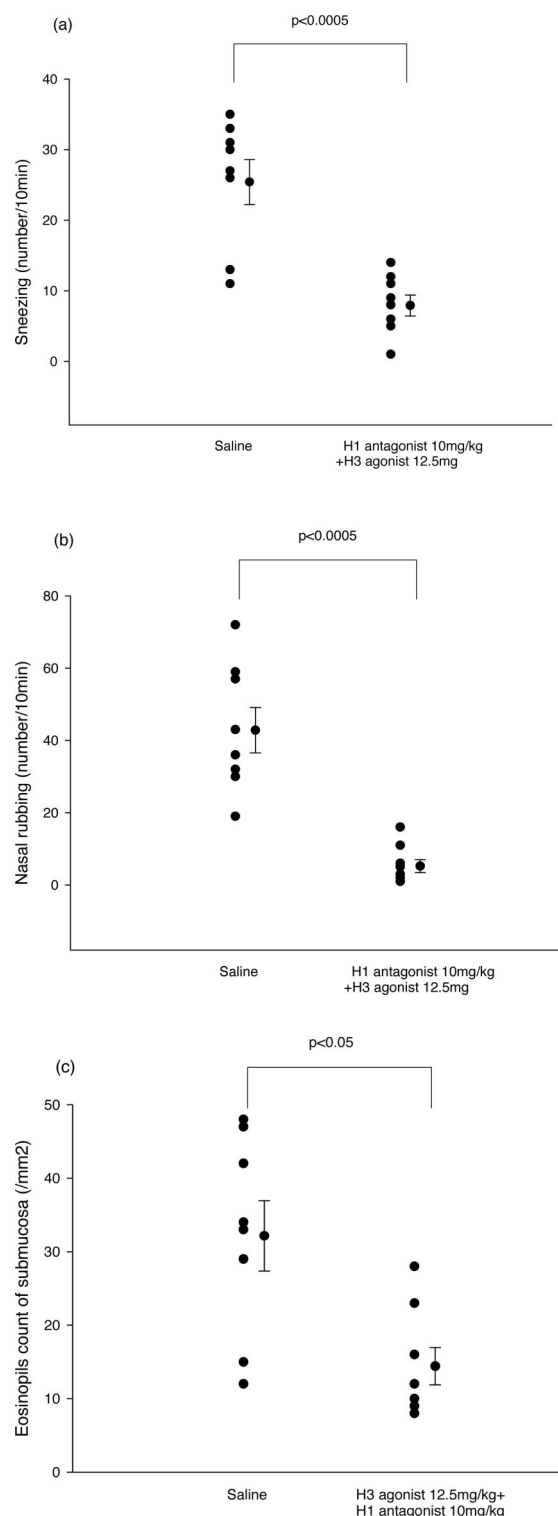


Fig. 4. Inhibition of nasal symptoms in murine allergic model by H₃ agonist and H₁ antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or (R)-methylhistamine (12.5 mg/kg) and d-chlorpheniramine maleate (10 mg/kg). The nasal symptoms of (A) sneezing and (B) nasal rubbing were counted over 10 minutes after intranasal drip of OVA. (C) Decrease of eosinophils in the submucosa of murine allergic model by H₃ agonist and H₁ antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or (R)-methylhistamine (12.5 mg/kg) and d-chlorpheniramine maleate (10 mg/kg). Eosinophils were counted after intranasal drip of OVA. Each bar represents the mean ± SE.

inhibited nasal allergic symptoms, such as sneezing and nasal rubbing, in OVA-sensitized mice. After intraperitoneal injection of 12.5 mg/kg (R)- α -methylhistamine and 10 mg/kg d-chlorpheniramine maleate and intranasal provocation, mean sneezing count was 8.25 ± 4.21 , and mean rubbing count was 5.88 ± 5.14 ($n = 8$). Mean sneezing and rubbing counts in the (R)- α -methylhistamine (12.5 mg/kg) and d-chlorpheniramine maleate (10 mg/kg) groups were significantly lower ($P < .0005$) versus the saline group. In addition, mean sneezing and rubbing counts in the (R)- α -methylhistamine (12.5 mg/kg) and d-chlorpheniramine maleate (10 mg/kg) groups were significantly lower ($P < .001$) versus the (R)- α -methylhistamine (25 mg/kg) or d-chlorpheniramine maleate (20 mg/kg) groups.

Exacerbation of Nasal Symptoms in Murine Allergic Model by H_3 Receptor Antagonist

As shown in Figure 5, the intraperitoneal injection of thioperamide exacerbated sneezing of OVA-sensitized mice. The mean numbers of sneezing were 39.88 ± 11.58 , and the mean times of rubbing were 49.13 ± 18.77 by intranasal provocation after 50 mg/kg of thioperamide intraperitoneal injections ($n = 8$). The mean numbers of sneezing in the thioperamide (50 mg/kg) group were significantly higher compared with that of the saline group ($P < .05$).

Eosinophil in Nasal Mucosa after Drug Stimulation and Nasal Challenge with OVA

Data are shown in Figures 2C, 3C, 4C, and 5C. Eosinophil count after stimulation by (R)- α -methylhistamine (50 mg/kg) ($n = 8$) was significantly decreased ($P < .05$) versus the saline group ($n = 8$). Eosinophil count after stimulation by d-chlorpheniramine maleate (40 mg/kg) ($n = 8$) was also significantly decreased ($P < .05$) versus the saline group ($n = 8$). Eosinophil count after stimulation by (R)- α -methylhistamine (12.5 mg/kg) and d-chlorpheniramine maleate (10 mg/kg) ($n = 8$) was also significantly decreased ($P < .01$) versus the saline group ($n = 8$). Eosinophil count after stimulation by thioperamide (50 mg/kg) ($n = 8$) was increased versus the saline group ($n = 8$), although not significantly ($P = .15$).

Immunocytochemistry

H_1 receptor was most excessively positive around the arteries and the veins in the epithelium (Fig. 6), and H_3 receptor was most densely distributed on the epithelium (Fig. 7). In the serial section, H_1 receptor and H_3 receptor coexisted in the epithelium.

DISCUSSION

The H_3 receptor was first identified as an autoreceptor controlling histamine synthesis and release in the brain.^{2,3} The inhibition mediated by H_3 autoreceptors constitutes a major regulatory mechanism of histaminergic neurons in vivo.¹¹ Recently, some studies have shown that H_3 receptors are also present in various peripheral tissues other than the brain,⁵⁻⁸ but to date, there has been no study of H_3 receptor mRNA in the nasal mucosa. We therefore used an immunohistochemical method to investigate the presence of H_3 receptors in human nasal mucosa.⁹

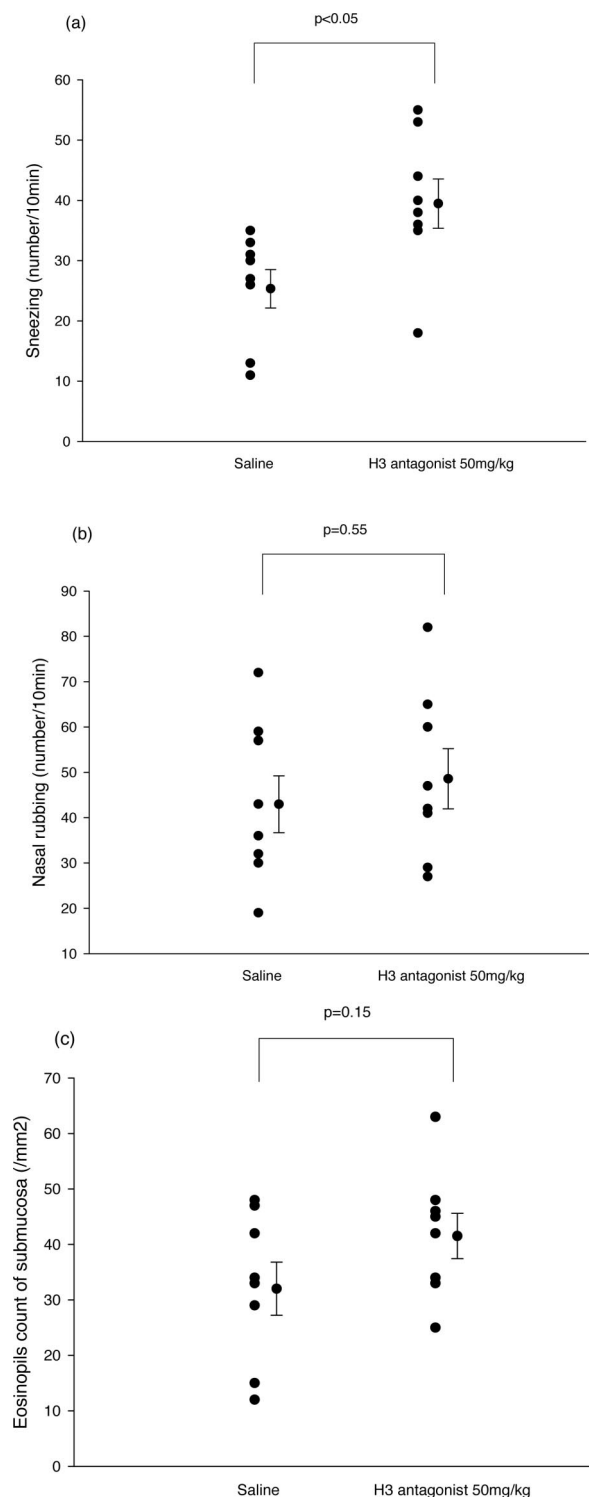


Fig. 5. Exacerbation of nasal symptoms in murine allergic model by H_3 antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or thioperamide (50 mg/kg). The nasal symptoms of (A) sneezing and (B) nasal rubbing were counted over 10 minutes after intranasal drip of OVA. (C) Increase of eosinophils in the submucosa of murine allergic model by H_3 antagonist. The BALB/c nasal allergic mice were given an intraperitoneal injection of saline or thioperamide (50 mg/kg). Eosinophils were counted after intranasal drip of OVA. Each bar represents the mean $\pm$ SE.

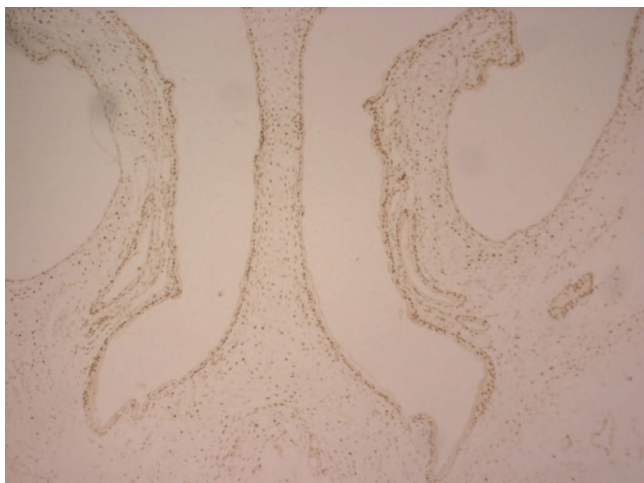


Fig. 6. H_1 receptors in murine nasal mucosa (10×10). H_1 receptor was most excessively positive around the arteries and the veins in the epithelium.

The existence of mouse H_3 receptor isoforms was reported recently.¹² The retention/detention of a fragment in the third intracellular loop of the mouse receptor leads to the existence of three isoforms designated $mH_{3(445)}$, $mH_{3(413)}$, and $mH_{3(397)}$ according to the length of their deduced amino acid sequence. In our study, we confirmed the existence of H_3 receptor mRNA in the mouse nasal mucosa for the first time, demonstrating $mH_{3(445)}$ H_3 receptor mRNA in the mouse nasal mucosa.

In peripheral tissue, it has been suggested that H_3 receptors exist in perivascular nerve terminal and regulate the release of histamine,³ acetylcholine,⁸ and noradrenaline.¹³ Furthermore, Varty and Hey¹³ reported H_3 receptor modulates vascular contractile responses in human nasal mucosa most likely by inhibiting noradrenaline release from sympathetic nerve terminals in nasal mucosa by pharmacologic method. It is also well known that histamine by way of H_3 receptors regulates substance-P release from peripheral ending sensory nerves.⁹ Previous

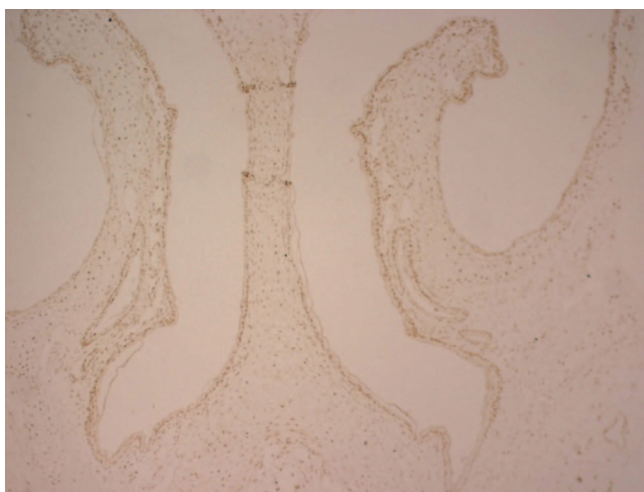


Fig. 7. H_3 receptors in murine nasal mucosa (10×10). H_3 receptor was most densely distributed on the epithelium.

studies have demonstrated the existence of H_3 receptors on the C-fibers of peripheral endings of the sensory nerves.^{3,7} Nemmar et al.¹⁴ reported that C-fibers of nerve terminals in afferent neurons contain certain neuropeptides, such as substance-P, that evoke skin reactions.¹⁴ It has also been reported that pretreatment with the H_3 receptor agonist imepit prevented capsaicin induced substance-P release.¹⁵

Using an immunohistochemical method, we reported that H_3 receptors localize on H_1 receptor positive nerves in human nasal mucosa.⁹ In this study, an immunohistochemical method showed that H_1 receptors and H_3 receptors are adjoined in the epithelium. This result indicates that both H_1 receptors and H_3 receptors adjoin and regulate each other. However, the mechanisms involved are unclear and require further study.

(R)- α -methylhistamine and thioperamide, respectively, the first H_3 receptor agonist and antagonist to be recognized, were used to characterize the H_3 receptors definitively; these substances were also used in various experiments to investigate the physiologic and pharmacologic roles of the H_3 receptors. In the present study, we found that the intraperitoneal injection of the H_3 receptor agonist (R)- α -methylhistamine inhibited the clinical nasal allergic symptoms such as sneezing and nasal rubbing in OVA-sensitized mice. Eosinophil count was also inhibited by this H_3 receptor agonist. The inhibitory effect on clinical allergic symptoms in the murine allergic model was the same using the H_1 receptor antagonist d-chlorpheniramine maleate, although the dose of each drug was different. Both the H_3 receptor agonist and the H_1 receptor antagonist had a stronger inhibitory effect on nasal allergic symptoms than each drug alone. On the other hand, intraperitoneal injection of the H_3 receptor antagonist thioperamide exacerbated sneezing in the murine allergic model.

This mechanism of H_3 receptor agonist and H_1 receptor antagonist interaction is uncertain, but it may be related to the finding that H_1 receptors and H_3 receptors adjoin in the epithelium, and this suggests a possible effect such as downregulation of H_1 receptors by H_3 receptors stimulation and upregulation of H_1 receptors by H_3 receptor blockade.

There has been no prior study of H_3 receptor agonists and antagonists using a murine allergic model. Sugimoto et al.¹⁰ reported that the H_3 receptor antagonist thioperamide increased the incidence of scratching behavior in normal ICR mice and that the H_3 receptor agonist (R)- α -methylhistamine inhibited thioperamide-induced scratching behavior. Sugimoto et al.¹⁰ indicated that H_3 receptors were involved in the modulation of dermal pruritus and that an H_3 receptor agonist may be a useful drug against pruritus. The results of this study support their findings.

In summary, H_3 receptors are involved in nasal allergy modulation. An H_3 receptor agonist may therefore be useful as a novel therapeutic agent in nasal allergy. Both H_3 receptor agonist and H_1 receptor antagonist may also be more effective than a single drug.

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BIBLIOGRAPHY

1. Naclerio RM, Togias AG. The nasal allergic reaction: observations on the role of histamine. *Clin Exp Allergy* 1991;21:13–19.
2. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H₃) of histamine receptor. *Nature* 1983;302:832–837.
3. Arrang JM, Garbarg M, Lancelot JC, et al. Highly potent and selective ligands for histamine H₃-receptors. *Nature* 1987;327:117–123.
4. Nakamura T, Itadani H, Hidaka Y, et al. Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun* 2000;279:615–620.
5. Ishikawa S, Sperelakis N. A novel class (H₃) of histamine receptors on perivascular nerve terminals. *Nature* 1987;327:158–160.
6. Dimitriadou V, Rouleau A, Dam Trung Tuong M, et al. Functional relationship between mast cells and C-sensitive nerve fibers evidenced by histamine H₃-receptor modulation in rat lung and spleen. *Clin Sci* 1994;87:151–163.
7. Delaunoy A, Gustin P, Garbarg M, et al. Modulation of acetylcholine, capsaicin and substance P effects by histamine H₃ receptors in isolated perfused rabbit lungs. *Eur J Pharmacol* 1995;277:243–250.
8. Ichinose M, Barnes PJ. Inhibitory histamine H₃-receptors on cholinergic nerves in human airways. *Eur J Pharmacol* 1989;163:383–386.
9. Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann Oto Rhino Laryngol* 2004;113:552–557.
10. Sugimoto Y, Iba Y, Nakamura Y, et al. Pruritus-associated response mediated by cutaneous histamine H₃ receptors. *Clin Exp Allergy* 2004;34:456–459.
11. Schwartz JC, Arrang JM, Garbarg M, et al. Histaminergic transmission in the mammalian brain. *Physiol Rev* 1991;71:1–51.
12. Rouleau A, Heron A, Cochois V, et al. Cloning and expression of the mouse histamine H₃ receptor: evidence for multiple isoforms. *J Neurochem* 2004;90:1331–1338.
13. Varty LM, Hey JA. Histamine H₃ receptor activation inhibits neurogenic sympathetic vasoconstriction in porcine nasal mucosa. *Eur J Pharmacol* 2002;452:339–345.
14. Nemmar A, Delaunoy A, Beckers JF, et al. Modulatory effect of imepit, a histamine H₃ receptor agonist, on C-fibers, cholinergic fibers and mast cells in rabbit lungs in vitro. *Eur J Pharmacol* 1999;371:23–30.
15. Foreman JC. Substance P and calcitonin gene-related peptide: effects on mast cells and in human skin. *Int Arch Allergy Appl Immunol* 1987;82:366–371.