Localization and function of histamine H_3 receptor in the nasal mucosa

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Summary

Background Histamine is an important chemical mediator of allergic rhinitis. Histamine H_3 receptors are located on cholinergic and NANC neurons of the myenteric plexus, and activation of histamine H_3 receptor regulates gastric acid secretion. However, little is known about the localization and function of H_3R in the upper airway.

Objective The objective of this study was to examine the localization and possible function of H_3R in the nasal mucosa.

Methods We extracted total RNA from the inferior turbinate mucosa of patients with allergic rhinitis. Histamine H_3 receptor mRNA and β-actin mRNA were amplified by RT-PCR. We used immunohistochemistry to examine localization of histamine H_3 receptor protein in inferior turbinate mucosa excised during clinically indicated surgery. We used alcian blue/ periodic acid-shiff (AB/PAS) staining to examine the effects of the histamine H_3 receptor agonist (R)-α-methylhistamine and the histamine H_3 receptor antagonist thioperamide on secretion from rat submucosal glands.

Results Histamine H_3 receptor protein was expressed around submucosal gland cells. Thioperamide induced degranulation in the submucosal gland in the nasal septum.

Conclusion The present results suggest that histamine H_3 receptor is localized mainly around submucosal glands, and that histamine H_3 receptor plays an important part in secretion of submucosal glands in the nose.

Key Words: histamine H_3 receptor, (R)-α-methylhistamine, thioperamide, submucosal gland

Introduction

Histamine plays an important role in eliciting the nasal symptoms of allergic

rhinitis; i.e., pruritus, sneezing, rhinorrhea, and congestion [1]. Histamine receptors are classified as H_1, H_2 [2], H_3 [3] and H_4 [4]. Stimulation of the histamine H_1 receptor (H_1R) leads to contraction of tracheal and vascular smooth muscles, increases vascular permeability, and stimulates sensory nerve endings [5]. The histamine H_2 receptor (H_2R) mainly mediates gastric acid secretion and relaxes several tissues, including airway and vascular smooth muscles [5]. Stimulation of the histamine H_3 receptor (H_3R) inhibits synthesis and release of histamine [5]. The human H_1R and H_2R genes have been isolated and cloned by screening a human genomic library with a bovine H_1R and H_2R probe, respectively [6,7]. The H_3R and H_4R genes have also been cloned. The nucleotide sequence of the human H_3R gene is very similar to that of H_4R, but shows little similarity to that of human H_1R or H_2R.

Previously, we reported that H_2R mRNA was localized in the epithelium, serous cells and mucous cells of nasal mucosa, and that expression of H_2R mRNA was increased in nasal mucosa from patients with allergic rhinitis, compared with normal subjects [8].

It has been reported that H_3R is expressed on sympathetic nerve terminals, and that stimulation of H_3R inhibits release of norepinephrine from those nerves [9]. In the absence of histamine, norepinephrine released from sympathetic nerve terminals helps to maintain normal vascular tone. The presence of histamine decreases norepinephrine levels, causing vasodilation that results in nasal obstruction [9].

In the human lower airway, H_3R regulates cholinergic nerve transduction [10]. In guinea pigs, H_3R is present on the vagus nerve, which modulates cholinergic neurotransmission in the airways [11]. An extensive body of evidence indicates that H_3R modulates sympathetic and parasympathetic function throughout the peripheral autonomic system [12-15]. Previous findings suggest that H_3R plays a role in allergic rhinitis, and that expression of H_3R increases during allergic rhinitis. However, to our knowledge, there have been few reported studies of the localization and expression of H_3R in the human nasal mucosa. We hypothesized that H_3R in the nose plays a role in mucus secretion. The purpose of the present study was to clarify the localization of H_3R protein in the nasal mucosa, and to clarify the function of H_3R in the nose.

Materials and Methods

Subjects

To examine the expression of H_3R mRNA and H_3R protein, we obtained nasal

mucosae from 10 allergic rhinitis patients (age range, 16 to 54 years; mean age, 32.2 years) at the time of clinically indicated surgery. All 10 patients were allergic to the house dust mite (HDM) and had severe nasal obstruction which was not responsive to conservative therapy. The diagnosis of allergic rhinitis was based on a compatible history, anterior rhinoscopic examination, and the following 3 criteria: 1) eosinophilia in nasal smears; 2) RAST class 2 or higher for HDM; and 3) a positive nasal provocation test response for HDM. Tests were performed several weeks before obtaining samples. The patients with allergic rhinitis were moderately symptomatic, and received neither antihistamine therapy nor topical steroids in the 6 months before the study. The experimental procedures followed the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all patients.

Human Tissue Preparation

At the time of surgery, 1% lidocaine hydrochloride and 0.02% epinephrine were applied topically in nasal packs. The turbinates were injected with 2 to 4 ml of 1% lidocaine with 1:100,000 epinephrine. An incision was made along the conchal bone from the lateral wall of the inferior turbinate. Nasal mucosal tissue samples for immunohistochemistry (n=5) were embedded in optimal cutting temperature compound and snap-frozen in liquid nitrogen. Frozen samples were stored at -80°C until used.

RT-PCR analysis for histamine H_3 receptor mRNA

Inferior turbinate mucosa excised at the time of the clinically indicated surgery (n=19) was homogenized in denaturing solution (4 M guanidium thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol). Then, we performed single-step RNA isolation, using the method of Chomczynski [16]. RNA pellets were resuspended in 20 µl of 10X buffer and incubated with 1 U of DNase I at 37°C for 30 minutes, to distinguish RNA from contaminating genomic DNA. The RNA was extracted with phenol/chloroform/isoamyl alcohol (25/24/1; vol./vol./vol.), ethanol precipitated, centrifuged at 15,000 rpm for 10 minutes, rinsed with 70% ethanol and dried. The RNA pellets thus prepared were reverse transcribed into cDNA using the GeneAmp RNA PCR Kit (Takara Biomedicals, Tokyo, Japan) and an oligo d(T) primer. After incubation at 42°C for 30 minutes, the samples were heated for 5 minutes at 99°C

to terminate the reaction, and were stored at -20°C until used. Oligonucleotide primers were designed based on the published cDNA sequences [17, 18]. The sequences of primers were as follows: H_3R sense (nt 754-778), 5'-AGTGCGGAAGATGCTGCTGGTGTGG-3'; H_3R antisense (nt 1383-1406), 5'-AGAGCCCAAAGATGCTCACGATGA -3'; β-actin sense, 5'-ATGGATGATGATATCGCCGCG-3'; β-actin antisense, 5'-ATGTCGTCCCAGTTGGTGACGAT-3'. We heated fresh tubes containing the upstream and downstream primers (25 μM of each primer) spanning the sequence to be amplified, 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 1 U of Thermus aquaticus (Taq) DNA polymerase at 94°C, and then added 1 μl of cDNA, for a final volume of 50 μl. To this reaction mixture, we added 1 drop of mineral oil, and then performed amplification using a Perkin-Elmer/Cetus (Norwalk, CT, USA) thermal cycler. The amplification thermal cycling consisted of 30 cycles of denaturation at 94°C for 45 seconds, primer annealing at 62°C for 2 minutes, and extension at 72°C for 1 minute. The reaction was terminated by chilling at 4°C. The PCR products were electrophoresed through a 2% (wt/vol) agarose gel.

RT-PCR analysis was performed on rat nasal mucosa in the similar manner using the following primers: rat sense: CCAGAACCCCCACCAGATG; rat antisense CCAGCAGAGCCCAAAGATG [19].

Reagents

(R)-α-methylhistamine dihydrochloride was purchased from Tocris (UK). Thioperamide was purchased from Sigma (St. Louis, MO, USA). (R)-α-methylhistamine and thioperamide were dissolved in physiological saline.

Animals

Specific-pathogen-free male Fisher 344 rats (weight, 230-250 g; Japan SLC, Inc., Shizuoka, Japan) were used in this study. The experiments were approved by the committee of Mie University School of Medicine for the care and use of laboratory animals.

Immunohistochemistry

Immunohistochemistry for H_3R was performed using the Vectastain ABC-AP Rabbit IgG kit (Vector Laboratories, Burlingame, CA) and Dako Fast Red substrate system (Dako, Carpinteria, CA). Frozen sections (thickness, 5 μm) were cut using a cryostat, mounted on poly-L-lysine-coated slides, air-dried and fixed in acetone. After washing in phosphate-buffered saline (PBS), the sections were treated with normal goat serum (blocking serum) for 30 min at room temperature to block non-specific binding of antibodies. After blocking, the sections were incubated overnight with polyclonal primary antibody against human H_3R (dilution 1:100; Chemicon, USA). The sections were washed with PBS, followed by addition of secondary antibodies (biotinylated goat anti-rabbit IgG) and incubation for 30 min at room temperature. After washing with PBS, the avidin-biotin-alkaline phosphatase complex was added, and the sections were incubated for 30 min at room temperature. After a final wash, the color was developed using the chromogen Dako Fast Red substrate system according to the manufacturer's instructions. The sections were counterstained with Mayer's hematoxylin for 3 min.

For rat immunohistochemistry, we used mucosa from the nasal septum of F344 male rats and rabbit anti-rat H_3R polyclonal antibody (1:800, Chemicon).

Immunohistochemistry for neurofilament protein was performed using a similar method. The sections were incubated with Peroxo Block (Zymed, Laboratories, South San Francisco, CA.) for 45 sec to suppress the endogenous peroxidase activity. The sections were incubated overnight with the monoclonal primary antibody against neurofilament (dilution 1:100; Dako, Carpinteria, CA). After rinsing, the sections were incubated in diluted biotinylated secondary antibody solution for 30 min, followed by incubation with Vectastain Elite avidin-biotinylated horseradish complex (ABC) Reagent (Vector Laboratories, Burlingame, CA) and staining with Diaminobenzine (DAB) substrate solution (DAB substrate kit; Vector Laboratories, Burlingame, CA) for 1.5 min. The sections were counterstained with Methylgreen solution for 3 min. The specificity of the immunohistochemical reaction was checked by omitting the primary antibody. The sections were evaluated using optical microscopy.

Protocol of Experiments and Rat Tissue Preparation

The rats were divided into 3 groups: the (R)-α-methylhistamine group received an intraperioneal (i.p.) injection of (R)-α-methylhistamine (10 mg/kg); the thiopermide

group received an i.p. injection of thioperamide (5 mg/kg); and the control group received an i.p. injection of saline. The concentration of (R)-α-methylhistamine was decided on the basis of the previous reports [20-22]. At 1 hour after those injections, the rats were killed with an i.p. injection of an overdose of sodium pentobarbital. The head of each rat was removed, fixed in 10% neutral buffered formalin for 3 days, and then decalcified in 5% trichloroacetic acid for 6 days. The nasal cavity was sectioned transversely from the incisor teeth to the incisive papilla of the nasal plate. The tissue block was embedded in paraffin and cut into sections 5 μm thick.

Morphometric Analysis

The paraffin sections were stained with Alcian blue (pH 2.5)/ periodic acid-shiff (AB/PAS) or hematoxyline. In the control group, the acini of submucosal glands were packed with granules and were positive for AB/PAS staining. To quantify submucosal glands, we used a method described elsewhere [23] with some modifications. The total area and the area of AB/PAS-positive submucosal glands in the nasal septum were manually circumscribed and analyzed using an image analyzer (SP 500; Olympus, Tokyo, Japan). Data are expressed as the percentage of the total area of gland tissue that was AB/PAS-positive. We counted the average of 3 places on each side of the septum. Measurements were performed in high-power fields at 400-times magnification.

Statistical analysis

The results are expressed as means ± S.E. of 4 different experiments. The statistical significance of differences between groups was evaluated using the Mann-Whitney U test.

Results

RT-PCR of histamine H_3R mRNA in the nasal mucosa

Figure 1 shows the results of RT-PCR of the 6 representative samples. Out of 10

samples of inferior turbinate mucosa, only 1 sample had a band between 500 bp and 750 bp. β-actin mRNA was detected in all samples. RT-PCR analysis on rat nasal mucosa did not reveal appropriate bands for rat H3R mRNA (data not shown).

Immunohistochemistry for human histamine H_3 receptor and neurofilament protein

The results clearly show that H_3R was present in the periphery of the human submucosal gland (Fig. 2). Signals indicating the presence of neurofilament were observed beneath the submucosal glands (Fig. 3).

Immunohistochemistry for rat histamine H_3 receptor

The results clearly show that H_3R was present in the periphery of the rat submucosal gland (Fig. 4). Signals indicating the presence of neurofilament were observed beneath the submucosal glands.

Effect of histamine H_3 receptor agonist and antagonist on the area of rat nasal glands that was AB/PAS-positive

In the control group (Fig. 5A) and RAM group (Fig. 5B), the submucosal glands were filled with AB/PAS-positive granules. In contrast, in the TPA group (Fig. 5C), cytoplasmic vacuoles were observed, and acinar ducts were enlarged. The findings in the TPA group indicate the presence of degranulation in submucosal glands, with a consequent decrease in the area of granulation by approximately 50% (Fig. 6).

Discussion

The present localization of H_3R expression signals on the periphery of the submucosal gland suggests 2 possibilities: 1) H_3R is expressed on the basolateral membranes of gland cells; 2) H_3R is expressed on the presynaptic region of the nerves innervating those glands cells. The absence of H_3R mRNA in the nasal tissues supports the latter possibility, which assumes that H_3R is produced in the sphenopalatine ganglion of the parasympathetic nerves or in the superior cervical ganglion of the sympathetic nerves and is transported from there to the nerve terminals.

Nakaya et al. [24] used immunohistochemistry to examine distribution of histamine receptor subtypes in the human inferior turbinates, and reported that H_3R and H_4R were clearly expressed on nerves in the human inferior turbinates. They

demonstrated that H_3R protein was expressed on the nerve bundle, rather than on the nerve terminals [24]. In the present study, to examine distribution of nerves in the inferior turbinate, we performed immunohistochemistry using a monoclonal antibody against neurofilament. Neurofilament-positive signals were observed beneath the submucosal glands. Neurofilament was present in bundles beneath the mucosal glands, and the peripheral portions of the nerve bundles were distributed to the glands. This suggests that H_3R may be expressed on the nerve terminals, but we did not observe any H_3R signals on the nerve bundles. The reason for this discrepancy between previous and present results is unclear.

Most studies [9, 25] of the possible function of H_3R in the nose have concentrated on regulation of release of norepinephrine from the terminal of sympathetic post-ganglionic neurons. To our knowledge, the present study is the first to specifically indicate a possible role for H3R in the regulation of mucus secretion from the submucosa gland cells. "Degranulation" in the present study is a histological finding in the submucosal glands by which we assume that mucus granules have been secreted by some stimuli, which results in the increased nasal discharge. In this sense, the degranulation of the submucosal gland is completely different from degranulation of mast cells often observed in the allergic reaction.

It is rather difficult to guess the exact function of H_3R in secretion of mucus from gland cells. In the present study, treatment with the H_3R agonist (R)- α -methylhistamine did not cause any significant change in gland cells, but the H_3R antagonist thioperamide caused degranulation. One possibility is that histamine inhibits degranulation from submucosal glands via H_3R. H_3R is probably expressed on the terminal of parasympathetic nerves, and activation of H_3R usually inhibits release of acetylcholine from the nerve terminals. Blocking H_3R (e.g., using thioperamide) stimulates release of acetylcholine, resulting in increased mucus secretion and degranulation from the gland cells. However, the precise mechanism responsible for this effect of blocking H_3R is unclear.

Another possible explanation for an effect of thioperamide on the degranulation of glands is its inverse agonist activity on H_3R. It is now well-documented that G-protein-coupled receptors can be spontaneous active, and this agonist-independent receptor activity is often referred to as constituitive receptor activity [26]. It is reported that both the rat and human H_3R show a high degree of constitutive activity and that thioperamide act as an inverse agonist [27]. Lack of effect of (R)-α-methylhistamine on the degranulation of glands can be explained by the constitutive receptor activity.

There is conflicting evidence regarding the role of H_3R in the nose and

potential clinical therapies involving H_3R. Some findings suggest that use of an H_3R agonist with an H_1R antagonist can alleviate the nasal symptoms of allergic rhinitis, and others suggest that use of an H_3R antagonist with an H_1R antagonist would be more effective. Nakaya et al. [28] used a murine allergic model to investigate the effects of H_3R agonists and antagonists on clinical nasal allergic symptoms. They administered i.p. injections of an H_3R agonist or antagonist to OVA-sensitized mice, and scored clinical nasal allergic symptoms over a 10-minute period after nasal provocation with OVA. They found that the H_3R agonist (R)-α-metylhistamine significantly inhibited clinical nasal allergic symptoms of OVA-sensitized mice. Their findings suggest that use of an H_3R agonist with an H_1R antagonist can reduce allergic symptoms in the nose. In contrast, a study by McLeod et al. [29] suggests that a combination of an H_3R antagonist and an H_1R antagonist can relieve nasal decongestion.

The human H_3R is related most closely to the human H_4R, which shares 31% sequence identity at the protein level [30]. Because of this sequence similarity, H_4R is activated by several H_3R agonists including (R)-α-metylhistamine and thioperamide, an inverse agonist at H_3R, is also an inverse agonist at the H_4R [30]. (R)-α-methylhistamine is a weak ligand at rat H_4R, but thioperamide has roughly the same affinity for H_4Rs at all species [31]. Thus, possible involvement of H_4R in the effect of thioperamide on the degranulation of glands cannot be ruled out. This point should be clarified in the future study.

In summary, the present findings indicate that H_3R protein is expressed around submucosal gland cells. Thioperamide induced degranulation in the submucosal gland in the nasal septum. These results suggest that H_3R plays a role in mucus secretion in the nasal submucosal gland.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (C) 14571615, (C) 16591705 and (C)18591864 from the Ministry of Education, Science, and Culture of Japan.

References

1. Gruber BL. Mast cells and basophils. In: Sigal LH, Ron Y, editors. Immunology and inflammation: basic mechanisms and clinical consequences New York: McGraw-Hill;1994;337-51
2. Black JW, Duncan WAM, Durant CJ, Ganellin CR, Parsons EM. Definition and

antagonism of histamine H2-receptors. Nature 1972;236:385-90
3. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. Nature 1983;302:832-7
4. Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R, et al. Discovery of a novel member of the histamine receptor family. Mol Pharmacol. 2001;59:427-33.
5. Hill SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol Rev 1990;42:45-83
6. De Backer MD, Gommeren W, Moereels H, Nobels G, Van Gompel P, Leysen JE, et al. Genomic cloning, heterologous expression and pharmacological characterization of a human histamine H1 receptor. Biochem Biophys Res Commun 1993;197:1601-8
7. Gantz I, Munzert G, Tashiro T, Schaffer M, Wang L, DelValle J, et al. Molecular cloning of the human histamine H2 receptor. Biochem Biophys Res Commun 1991;178:1386-92
8. Hirata N, Takeuchi K, Ukai K, Jin C, Yoshida T, Sakakura Y. Expression and localization of histamine H2 receptor messenger RNA in human nasal mucosa. J Allergy Clin Immunol. 1999;103:944-9.
9. Varty LM, Gustafson E, Laverty M, Hey JA. Activation of histamine H_3 receptors in human nasal mucosa inhibits sympathetic vasoconstriction. Eur J Pharmacol 2004;484:83-9.
10. Ichinose M, Barnes PJ. Inhibitory histamine H_3-receptors on cholinergic nerves in human airways. Eur J Pharmacol 1989;163:383-6.
11. Ichinose M, Stretton CD, Schwartz JC, Barnes PJ. Histamine H_3-receptors inhibit cholinergic neurotransmission in guinea-pig airways. Br J Pharmacol 1989;97:13-5.
12. Coruzzi G, Morini G, Adami M, Grandi D. Role of histamine H_3 receptors in the regulation of gastric functions. J Physiol Pharmacol 2001;52:539-53.
13. Koss MC, Hey JA. Activation of histamine H_3 receptors produces presynaptic inhibition of neurally evoked cat nictitating membrane responses in vivo. Naunyn Schmiedebergs Arch Pharmacol 1992:346:208-12.
14. Hey JA, del Prado M, Egan RW, Kreutner W, Chapman RW. (R)-alpha-methylhistamine augments neural, cholinergic bronchospasm in guinea pigs by histamine H_1 receptor activation. Eur J Pharmacol 1992;211:421-6.
15. Gothert M, Garbarg M, Hey JA, Schlicker E, Schwartz JC, Levi R. New aspects of the role of histamine in cardiovascular function: identification, characterization, and potential pathophysiological importance of H3 receptors. Can J Physiol Pharmacol 1995;73:558-64.

16. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9
17. Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, et al. Cloning and functional expression of the human histamine H3 receptor. Mol Pharmacol 1999;55:1101-7.
18. Ponte P, Ng SY, Engel J, Gunning P, Kedes L. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. Nucleic Acids Res 1984;12:1687-96
19. Azuma H, Sawada S, Takeuchi S, Higashiyama K, Kakigi A, Takeda T. Expression of mRNA encoding the H1,H2,and H3 histamine receptors in the rat cochlea. Neuroreport 2003;14:423-5
20. Clapham J, Kilpatrick GJ. Histamine H3 receptor-mediated modulation of water consumption in the rat. Eur J Pharmacol. 1993;232:99-103.
21. Rubio S, Begega A, Santín LJ, Miranda R, Arias JL. Effects of histamine precursor and (R)-alpha-methylhistamine on the avoidance response in rats. Behav Brain Res. 2001 ;124:177-81.
22. Sugimoto Y, Iba Y, Nakamura Y, Kayasuga R, Kamei C. Pruritus-associated response mediated by cutaneous histamine H3 receptors. Clin Exp Allergy. 2004;34:456-9.
23. Kim ST, Takashi N, Ueki I, Nadel JA. fMLP causes degranulation followed by regranulation in rat nasal glands. Laryngoscope 2003;113:1998-2003.
24. Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. Ann Otol Rhinol Laryngol 2004;113:552-7.
25. Taylor-Clark T, Sodha R, Warner B, Foreman J. Histamine receptors that influence blockage of the normal human nasal airway. Br J Pharmacol 2005;144:867-74.
26. Milligan G, Bond RA, Lee M. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? Trends Pharmacol Sci. 1995;16:10-3.
27. Wieland K, Bongers G, Yamamoto Y, Hashimoto T, Yamatodani A, Menge WM, et al. Constitutive activity of histamine H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. J Pharmacol Exp Ther. 2001;299:908-14.
28. Nakaya M, Fukushima Y, Takeuchi N, Kaga K. Nasal allergic response mediated by histamine H_3 receptors in murine allergic rhinitis. Laryngoscope 2005;115:1778-84.
29. McLeod RL, Mingo GG, Herczku C, DeGennaro-Culver F, Kreutner W, Egan RW,

et al. Combined histamine H1 and H3 receptor blockade produces nasal decongestion in an experimental model of nasal congestion. Am J Rhinol 1999;13:391-9.

30. de Esch IJ, Thurmond RL, Jongejan A, Leurs R. The histamine H4 receptor as a new therapeutic target for inflammation. Trends Pharmacol Sci. 2005;26:462-9.
31. Liu C, Wilson SJ, Kuei C, Lovenberg TW. Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. J Pharmacol Exp Ther. 2001;299:121-30.

Figures

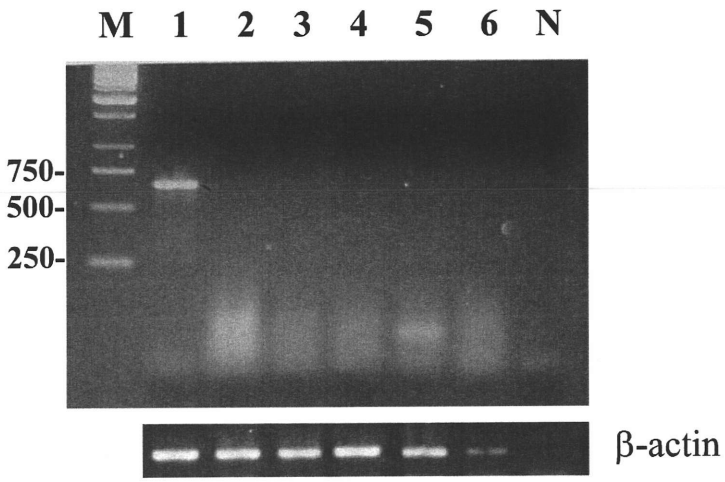

Fig. 1.

Fig. 1. Expression of histamine H_3 receptor mRNA in the inferior turbinate mucosa of patients with allergic rhinitis. The slide shows the results of RT-PCR of the 6 representative samples. Out of 10 inferior turbinate mucosa samples, only 1 sample had a band between 500 bp and 750 bp. β-actin mRNA was expressed in all 10 samples. N refers to negative control (no cDNAs).

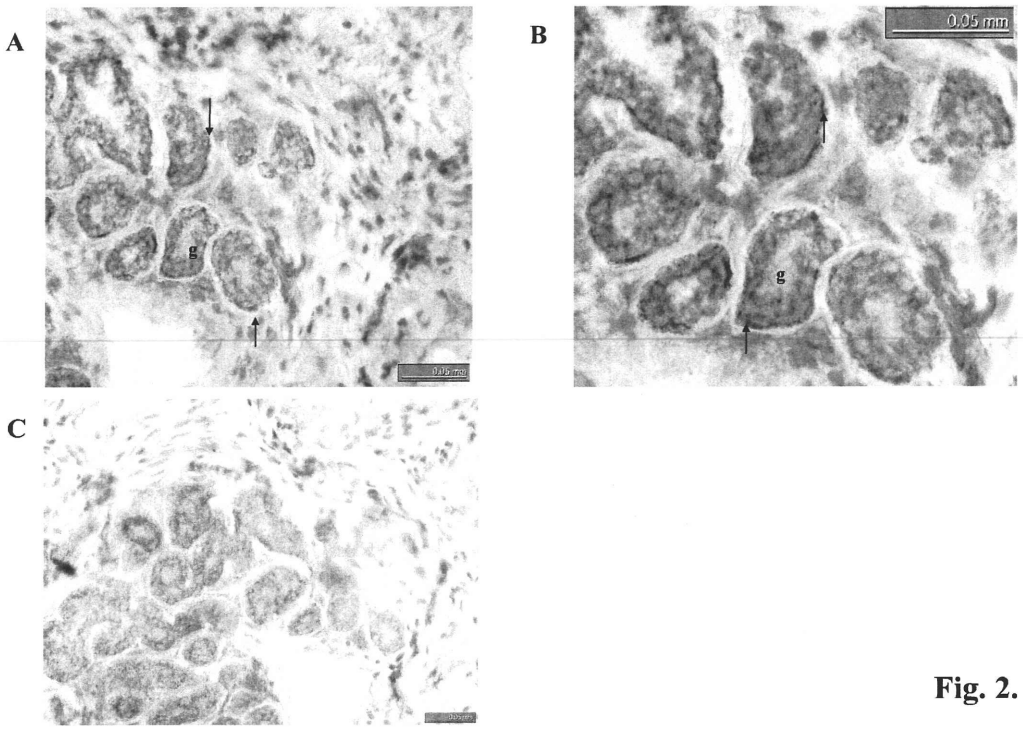

Fig. 2.

Fig. 2. Immunohistochemistry for histamine H_3 receptor in human nasal mucosa with allergic rhinitis (A,B). Histamine H_3 receptor (arrows in A,B) in human nasal mucosa. All 5 experiments produced similar results. Negative control (C). g; submucosal grand

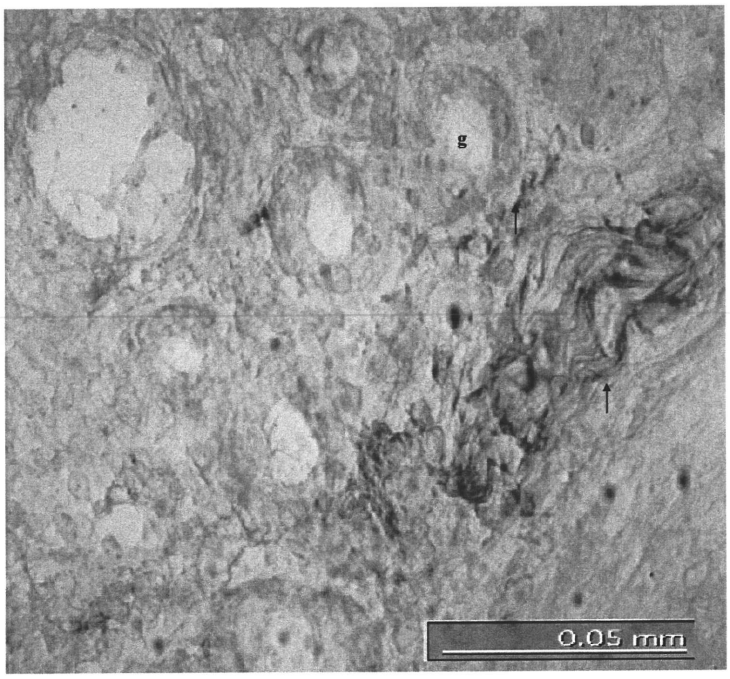

Fig. 3.

Fig. 3. Immunohistochemistry for neurofilament protein in human nasal mucosa. Neurofirament (arrows) in human nasal mucosa. All 5 experiments produced similar results. g; submucosal grand

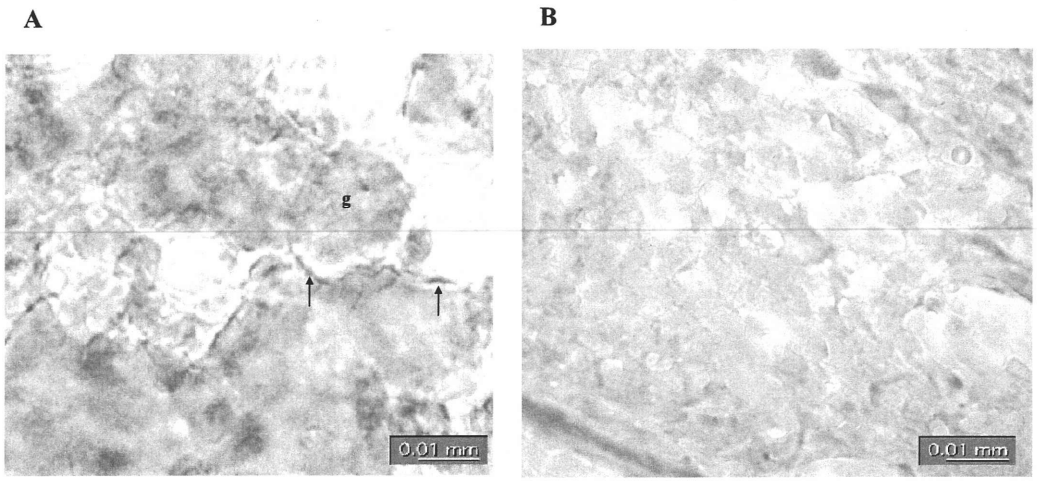

Fig. 4.

Fig. 4. Immunohistochemistry for rat histamine H_3 receptor.
Histamine H_3 receptor (arrows) in rat nasal mucosa (A). All 5 experiments produced similar results. Negative control (B). g; submucosal grand

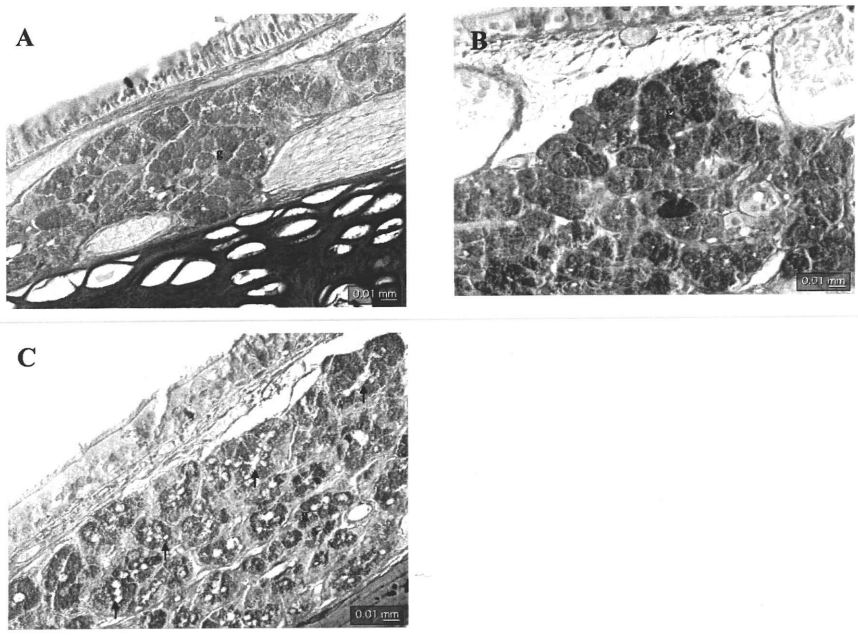

Fig. 5.

Fig. 5. Photomicrographs of the effects of H_3R agonist and antagonist in rat nasal glands. In the control group (A), the submucosal glands were filled with AB/PAS-positive granules. One group was treated with (R)-α-methylhistamine (B). Treatment with thioperamide (C) caused degranulation; cytoplasmic vacuoles were observed, and acinar ducts were enlarged (arrows in C). The images shown are representative of the experimental results.

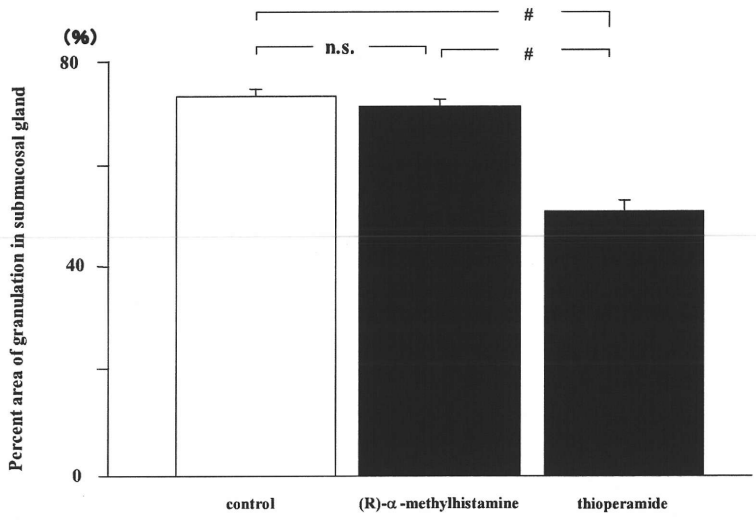

Fig. 6.

Fig. 6. Effects of H_3R agonist and antagonist on the AB/PAS-positive area of rat nasal glands. Data are expressed as the percentage of total gland area that is AB/PAS-positive; reduced percentage indicates submucosal gland degranulation. The results are expressed as means ± S.E. of 4 different experiments. Significance: # $p<0.05$, significantly different from control. n.s. indicates data not significantly different.