

Full Paper

Participation of Histamine H₃ Receptors in Experimental Allergic Rhinitis of Mice

Emiko Yokota¹, Shoji Kuyama¹, Yukio Sugimoto¹, Masami Ogawa², and Chiaki Kamei^{1,*}¹Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima-naka 1-1-1, Okayama 700-8530, Japan²Preclinical Research Department, Research and Development Division, Schering-Plough K.K., Hiranomachi 2-3-7, Chuo-Ku, Osaka 541-0046, Japan

Received June 24, 2008; Accepted August 19, 2008

Abstract. The present study was performed to study the participation of histamine H₃ receptors in nasal symptoms using Sch 50971, a potent and selective agonist of the H₃ receptor. Repeated topical application of antigen caused an increase in sneezing and nasal rubbing in sensitized mice. Oral administration of Sch 50971 and imetit, specific H₃-receptor agonists, resulted in an inhibition of nasal symptoms induced by an antigen similar to an H₁-receptor antagonist, cetirizine. Furthermore, simultaneous use of H₃-receptor agonists, Sch 50971 or imetit, and an H₁-receptor antagonist, cetirizine, caused a significant inhibitory effect on nasal symptoms at doses that showed no effect when used separately. The number of eosinophils in the nasal mucosa of mice sensitized with antigen was significantly decreased by cetirizine; however, Sch 50971 and imetit had no effect on eosinophil infiltration. These results clearly indicate that H₃ receptors are involved in the etiology of nasal allergy, and the stimulation of H₃ receptors may be useful as a novel therapeutic approach in nasal allergy.

Keywords: histamine H₃ receptor, histamine H₃-receptor agonist, cetirizine, allergic rhinitis, eosinophil infiltration

Introduction

Allergic rhinitis is a chronic inflammatory disease of the upper airway, and its prevalence is progressively increasing (1). Histamine has been recognized as one of the most important chemical mediators in allergic reactions, and histamine H₁-receptor antagonists are widely used in the treatment of allergic rhinitis; however, current H₁-receptor antagonists are not effective in controlling the disease completely (2, 3). From these findings, it is reasonable to presume that mechanisms other than H₁ receptors are involved in allergic rhinitis.

H₃ receptors were first found in the brain by Arrang et al. (4), and the receptors have been considered autoreceptors in presynaptic terminals of histaminergic neurons that regulate histamine synthesis and release. Not only in the central nervous system but also in the

peripheral system, they regulate the release of other chemical mediators such as acetylcholine, noradrenaline, dopamine, serotonin, and substance P. At present, it is clear that H₃ receptors exist in human and animal peripheral tissues (5–8). In addition, Nakaya et al. (9) reported that H₃ receptors were localized on the epithelium and nerves in human nasal mucosa.

On the other hand, we have reported that H₃-receptor antagonists induced scratching behavior (10) and an increase of skin vascular permeability (11) in mice. Furthermore, H₃-receptor agonists, Sch 50971, BP 2-94, and N^m-methylhistamine have been shown to be effective in inhibiting inflammation and migraine in guinea pigs, mice, and humans (12–14); however, very little information is available about the role of H₃ receptors in allergic diseases, particularly nasal allergy.

The purpose of the present study is to study the participation of histamine H₃ receptors in allergic rhinitis of mice using Sch 50971, a potent histamine H₃-receptor agonist with excellent in vivo receptor selectivity (12).

*Corresponding author. kamei@pheasant.pharm.okayama-u.ac.jp
Published online in J-STAGE on October 9, 2008 (in advance)
doi: 10.1254/jphs.08164FP

Materials and Methods

Animals

Six-week-old female BALB/c mice were obtained from Japan SLC, Shizuoka. The animals were housed in an air-conditioned room maintained at $24 \pm 2^\circ\text{C}$ with a relative humidity of $55 \pm 15\%$. Food and water were provided ad libitum. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Reagents and drugs

The following drugs were obtained from the sources shown in parentheses: ovalbumin (Grade VII, crystallized and lyophilized, essentially salt-free; Sigma, St. Louis, MO, USA), aluminum hydroxide hydrate gel (alum; LSL, Tokyo), Sch 50971 dihydrochloride (Schering-Plough K.K., Osaka), imetit dihydrobromide (Sigma), and cetirizine dihydrochloride (UCB; SA, Brussels, Belgium). Sch 50971 dihydrochloride, imetit dihydrobromide, and cetirizine dihydrochloride were dissolved in distilled water and administered orally. The other drugs were dissolved in physiological saline.

Sensitization

BALB/c mice were sensitized by intraperitoneal injection of 0.2 ml of physiological saline containing ovalbumin (1 μg) and alum (100 μg) on days 0, 5, 14, and 21. Then, local sensitization was performed 3 times a week from day 28 by instilling the ovalbumin in physiological saline (100 mg/ml, 2 μl) into the bilateral nasal cavities using a micropipette.

Evaluation of nasal symptoms in mice

Before the experiment, the animals were placed in an observation cage (31 \times 25 \times 18 cm) for about 10 min for acclimatization. After nasal instillation of 2 μl of ovalbumin dissolved in physiological saline (100 mg/ml) into the bilateral nasal cavities, the animals were returned to the observation cage (one animal/cage), and the frequency of sneezing and nasal rubbing was counted for 30 min. For the drug tests, the mice that showed remarkable sneezing and nasal rubbing by sensitization for 56 days or more were used.

Assessment of eosinophils in the nasal mucosa

Twenty-four hours after the challenge on day 56, mice were anesthetized with diethylether. The animals were sacrificed by exsanguination, and then their heads were removed. They were fixed with 10% neutral-buffered formalin for several days, and decalcified with 10% EDTA solution (pH 7.4) for 1 week. Samples were

embedded in paraffin, and frontal sections of the nose, 4- μm -thick, were stained with hematoxylin and eosin to evaluate the number of eosinophils. All eosinophils that had infiltrated the nasal mucosa at both sides of the nasal septum were determined microscopically.

Effects of drugs on nasal symptoms and the increase in nasal eosinophils

In this study, Sch 50971 and cetirizine were administered orally 1 h before the topical application of antigen. Imetit was administered orally 2 h before antigen challenge. Sneezing and nasal rubbing induced by ovalbumin were observed for 30 min and the number of eosinophils was counted under a light microscope.

Statistical analyses

All experimental data are shown as the mean \pm S.E.M. Statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison. Student's *t*-test was used for comparison between two groups. A probability value of less than 0.05 was considered significant.

Results

Changes in sneezing and nasal rubbing induced by antigen in sensitized mice

Figure 1 shows the changes in sneezing and nasal rubbing after antigen challenge. The number of sneezing and nasal rubbing were increased by repeated topical application of antigen. Sneezing was significantly increased from day 35, and nasal rubbing was significantly increased from day 28.

Effects of drugs on sneezing and nasal rubbing induced by antigen in sensitized mice

As shown in Fig. 2, histamine H₃-receptor agonists, Sch 50971 and imetit, dose-dependently inhibited sneezing and nasal rubbing induced by antigen, and a significant effect was observed at doses of 30 and 100 mg/kg. The histamine H₁-receptor antagonist cetirizine also caused a dose-related inhibition of this response and, at a dose of 10 mg/kg, it significantly decreased sneezing and nasal rubbing.

Effects of the simultaneous use of H₃ agonists and H₁ antagonist on sneezing and nasal rubbing induced by antigen in sensitized mice

Figure 3 shows the effects of the simultaneous use of Sch 50971 and cetirizine or imetit and cetirizine on sneezing and nasal rubbing induced by antigen. Sch 50971 (10 mg/kg), imetit (10 mg/kg), and cetirizine (3 mg/kg) had no effect on sneezing and nasal rubbing

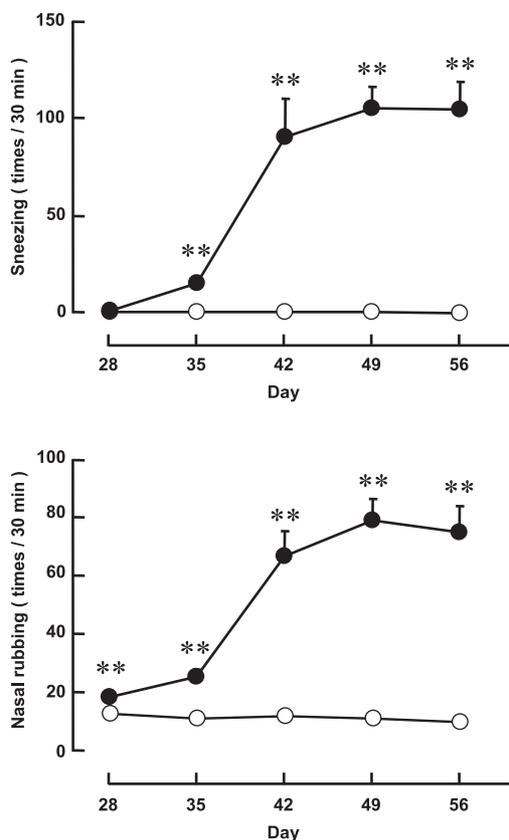


Fig. 1. Changes in sneezing and nasal rubbing induced by antigen in sensitized mice. Mice were immunized with saline (open circle) or ovalbumin (closed circle), and sneezing and nasal rubbing were counted for 30 min. Each point and vertical bar shows the mean \pm S.E.M. of 10 experiments. **: Significantly different from the saline-treated group with $P < 0.01$.

when used alone. The simultaneous use of Sch 50971 (10 mg/kg) and cetirizine (3 mg/kg) significantly inhibited nasal symptoms (sneezing and nasal rubbing). The simultaneous use of imetit (10 mg/kg) and cetirizine (3 mg/kg) also caused a significant decrease in this response. In addition, the simultaneous use of Sch 50971 and cetirizine or imetit and cetirizine was more potent than these drugs used separately.

Effects of drugs on the increase in nasal eosinophils induced by antigen

The results are shown in Table 1. Neither Sch 50971 nor imetit significantly decreased the number of eosinophils induced by antigen even at a dose of 100 mg/kg. On the other hand, cetirizine significantly decreased the number of eosinophils at a dose of 10 mg/kg.

Discussion

In the present study, it was found that marked sneezing and nasal rubbing were observed after repeated topical application of antigen in sensitized BALB/c mice. In addition, an increase in PCA titer, indicating the production of antigen-specific IgE antibody, was also noted (data not shown). Almost the similar findings in rats were reported by Sugimoto et al. (15), who showed that the topical instillation of antigen resulted in a significant increase of sneezing and nasal rubbing as well as PCA titer in sensitized rats.

Sch 50971 is a potent histamine H_3 -receptor agonist with excellent *in vivo* receptor selectivity (12, 16).

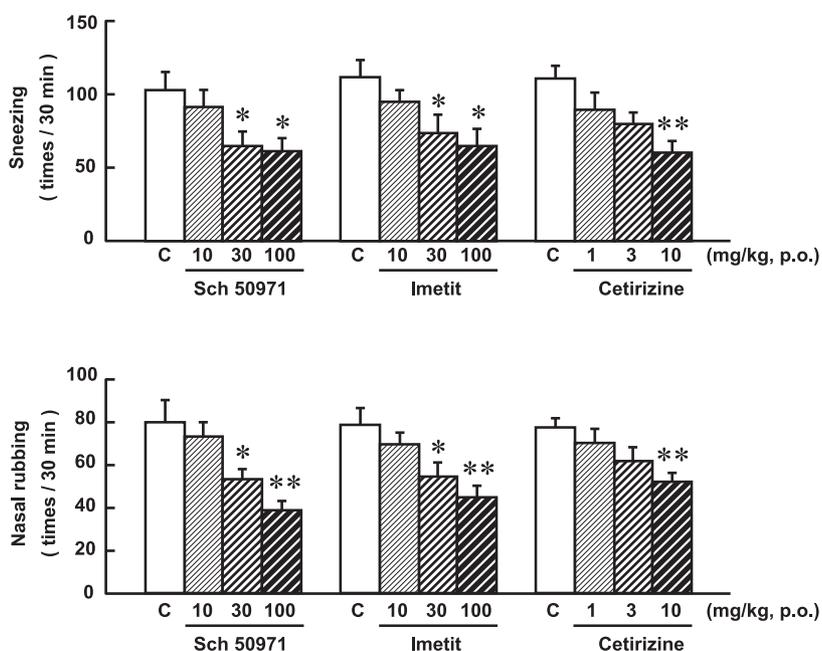


Fig. 2. Effects of histamine H_3 -receptor agonists and H_1 -receptor antagonist on sneezing and nasal rubbing induced by antigen in sensitized mice. Sch 50971 and cetirizine were orally administered 1 h before the measurement of nasal symptoms. Imetit was orally administered 2 h prior to measurement. Column and vertical bar of imetit group shows the mean \pm S.E.M. of 8 experiments, and the column and vertical bar of the Sch 50971 group and cetirizine group show the mean \pm S.E.M. of 10 experiments. *, **: Significantly different from the control group with $P < 0.05$ and $P < 0.01$, respectively.

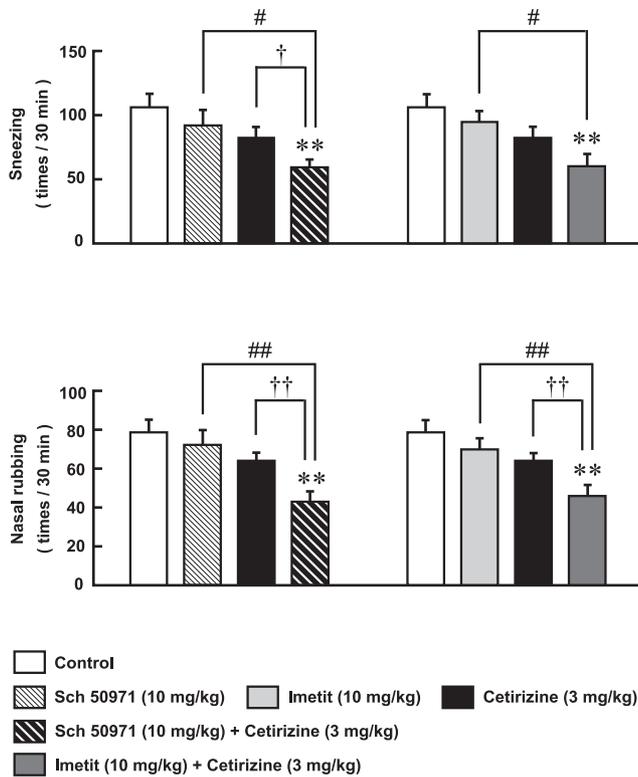


Fig. 3. Effects of the simultaneous use of H₃ agonists and H₁ antagonist on sneezing and nasal rubbing induced by antigen in sensitized mice. Sch 50971 and cetirizine were orally administered 1 h before the measurement of nasal symptoms. Imetit was orally administered 2 h prior to measurement. Column and vertical bar of imetit group shows the mean ± S.E.M. of 8 experiments, and the column and vertical bar of the other groups show the mean ± S.E.M. of 10 experiments. **: Significantly different from the control group with *P*<0.01. #, ##: Significantly different from the Sch 50971 (10 mg/kg) group or imetit (10 mg/kg) group with *P*<0.05 and *P*<0.01, respectively. †, ††: Significantly different from the cetirizine (3 mg/kg) group with *P*<0.05 and *P*<0.01, respectively.

Table 1. Effects of certain drugs on the increase in nasal eosinophils after antigen challenge in sensitized mice

Drugs	Dose (mg/kg)	Eosinophil influx
Control		111.1 ± 16.2
Sch 50971	30	99.6 ± 23.2
	100	107.8 ± 23.9
Imetit	30	98.7 ± 13.8
	100	105.0 ± 15.7
Cetirizine	3	79.0 ± 6.0
	10	69.0 ± 2.1*

Mice were immunized with ovalbumin, and 24 h after final challenge, the number of eosinophils was counted on both sides of the nasal septum. Sch 50971 and cetirizine were orally administered 1 h before the topical application of antigen. Imetit was orally administered 2 h before antigen challenge. Each value represents the mean ± S.E.M. of 5–7 experiments. *: Significantly different from the control group with *P*<0.05.

Imetit also displayed high affinity and efficacy as an H₃-receptor agonist (17, 18). As shown in the present data, oral administration of Sch 50971 and imetit significantly inhibited sneezing and nasal rubbing induced by an antigen, similar to cetirizine. These results are consistent with the findings that (*R*)- α -methylhistamine, a classical H₃-receptor agonist, inhibited nasal symptoms induced by antigen in mice (19). Cetirizine has high specificity for the histamine H₁ receptor and is well known to be effective in the treatment of allergic diseases (20). We have also demonstrated that the inhibitory effect of cetirizine on nasal signs occurred only through histamine H₁ receptors from the findings that the drug caused no inhibition of nasal symptoms in histamine H₁-receptor-deficient mice (21).

It has been thought that H₃ receptors are involved in allergic and inflammatory reactions, and H₃-receptor agonists might represent a novel class of anti-inflammatory agents. For instance, MacLeod et al. (12) reported that Sch 50971 might act to ameliorate the sequelae of migraine. In addition, it was also demonstrated that BP 2-94, an H₃-receptor agonist, appeared to be a promising novel therapeutic agent for disorders such as asthma, migraine, or a variety of inflammatory diseases (14, 22). These effects were supposed to be attributable to the inhibition of neuropeptide release from primary sensory endings. In addition, it is well known that H₃ receptors exist in nerve terminals and regulate the release of various chemical mediators (4). Nemmar et al. (23) reported that C-fibers of nerve terminals in afferent neurons contain neuropeptides such as substance P and that pretreatment with imetit inhibited capsaicin-induced substance P release. It was also demonstrated that (*R*)- α -methylhistamine inhibited the increase in substance P release induced by antidromic electrical stimulation (8). Furthermore, we have reported that substance P plays an important role in allergic rhinitis (24). From these findings, it seems likely that the activation of H₃ receptors with Sch 50971 or imetit decreased antigen-induced nasal signs resulting from the inhibition of substance P release from sensory nerve endings. In addition, H₃ receptors also exist in the central nervous system; therefore, H₃-receptor agonists can influence the general behavior of mice. However, there are no remarkable behavioral changes during the present experiments. Therefore, we assume that an inhibition of H₃ agonists on nasal allergy occurs through a peripheral effect.

As shown in the text, the simultaneous use of H₃-receptor agonists and H₁-receptor antagonist potentiated the inhibitory effects compared with when both drugs were used separately. In chronic allergic rhinitis, not only histamine but also other chemical mediators

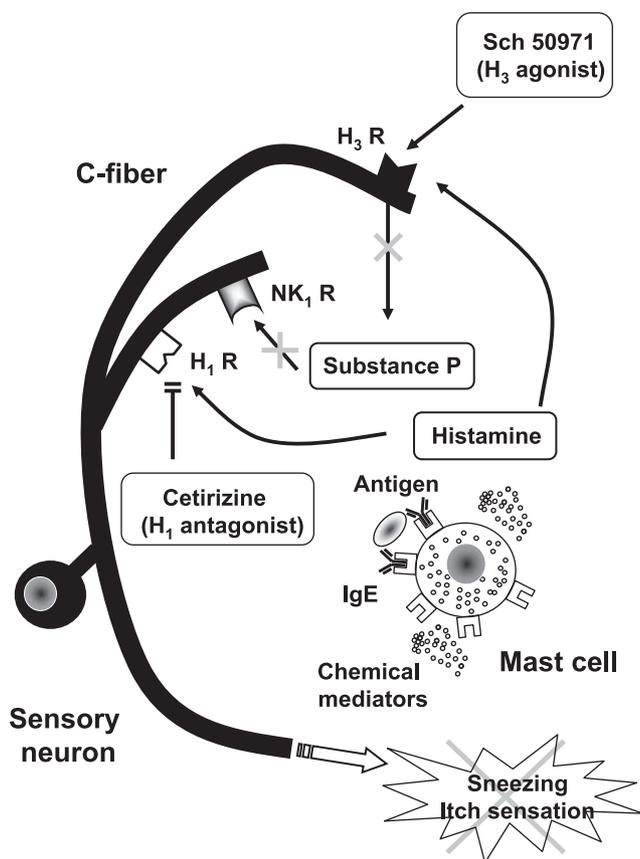


Fig. 4. A schematic diagram of the relationship between H_1 antagonism and H_3 stimulation in inhibition of substance P release from sensory nerve endings.

such as thromboxane, leukotriene, and substance P are responsible for allergic symptoms (3). Based on these findings, the combination of H_3 -receptor agonists and an H_1 -receptor antagonist may provide an additional benefit by inhibiting substance P release from the C-fiber of the sensory nerves via H_3 receptors that is not blocked by an H_1 -receptor antagonist. We show a schematic diagram about simultaneous use of H_3 -receptor agonists and H_1 -receptor antagonist (Fig. 4).

Eosinophils play a significant role in the pathophysiology of various allergic diseases and it is well known that they accumulate in the nasal mucosa in patients suffering from allergic rhinitis (25, 26). To investigate the role of H_3 receptors in the increase of nasal eosinophils, we studied the effects of H_3 -receptor agonists. As shown in the data, no effect was observed with Sch 50971 and imetit, even at a high dose. Nakayama et al. (27) reported that human and mouse eosinophils did not express H_3 receptors. In addition, H_3 receptors did not induce eosinophil chemotaxis, cell-shape change, and upregulation of adhesion molecules in an in vitro study (28). Taken together, these data indicate

that H_3 receptors are not involved in eosinophil influx into the nasal mucosa.

In conclusion, H_3 receptors are involved in the etiology of nasal allergy, and their stimulation may be useful as a novel therapeutic approach in allergic rhinitis.

References

- 1 Naclerio RM. Allergic rhinitis. *N Engl J Med.* 1991;325:860–869.
- 2 Bachert C. Histamine – a major role in allergy? *Clin Exp Allergy.* 1998;28 S6:15–19.
- 3 Howarth PH, Salagean M, Dokic D. Allergic rhinitis: not purely a histamine-related disease. *Allergy.* 2000;55:7–16.
- 4 Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature.* 1983;302:832–837.
- 5 Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International union of pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev.* 1997;49: 253–278.
- 6 Ichinose M, Barnes PJ. Inhibitory histamine H_3 -receptors on cholinergic nerves in human airways. *Eur J Pharmacol.* 1989; 163:383–386.
- 7 Koss MC, Hey JA. Prejunctional inhibition of sympathetically evoked pupillary dilation in cats by activation of histamine H_3 receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 1993;348: 141–145.
- 8 Ohkubo T, Shibata M, Inoue M, Kaya H, Takahashi H. Regulation of substance P release mediated via prejunctional histamine H_3 receptors. *Eur J Pharmacol.* 1995;273:83–88.
- 9 Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. *Ann Otol Rhinol Laryngol.* 2004;113:552–557.
- 10 Hossen MA, Sugimoto Y, Kayasuga R, Kamei C. Involvement of histamine H_3 receptors in scratching behaviour in mast cell-deficient mice. *Br J Dermatol.* 2003;149:17–22.
- 11 Hossen MA, Fujii Y, Sugimoto Y, Kayasuga R, Kamei C. Histamine H_3 receptors regulate vascular permeability changes in the skin of mast cell-deficient mice. *Int Immunopharmacol.* 2003;3:1563–1568.
- 12 McLeod RL, Aslanian R, del Prado M, Duffy R, Egan RW, Kreutner W, et al. Sch 50971, an orally active histamine H_3 receptor agonist, inhibits central neurogenic vascular inflammation and produces sedation in the guinea pig. *J Pharmacol Exp Ther.* 1998;287:43–50.
- 13 Millan-Guerrero RO, Isais-Millan R, Benjamin TH, Tene CE. N^{α} -Methyl histamine safety and efficacy in migraine prophylaxis: phase III study. *Can J Neurol Sci.* 2006;33:195–199.
- 14 Rouleau A, Stark H, Schunack W, Schwartz JC. Anti-inflammatory and antinociceptive properties of BP 2-94, a histamine H_3 -receptor agonist prodrug. *J Pharmacol Exp Ther.* 2000;295:219–225.
- 15 Sugimoto Y, Kawamoto E, Chen Z, Kamei C. A new model of allergic rhinitis in rats by topical sensitization and evaluation of H_1 -receptor antagonists. *Immunopharmacology.* 2000;8:1–7.
- 16 Hey JA, Aslanian R, Bolser DC, Chapman RW, Egan RW, Rizzo CA, et al. Studies on the pharmacology of the novel histamine H_3 receptor agonist Sch 50971. *Arzneimittelforschung.*

- 1998;48:881–888.
- 17 Garbarg M, Arrang JM, Rouleau A, Ligneau X, Tuong MD, Schwartz JC, et al. S-[2-(4-imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H₃ receptor agonist. *J Pharmacol Exp Ther.* 1992;263:304–310.
- 18 van der Goot H, Timmerman H. Selective ligands as tools to study histamine receptors. *Eur J Med Chem.* 2000;35:5–20.
- 19 Nakaya M, Fukushima Y, Takeuchi N, Kaga K. Nasal allergic response mediated by histamine H₃ receptors in murine allergic rhinitis. *Laryngoscope.* 2005;115:1778–1784.
- 20 Du Buske LM. Clinical comparison of histamine H₁-receptor antagonist drugs. *J Allergy Clin Immunol.* 1996;98:S307–S318.
- 21 Kayasuga R, Sugimoto Y, Watanabe T, Kamei C. Participation of chemical mediators other than histamine in nasal allergy signs: a study using mice lacking histamine H₁ receptors. *Eur J Pharmacol.* 2002;449:287–291.
- 22 Rouleau A, Garbarg M, Ligneau X, Manton C, Lavie P, Advenier C, et al. Bioavailability, antinociceptive and anti-inflammatory properties of BP 2-94, a histamine H₃ receptor agonist prodrug. *J Pharmacol Exp Ther.* 1997;281:1085–1094.
- 23 Nemmar A, Delaunois A, Beckers JF, Sulon J, Bloden S, Gustin P. Modulatory effect of imetit, 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.
- 24 Rahman MA, Inoue T, Kamei C. Role of substance P in allergic nasal symptoms in rats. *Eur J Pharmacol.* 2006;532:155–161.
- 25 Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol.* 2000;105:651–663.
- 26 Klementsson H. Eosinophils and the pathophysiology of allergic rhinitis. *Clin Exp Allergy.* 1992;22:1058–1064.
- 27 Nakayama T, Kato Y, Hieshima K, Nagakubo D, Kunori Y, Fujisawa T, et al. Liver-expressed chemokine/CC chemokine ligand 16 attracts eosinophils by interacting with histamine H₄ receptor. *J Immunol.* 2004;173:2078–2083.
- 28 Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L, Fung-Leung WP. Histamine H₄ receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule up-regulation. *Br J Pharmacol.* 2004;142:161–171.