Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 476 (2003) 229–237
www.elsevier.com/locate/ejphar

Kinins are involved in the development of allergic nasal hyperresponsiveness in guinea pigs

Shingo Sugahara[a], Takeshi Nabe[a], Nobuaki Mizutani[a], Hiroshi Takenaka[b], Shigekatsu Kohno[a,*]

[a] Department of Pharmacology, Kyoto Pharmaceutical University, 5 Nakauchi, Misasagi, Yamashina, Kyoto 607-8414, Japan
[b] Department of Otorhinolaryngology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki, Osaka 569-8686, Japan

Received 15 May 2003; received in revised form 21 July 2003; accepted 29 July 2003

Abstract

We evaluated roles of kinins in allergen-induced nasal blockage and sneezing, and development of nasal hyperresponsiveness to leukotriene D_4 in a Japanese cedar pollen-induced allergic rhinitis model of guinea pigs. Sensitised guinea pigs were repeatedly challenged by pollen inhalation once every week. Neither a bradykinin B_1 receptor antagonist, des-Arg^9-[Leu^8]bradykinin nor a bradykinin B_2 receptor antagonist, icatibant suppressed allergen-induced sneezing and nasal blockage. However, development of nasal hyperresponsiveness to leukotriene D_4 was significantly suppressed by them. The amount of bradykinin in nasal cavity lavage fluid was immediately increased after the challenge. In non-sensitised animals, hyperresponsiveness to leukotriene D_4 was developed by a bradykinin B_2 receptor agonist, bradykinin, but not by a bradykinin B_1 receptor agonist, des-Arg^{10}-kallidin, while in the sensitised-challenged animal, both agonists developed hyperresponsiveness. In conclusion, the nasal hyperresponsiveness appeared to be induced by kinins produced in response to the antigen challenge through activation of not only bradykinin B_2 but also B_1 receptors.

Keywords: Allergic rhinitis; Nasal blockage; Hyperresponsiveness; Bradykinin; Bradykinin B_1 receptor; Bradykinin B_2 receptor

1. Introduction

Characteristic symptoms of patients with allergic rhinitis are sneezing, rhinorrhea and nasal blockage (Naclerio, 1991). When specific allergens are applied to the nasal cavities of such patients, over 90% of them immediately start sneezing, and develop rhinorrhea and nasal blockage (Iliopoulos et al., 1990). About 50% of these patients further develop a late phase reaction with the predominant symptom being nasal blockage (Iliopoulos et al., 1990; Pelikan, 1978). Furthermore, the nasal responsiveness of patients with allergic rhinitis to stimuli other than a specific allergen increases compared with those of healthy individuals (Naclerio, 1991; Kanthawatana et al., 1997). The increase in nasal reactivity to stimuli that occurs after allergen provocation may resemble the reaction provoked by increased sensitivity to histamine (Walden et al., 1991).

We established a Japanese cedar pollen-induced allergic rhinitis model of guinea pigs that develop symptoms similar to those described above. Following intranasal active sensitisation by instillation with pollen extracts and aluminium hydroxide adjuvant, frequent sneezing is induced immediately (within 0–20 min), antigen-specific immunoglobulin (Ig) E antibody is produced, and allergen-induced biphasic nasal blockage develops (Nabe et al., 1997a, 1998). Furthermore, the nasal blockage response of sensitised animals to intranasal instillation of not only histamine (Mizutani et al., 1999) but also leukotriene D_4 (Mizutani et al., 2001) appears to be enhanced in proportion to the number of challenges. We have pharmacologically examined the induction mechanisms of these symptoms in regard to chemical mediators and found the following: (1) sneezing is mainly induced by histamine that is released, probably from mast cells (Nabe et al., 2001; Yamasaki et al., 2001a,b); (2) cysteinyl leukotrienes and thromboxane A_2 are involved in the induction of the late, but not early phase of nasal blockage induced by allergen (Yamasaki et al., 2001a,b); (3) exposure to anti-histaminics, a cysteinyl leukotriene antagonist and a thromboxane A_2 antagonist before the

* Corresponding author. Tel.: +81-75-595-4667; fax: +81-75-595-4764.
E-mail address: kohno@mb.kyoto-phu.ac.jp (S. Kohno).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02185-X

allergen challenge does not affect the development of nasal hyperresponsiveness when assessed 2 days after the challenge although a corticosteroid is effective (Yamasaki et al., 2001a,b). These findings indicate that, at least some of the chemical mediators that induce sneezing and the allergen-induced biphasic nasal blockage were elucidated, whereas how nasal hyperresponsiveness develops remains unclear.

A decrease in nasal patency (nasal blockage) is predominantly induced by (1) dilatation of the postcapillary venules and the cavernous venous sinusoids, in which the nasal mucosal tissue is rich, and (2) plasma leakage at the postcapillary venules followed by the induction of nasal mucosal oedema (Gerrelds et al., 1996). Indeed, we found that early and late nasal blockage induced by the pollen challenge in our model was potently suppressed by the vasoconstricting α-adrenoceptor agonist, naphazoline, and the constitutive nitric oxide synthase (cNOS) inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Imai et al., 2001). In addition, plasma leaked from the nasal tissue during the induction of allergen-induced nasal blockage (Mizutani, 2003). Thus, vasoactive mediators may induce nasal blockage.

Kinins such as bradykinin, kallidin, des-Arg^9-bradykinin and des-Arg^{10}-kallidin are potent vasoactive peptides. The bradykinin B_2 receptor agonists, bradykinin and kallidin, are generated in nasal secretions during allergic rhinitis (Proud et al., 1983). In addition, the intranasal application of these bradykinin B_2 receptor agonists increases nasal airway resistance in individuals with and without rhinitis (Rajakulasingam et al., 1991). The bradykinin B_2 receptor antagonist, icatibant, effectively suppresses the induction of nasal hyperresponsiveness to histamine assessed 24 h after allergen challenge but not allergen-induced nasal blockage determined 10 min after the challenge in individuals with seasonal allergic rhinitis (Turner et al., 2001). On the other hand, the bradykinin B_1 receptor agonist, des-Arg^{10}-kallidin, did not seem to cause any significant change after intranasal application even in patients with atopic rhinitis (Rajakulasingam et al., 1991). However, the bradykinin B_1 receptor is induced during allergic airway inflammation in animal models (Huang et al., 1999). As further support for the findings of animal studies, during the preparation of this manuscript, Christiansen et al. (2002) reported that patients with allergic rhinitis express significantly more bradykinin B_1 receptor mRNA than normal individuals. However, the involvement of bradykinin B_1 and B_2 receptor agonists in the induction of allergic rhinitis symptoms, especially late phase nasal blockage induced by allergen and nasal hyperresponsiveness to nonspecific stimuli, remains unclear.

We assessed whether kinins are involved in the induction of allergic rhinitis symptoms in our guinea pig model of allergic rhinitis. We initially evaluated the effects of the bradykinin B_2 receptor antagonist, icatibant, and the bradykinin B_1 receptor antagonist, des-Arg^9-[Leu^8]bradykinin, on the induction of allergen-induced sneezing and nasal blockage that is induced early and late after allergen challenge, and the development of nasal hyperresponsiveness to leukotriene D_4. In addition, we evaluated whether exogenous intranasal applications of the bradykinin B_1 receptor agonist, des-Arg^{10}-kallidin, and the bradykinin B_2 receptor agonist, bradykinin, induce nasal blockage and develop nasal hyperresponsiveness to leukotriene D_4 in sensitised-challenged and in non-sensitised animals.

2. Materials and methods

2.1. Animals

Male, 3-week-old, Hartley guinea pigs weighing 200–250 g purchased from Japan SLC (Hamamatsu, Japan) were housed in an air-conditioned room at 23 ± 1 °C and 60 ± 10% humidity and illuminated from 0800 to 2000 h. The animals were fed with a standard laboratory diet and given water ad libitum. The study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.2. Reagents

Reagents and their sources were as follows: Japanese cedar (*Cryptomeria japonica*) pollen (harvested in Gifu and Shiga prefectures in 1998), lidocaine hydrochloride (Fujisawa Pharmaceutical, Osaka, Japan), leukotriene D_4 (Cayman Chem., Ann Arbor, MI, USA), bradykinin, des-Arg^9-[Leu^8]bradykinin, des-Arg^{10}-kallidin and D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (HOE140, icatibant) (Peptide, Osaka, Japan), benzamidine hydrochloride, enalapril maleate, 1,10-phenanthroline, phenylmethylsulphonyl fluoride, phosphoramidon and soybean trypsin inhibitor (Sigma, St. Louis, MO, USA), aprotinin, bacitracin and 1,1,2-trichloro-1,2,2-trifluoroethane (Wako, Osaka, Japan), gelatin (Merck, Darmstadt, Germany), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, Nacalai Tesque, Kyoto, Japan).

Aluminium hydroxide [$Al(OH)_3$] gel was prepared with 0.5 N NaOH and 0.5 N $Al_2(SO_4)_3$ as described (Nabe et al., 1997b).

The cedar pollen extract used for sensitisation was prepared as described (Nabe et al., 1997a). In brief, the pollen was suspended in phosphate-buffered saline at 100 mg/ml and left at 4 °C for 18 h with mild stirring. The suspension was then centrifuged (1700 × *g*, 15 min), and the supernatant was stored at − 80 °C until use as the sensitisation antigen.

2.3. Sensitisation and challenge with cedar pollen

Guinea pigs were sensitised by intranasal instillation of cedar pollen extracts adsorbed on $Al(OH)_3$ gel into the bilateral cavities at a dose of 0.3 μg protein/0.3 mg

$Al(OH)_3$/3 μl/nostril twice each day for 7 days (Nabe et al., 1998). Prior to each sensitisation, the upper airway mucosal surface was topically anaesthetized by a 5-min inhalation of 4% lidocaine mist generated using an ultrasonic nebulizer (NE-U12, Omron, Osaka, Japan) to prevent the rapid elimination of antigen by ciliary movement. The sensitised animal was intranasally challenged once every week by inhalation of 1.8 mg of cedar pollen per nostril (3.6 mg/animal) using a hand-made inhalation apparatus (Nabe et al., 1997a). Upon application to both nostrils of the spontaneously breathing guinea pig, almost all inhaled pollens were trapped in the upper airways (Nabe et al., 1997a).

2.4. Measurement of specific airway resistance (sRaw)

As an indicator of respiratory function (nasal blockage), the sRaw in conscious guinea pigs was measured using a two-chambered, double-flow plethysmograph system (Pulmos-I, M.I.P.S., Osaka, Japan) as described by Pennock et al. (1979). In brief, an animal was placed with its neck extending through the partition of a two-chambered box. Sensors at both the front and rear chambers detected airflow, then the sRaw was measured using the data analyser Pulmos-I and a PC 9801 FA computer (NEC, Tokyo).

Because the guinea pig functionally respires through the nose but not through the mouth, sRaw can be taken as the total resistance of the upper and lower airways in the animal. However, when the pollen was forced to inhale to spontaneously breathing conscious guinea pigs as described above, almost all of the inhaled pollen was trapped in the upper airways, but less than 0.001% reached to the lower airways (Nabe et al., 1997a). Furthermore, although antigen inhalation-induced early bronchoconstrictor response has been characterised by rapid and shallow breathing in a guinea pig model of asthma (Iijima et al., 1987), the pollen inhalation challenge-induced elevation of sRaw correlated well with the decrease in respiratory frequency in the present experimental allergic rhinitis model (Nabe et al., 1998). In addition, eosinophil accumulation in the lung, which is a characteristic feature of asthmmatic response, was not observed at 5 h after the pollen inhalation challenge in this model (Yamasaki et al., 2001a) although eosinophils markedly increased in the nasal cavity lavage fluid at 5 h after the challenge (Yamasaki et al., 2001b). These findings indicate that change in sRaw induced by the pollen challenge can be considered to reflect upper airway obstruction in our model.

2.5. Effects of bradykinin B_1 and B_2 receptor antagonists on antigen-induced nasal symptoms

To evaluate involvement of kinins in antigen-induced sneezing, biphasic nasal blockage, and the development of nasal hyperresponsiveness to leukotriene D_4, the bradykinin B_1 and B_2 receptor antagonists, des-Arg^9-[Leu^8]bradykinin (60 nmol/kg) and icatibant (10 nmol/kg), or vehicle (physiological saline) were intravenously administered 5 min before the 8th pollen inhalation challenge. The doses of antagonists were sufficient to block the respective receptors and not much higher than those applied in other in vivo studies (Dray and Perkins, 1993; Wirth et al., 1991). The effects on the time-course of sRaw were examined before, then 10 min, 1, 2, 3, 4, 6, 8 and 10 h after the 8th challenge. The magnitudes of the respective early and late phase responses are expressed as the area under the response curve (AUC) from 0 (before) to 3 h and from 3 to 10 h after the antigen challenge. Sneezing frequency was counted between 0 and 10 min and between 10 and 60 min.

Effects of the bradykinin B_1 and B_2 receptor antagonists on the development of nasal hyperresponsiveness to leukotriene D_4 were evaluated 2 days after the 8th pollen challenge. As described (Mizutani et al., 2001; Nabe et al., 2001), 10 μl/nostril of 10^{-8} and 10^{-6} M of leukotriene D_4 were consecutively instilled into the bilateral nasal cavities at an interval of 20 min. The sRaw value was measured before and 10 min after each set of two leukotriene D_4 instillations. Because leukotriene D_4 induced dose-dependent increase of sRaw in the sensitised-challenged animals at 10^{-12}–10^{-6} M (Mizutani et al., 2001), we used 10^{-8} and 10^{-6} M in the present study.

As shown in our previous report (Mizutani et al., 2001) and the present literature, administration of leukotriene D_4, a well-known potent bronchoconstrictor, into the nasal cavities of the non-sensitised guinea pig produced no elevation of sRaw even at 10^{-6} M. In addition, Narita et al. (1997) reported that most Evans blue dye instilled intranasally was found within the nasal cavity. Thus, it can be concluded that the changes in sRaw induced by instillation of leukotriene D_4 in the present manner entirely reflect the nasal response and not the lower airway response.

2.6. Des-Arg^{10}-kallidin and bradykinin-induced nasal blockage and nasal hyperresponsiveness to leukotriene D_4

Des-Arg^{10}-kallidin (10^{-7}, 10^{-5} or 10^{-3} M) and bradykinin (10^{-7}, 10^{-5} or 10^{-3} M; 10 μl/nostril) were instilled into the bilateral nasal cavities of non-sensitised and sensitised guinea pigs. In the sensitised animal, the kinins were administered 7 days after the 9th pollen inhalation challenge. We reported that the allergen-induced nasal hyperresponsiveness to histamine and leukotriene D_4 almost completely disappears by 7 days after each pollen challenge (Mizutani et al., 1999, 2001). We measured sRaw before, then 2, 5, 10, 20, 40 and 60 min after the intranasal administration of des-Arg^{10}-kallidin or bradykinin. When evaluating dose-dependent responses of des-Arg^{10}-kallidin and bradykinin, 10^{-7}, 10^{-5} or 10^{-3} M of each were administered to different animals, and then sRaw was measured 5 min after the intranasal administration.

Table 1
Effects of des-Arg^9-[Leu^8]bradykinin and icatibant on sneezing and early and late phase nasal blockage induced by the 8th pollen inhalation challenge in sensitised guinea pigs

Drug	Number of animals	Sneezing frequency (numbers of episodes)		Nasal blockage (increase in AUC, cm H_2O ml/(ml/s) h)	
		0–10 min	10 min^{-1} h	Early phase ($AUC_{0-3\ h}$)	Late phase ($AUC_{3-10\ h}$)
Control	29	8.0 ± 1.1	10.2 ± 1.4	3.7 ± 0.5	3.9 ± 0.8
Des-Arg^9-[Leu^8]bradykinin	15	7.6 ± 1.3	11.5 ± 3.1	3.7 ± 0.8	4.8 ± 0.7
Icatibant	15	7.3 ± 2.0	11.3 ± 1.7	2.5 ± 0.5	4.3 ± 1.2
Des-Arg^9-[Leu^8]bradykinin + Icatibant	19	10.2 ± 2.1	12.1 ± 1.9	2.9 ± 0.4	5.0 ± 1.0

Des-Arg^9-[Leu^8]bradykinin (60 nmol/kg) and icatibant (10 nmol/kg) were administered intravenously 5 min before antigen challenge. Sneezing frequency was determined 0–10 min and 10 min–1 h later. Early and late phase increases in specific airway resistance (sRaw) were determined by calculating area under response curve (AUC) [cm H_2O ml/(ml/s) h] for change in sRaw from 0 (before) to 3 h and 3 to 10 h, respectively, after challenge. Values are means ± S.E. of indicated number of animals.

The acquisition of nasal hyperresponsiveness to leukotriene D_4 in guinea pigs was evaluated 4 h and 2 days after exposure to des-Arg^{10}-kallidin and bradykinin as described above.

2.7. Measurement of concentration of bradykinin in nasal cavity lavage fluid

The nasal cavity of the sensitised animal was lavaged with saline before, and 10, 20, 60, 120 and 240 min after the 11th pollen inhalation challenge. Because repeated nasal cavity lavage induces a nonspecific increase of neutrophils in the lavage fluid (Nabe et al., 1997a), we tested different animals at the respective time periods in this study. The nasal cavity lavage proceeded under pentobarbital anaesthesia (30 mg/kg, i.p.) as described (Nabe et al., 1997a). One side of a silicone tube (outside diameter: 3 mm, inside diameter: 1.5 mm), the other side of which was connected to an air pump, was positioned on the right nostril and under reduced pressure (−0.19 atmospheres at complete plugging) by the air pump, warm saline (1 ml at 37 °C) was aspirated from the left nostril via the silicone tube. We collected nasal cavity lavage fluid from the right nostril into a tube containing 200 μl of protease inhibitor cocktail (2.5 mg/ml soybean trypsin inhibitor, 2 mg/ml 1,10-phenanthroline, 5000 U/ml aprotinin, 10 μg/ml benzamidine, 1.7 μg/ml phenylmethylsulphonyl fluoride, 2 mg/ml EDTA, 10 μg/ml enalapril and 50 μg/ml phosphoramidon).

The supernatant from the nasal cavity lavage fluid was treated with 80% ethanol to remove contaminating proteins. Gelatin (0.2%, 40 μl/tube) was added and the supernatant was evaporated to dryness under reduced pressure and dissolved in 0.1 M phosphate buffer containing 0.1% bovine serum albumin. Lipids were removed with 1,1,2-trichloro-1,2,2-trifluoroethane then the amount of bradykinin in the sample was assayed using an enzyme immunoassay kit (Peninsula, San Carlos, CA, USA). Cross-reactivity of the anti-bradykinin antibody in the kit to bradykinin, kallidin and des-Arg^9-bradykinin is 100%, 100% and 0%, respectively. The antibody does not react with angiotensin II, Met-enkephalin, substance P or endothelin-1.

2.8. Statistical analyses

Data were statistically evaluated by the one-way analysis of variance. If a difference appeared significant, the individual group difference was determined using Bonferroni's multiple tests. A p value below 0.05 was considered statistically significant.

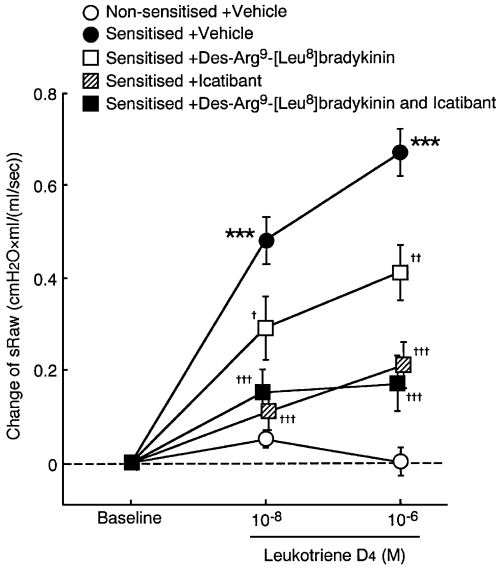

Fig. 1. Effects of icatibant and des-Arg^9-[Leu^8]bradykinin on development of nasal hyperresponsiveness to leukotriene D_4 induced by the 8th pollen inhalation challenge in sensitised guinea pigs. Des-Arg^9-[Leu^8]bradykinin (60 nmol/kg) and icatibant (10 nmol/kg) were administered intravenously 5 min before antigen challenge. Nasal hyperresponsiveness to leukotriene D_4 was evaluated 2 days after the 8th antigen challenge. Data are means ± S.E. of number of animals shown in parentheses. ***$P<0.001$ vs. non-sensitised + vehicle. †$P<0.05$, ††$P<0.01$ and †††$P<0.001$ vs. sensitised + vehicle.

3. Result

3.1. Effects of bradykinin B_1 and B_2 receptor antagonists on antigen-induced nasal symptoms

Table 1 shows the effects of des-Arg^9-[Leu^8]bradykinin and icatibant alone and in combination on sneezing and biphasic nasal blockage induced by the 8th pollen inhalation challenge. Eighteen sneezes were induced within 1 h after the pollen challenge, whereas sneezing was very infrequent between 1 and 10 h later. We have reported that the pollen inhalation induced three to four sneezes within 1 h after the exposure in non-sensitised guinea pigs (Nabe et al., 1998, 2001; Yamasaki et al., 2001a). On the other hand, allergen-induced nasal blockage is reproducibly biphasic with peaks at 1–2 (early phase) and at 4–6 (late phase) h. The pollen inhalation did not change sRaw of non-sensitised guinea pigs (Nabe et al., 1998, 2001; Yamasaki et al., 2001a). Therefore, we determined early and late phase increases in sRaw by calculating the area under the response curve (AUC). Neither des-Arg^9-[Leu^8]bradykinin nor icatibant affected the induction of sneezing and biphasic nasal blockage. A combination of the two antagonists also did not inhibit on the occurrence of these nasal symptoms.

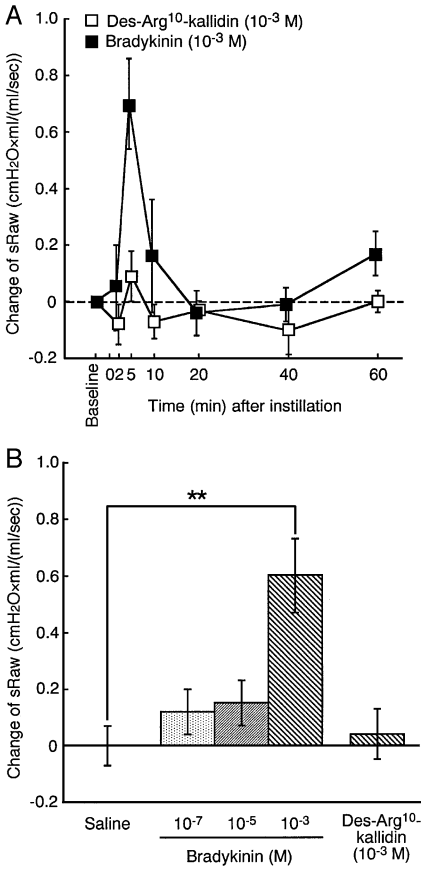

Fig. 2. Time-course (A) and dose–response (B) of changes in specific airway resistance (sRaw) induced by intranasal instillation of des-Arg^{10}-kallidin and bradykinin in non-sensitised guinea pigs. (B) sRaw measured 5 min after instillation. Data are means ± S.E. of five to seven guinea pigs. $^{**}P<0.01$.

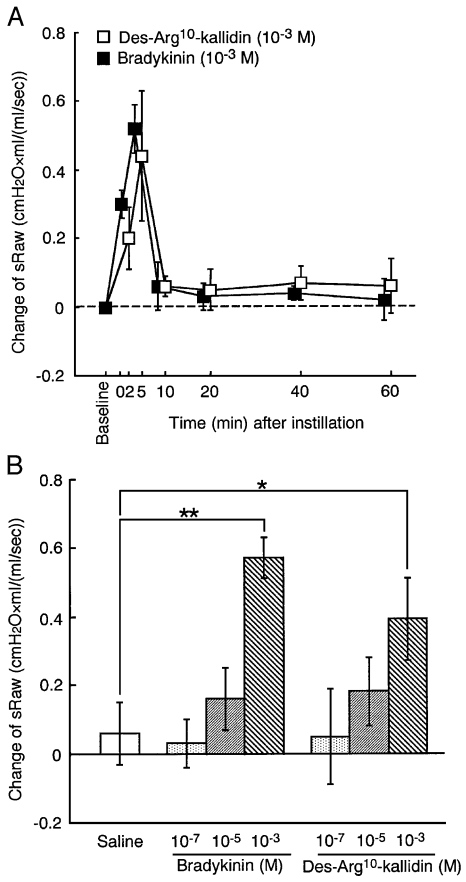

Fig. 3. Time-course (A) and dose–response (B) of changes in specific airway resistance (sRaw) induced by intranasal instillation of des-Arg^{10}-kallidin and bradykinin in sensitised guinea pigs. Kinins were administered 7 days after the 9th pollen inhalation challenge. (B) sRaw was measured 5 min after instillation. Data are means ± S.E. of five to eight guinea pigs. $^{*}P<0.05$ and $^{**}P<0.01$.

Fig. 1 shows the effects of the antagonists on the development of nasal hyperresponsiveness to leukotriene D_4 that developed 2 days after the pollen challenge. Both des-Arg^9-[Leu^8]bradykinin and icatibant significantly suppressed the induction of nasal hyperresponsiveness when administered before the pollen challenge but not before the leukotriene D_4 application by approximately 40% and 80%, respectively. Both antagonists inhibited the induction to a similar degree to the group given icatibant when administered simultaneously.

3.2. Des-Arg^{10}-kallidin and bradykinin-induced nasal blockage and nasal hyperresponsiveness to leukotriene D_4

Fig. 2A and B shows that bradykinin swiftly increased the sRaw of the non-sensitised guinea pigs peaking at 5 min after the application (Fig. 2A) and that the effect was dose-dependent at 10^{-7}–10^{-3} M (Fig. 2B). However, des-

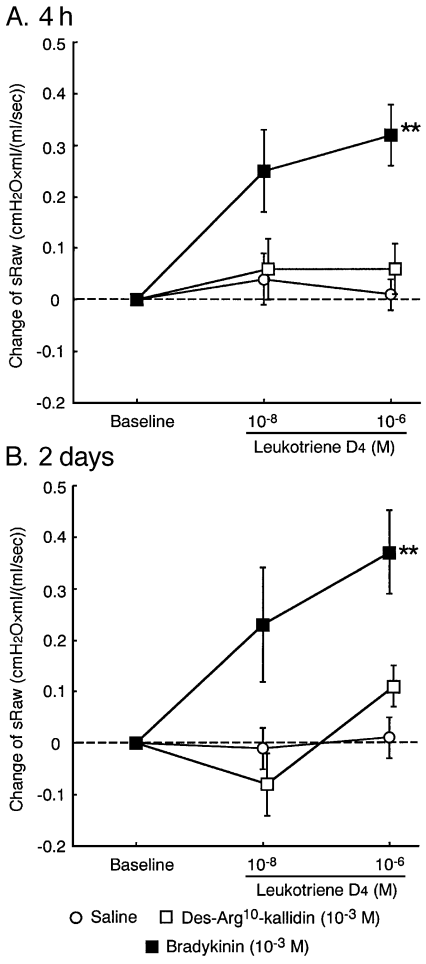

Fig. 4. Time-course of changes in nasal responsiveness to leukotriene D_4 4 h (A) and 2 days (B) after intranasal instillation of des-Arg^{10}-kallidin (10^{-3} M) and bradykinin (10^{-3} M) in the non-sensitised guinea pig. Data are presented as means ± S.E. of six to eight animals. $^{**}P<0.01$ vs. saline.

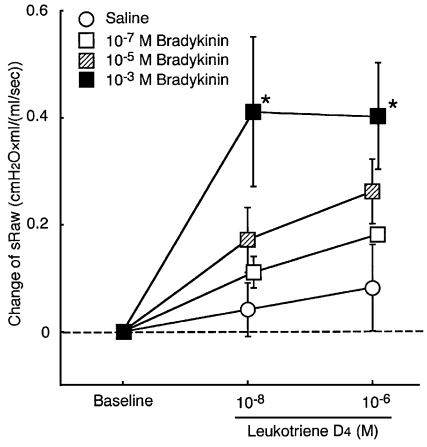

Fig. 5. Dose–response of changes in nasal hyperresponsiveness to leukotriene D_4 induced by intranasal instillation of bradykinin in non-sensitised guinea pigs. Nasal hyperresponsiveness to leukotriene D_4 was assessed 4 h after administration of bradykinin. Data are means ± S.E. of six to eight animals. $^{*}P<0.05$ vs. saline.

Arg^{10}-kallidin did not affect the sRaw of non-sensitised guinea pigs even at 10^{-3} M (Fig. 2A and B).

On the other hand, both bradykinin and des-Arg^{10}-kallidin induced dose-dependent increases of sRaw in the sensitised-challenged guinea pig (Fig. 3A and B). The time-course and magnitude of the des-Arg^{10}-kallidin-induced sRaw increase were similar to those of the bradykinin-induced temporal nasal blockage (Fig. 3A and B).

We assessed whether nasal hyperresponsiveness to leukotriene D_4 is induced by a single dose of bradykinin and des-Arg^{10}-kallidin 4 h and 2 days after application in non-sensitised and sensitised-challenged animals. Fig. 4A and B shows that nasal hyperresponsiveness to leukotriene D_4 apparently developed only in non-sensitised guinea pigs exposed to bradykinin both at 4 h and on day 2. The induction was dose-dependent at 10^{-7}–10^{-3} M (Fig. 5). However, in the sensitised-challenged guinea pig, both bradykinin and des-Arg^{10}-kallidin after 4 h and 2 days caused nasal hyperresponsiveness to leukotriene D_4 (Fig.

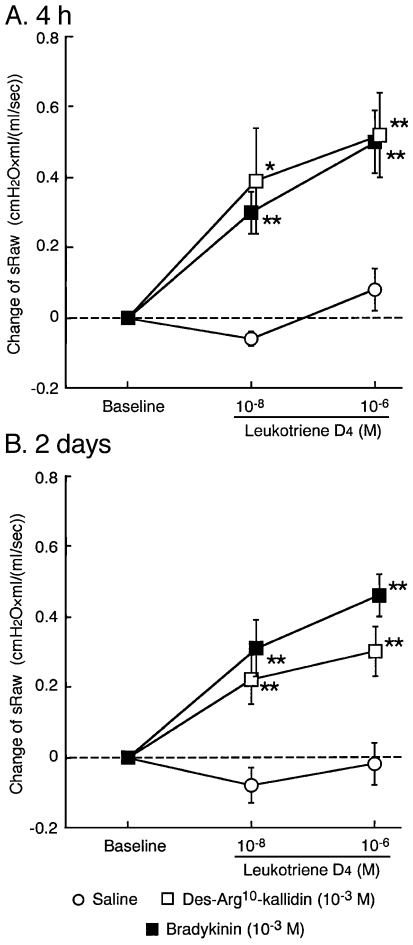

Fig. 6. Time-course of changes in nasal responsiveness to leukotriene D_4 4 h (A) and 2 days (B) after intranasal instillation of des-Arg^{10}-kallidin (10^{-3} M) and bradykinin (10^{-3} M) in sensitised guinea pigs. Kinins were administered 7 days after the 9th pollen inhalation challenge. Data are means ± S.E. of six or seven animals. $^{*}P<0.05$ and $^{**}P<0.01$ vs. saline.

6A and B). Like the non-sensitised animals, the bradykinin induction was dose-dependent in the sensitised-challenged animal (Fig. 7B). The bradykinin B_1 receptor agonist at 10^{-7} M caused no significant change in responsiveness to leukotriene D_4 even in the sensitised-challenged animal, yet the hyperresponsiveness induced by 10^{-5} and 10^{-3} M des-Arg^{10}-kallidin was of a similar magnitude (Fig. 7A).

3.3. Time-course change in amount of bradykinin in nasal cavity lavage fluid

Fig. 8 shows the time-course of the amount of bradykinin in nasal cavity lavage fluid after the pollen challenge. The allergen challenge immediately induced obvious production of bradykinin with a peak at 10 min. The increased amount of bradykinin gradually decreased over 240 min after the challenge. However, levels of the kinin at 180 and 240 min were still significantly higher than those before the challenge. We could not measure endogenous des-Arg^{10}-kallidin because a system with an antibody that can react with kinins lacking Arg at the carboxyl terminus is not yet commercially available.

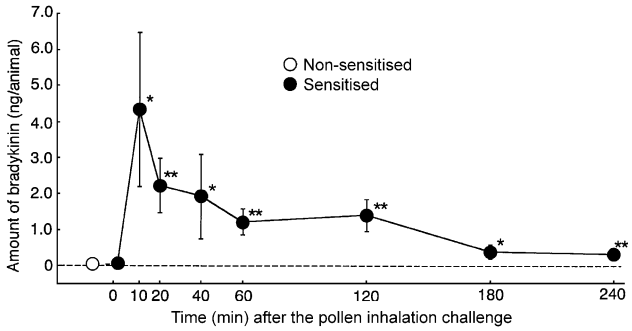

Fig. 8. Time-course of changes in amount of bradykinin in nasal cavity lavage fluid after the 11th pollen inhalation challenge in sensitised guinea pigs. Data are means ± S.E. of 5–10 animals. *$P<0.05$ and **$P<0.01$ vs. value before challenge.

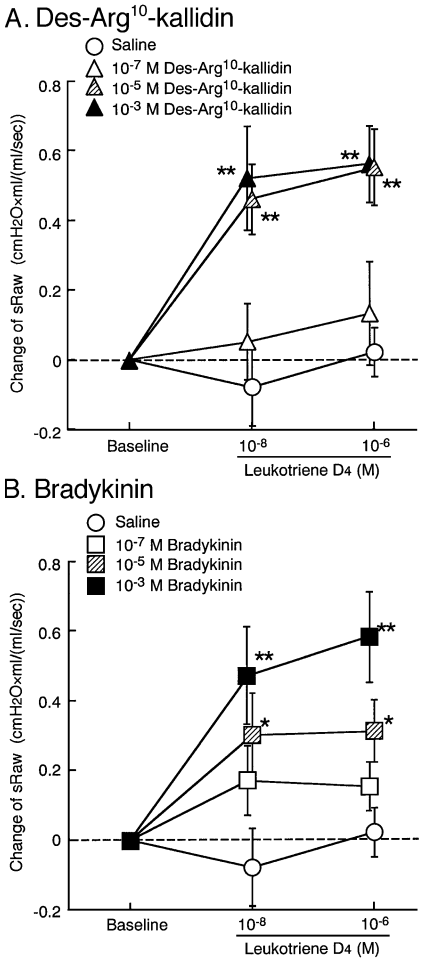

Fig. 7. Dose–response of changes in nasal hyperresponsiveness to leukotriene D_4 induced by intranasal instillation of des-Arg^{10}-kallidin (A) and bradykinin (B) in sensitised guinea pigs. Kinins were administered 7 days after the 9th pollen inhalation challenge then nasal hyperresponsiveness to leukotriene D_4 4 h was evaluated. Data are presented as means ± S.E. of five to eight animals. *$P<0.05$ and **$P<0.01$ vs. saline.

4. Discussion

In agreement with a clinical examination (Turner et al., 2001), the bradykinin B_2 receptor antagonist, icatibant, potently suppressed the development of nasal hyperresponsiveness to leukotriene D_4. The bradykinin B_1 receptor antagonist, des-Arg^9-[Leu^8]bradykinin, also significantly inhibited the induction of hyperresponsiveness. To our knowledge, we are the first to demonstrate that a bradykinin B_1 receptor antagonist can suppress induction of nasal hyperresponsiveness to a stimulus in allergic rhinitis. In addition, the intranasal instillation of not only the bradykinin B_2 receptor agonist, bradykinin, but also the bradykinin B_1 receptor agonist, des-Arg^{10}-kallidin, induced increased nasal responsiveness to leukotriene D_4 in the sensitised-challenged guinea pig. These results indicate that both bradykinin B_1 and B_2 receptor agonists that should be endogenously produced by the antigen-antibody reaction are significantly involved in the development of nasal hyperresponsiveness to leukotriene D_4.

Nasal hyperresponsiveness to leukotriene D_4 is induced even at 4 h after a pollen challenge, persists at least for 2 days and disappears by day 7 in our model of allergic rhinitis (Mizutani et al., 2001). Consistent with this time-course in the induction and remission, a single dose of bradykinin or des-Arg^{10}-kallidin increased responsiveness to leukotriene D_4 in guinea pig nasal tissue at 4 h and on day 2. In addition, the hyperresponsiveness induced by kinins disappeared 7 days after the application (data not shown). Furthermore, bradykinin was obviously produced immediately and persistently after the pollen challenge. These findings confirmed that kinins are key mediators that are involved in producing nasal hyperresponsiveness in the allergic rhinitis model. However, at present, we have no

evidence showing detailed mechanisms of nasal hyperresponsiveness induced by kinins. As demonstrated in lower airways of guinea pigs (Kamijo et al., 2001), the kinin-induced nasal hyperresponsiveness may also be mediated by release of neurokinin A at the nasal tissues.

In contrast to the finding that the bradykinin B_1 receptor agonist increased nasal responsiveness to leukotriene D_4 in the sensitised-challenged guinea pig, that of the non-sensitised animal was not increased even by a high concentration (10^{-3} M) of des-Arg^{10}-kallidin. On the other hand, the magnitude of the hyperresponsiveness induced by the bradykinin B_2 receptor agonist in the non-sensitised guinea pig was similar to that in the sensitised animal. Bradykinin B_2 receptors are constitutively expressed on many cell types (Hall, 1992) including airway epithelial cells (Lung et al., 1998), whereas bradykinin B_1 receptors are expressed at low levels in normal tissue but can be induced in response to pathophysiological stimuli (DeBlois et al., 1991; Marceau et al., 1998). In our allergic rhinitis model, the degree of the nasal hyperresponsiveness to leukotriene D_4 increases in proportion to the number of pollen inhalation challenges (Mizutani et al., 2001). Thus, bradykinin B_1 receptor expression should be upregulated in the nasal tissues by the inflammatory events elicited by repeated antigen challenge. In fact, bradykinin B_1 receptor mRNA is upregulated in patients with allergic rhinitis (Christiansen et al., 2002). On the other hand, the amount of inhibition caused by the combination of icatibant and des-Arg^9-[Leu^8]bradykinin was almost identical to that induced by a single dose of icatibant. Thus, the mechanisms of nasal hyperresponsiveness induced by bradykinin B_1 and B_2 receptor agonists may be similar or partly associated.

Although they inhibited development of the nasal hyperresponsiveness, neither icatibant nor des-Arg^9-[Leu^8]bradykinin affected allergen-induced early or late phase nasal blockage and sneezing. Because NO is closely involved in both the early and late phase nasal blockage induced by allergen challenge (Imai et al., 2001), we speculated that kinins, which are vasoactive mediators, could stimulate cNOS. However, bradykinin B_1 and B_2 receptor agonists produced endogenously after a pollen challenge are probably unrelated to the induction of biphasic nasal blockage. The present findings that intranasal doses of both bradykinin and des-Arg^{10}-kallidin can induce temporal but significant nasal blockage in sensitised-challenged animals seem to conflict with the notion that bradykinin B_1 and B_2 receptor antagonists do not affect allergen-induced early phase nasal blockage. However, a very high concentration (10^{-3} M) of kinins was required to induce significant changes in nasal patency. The amount of kinins produced after a pollen challenge should be insufficient to directly induce nasal blockage. The finding that bradykinin B_1 and B_2 receptor antagonists inhibit the induction of nasal hyperresponsiveness but do not affect biphasic nasal blockage and sneeze further indicates that the induction mechanisms of these nasal symptoms are quite different.

Bradykinin was produced and histamine was also released into the nasal cavity lavage fluid immediately after a pollen challenge in our model (Yamasaki et al., 2001a). When guinea pigs were passively sensitised with an antiserum containing high titre of IgE antibody against Japanese cedar pollen, and then challenged once by pollen inhalation, nasal responsiveness to leukotriene D_4 was significantly increased although to a lesser extent than that in the actively sensitised, repeatedly challenged animal (Nabe et al., 2001). This suggests that IgE and mast cells are required to induce nasal hyperresponsiveness to leukotriene D_4. Mast cells containing tryptase but not chymase (MC_T) are predominantly located at the mucosal tissues in the respiratory and gastrointestinal tracts (Walls, 2000). Furthermore, tryptase possesses kininogenase activity with both low and high molecular weight kininogens, leading kinin production (Proud et al., 1988; Walls et al., 1992; Imamura et al., 1996). Like the immediate increases in bradykinin and histamine after a pollen challenge in our model, the bronchoalveolar lavage fluid concentration of tryptase and histamine increases within minutes of introducing an allergen into the lower airways of patients with atopic asthma (Wenzel et al., 1988) and allergic rhinitis (Sedgwick et al., 1991). Thus, kinins may be produced through the kininogenase activity of tryptase, which is likely to be released from the nasal mucosal mast cells immediately after an antigen challenge.

In conclusion, kinins appear to be significantly involved in the allergen-induced development of nasal hyperresponsiveness to leukotriene D_4 via the stimulation of bradykinin B_1 and B_2 receptors in the nasal tissues. Thus, bradykinin B_1 and B_2 receptor antagonists might be useful therapeutic drugs for treating patients with allergic rhinitis.

Acknowledgements

This work was supported by the Promotion and Mutual Aid for Private Schools of Japan, and a Grant-in-Aid for Scientific Research (c) (15590080) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Christiansen, S.C., Eddleston, J., Woessner, K.M., Chambers, S.S., Ye, R., Pan, Z.K., Zuraw, B.L., 2002. Up-regulation of functional kinin B_1 receptors in allergic airway inflammation. J. Immunol. 169, 2054–2060.

DeBlois, D., Bouthillier, J., Marceau, F., 1991. Pulse exposure to protein synthesis inhibitors enhances vascular responses to des-Arg^9-bradykinin: possible role of interleukin-1. Br. J. Pharmacol. 103, 1057–1066.

Dray, A., Perkins, M., 1993. Bradykinin and inflammatory pain. Trends Neurosci. 16, 99–104.

Gerrelds, I.M., de Graaf-in't Veld, C., Gerth van Wijk, R., Zijlstra, F.J., 1996. Nasal hyperreactivity and inflammation in allergic rhinitis. Mediat. Inflamm. 5, 79–94.

Hall, J.M., 1992. Bradykinin receptors: pharmacological properties and biological roles. Pharmacol. Ther. 56, 131–190.

Huang, T.J., Haddad, E.B., Fox, A.J., Salmon, M., Jones, C., Burgess, G., Chung, K.F., 1999. Contribution of bradykinin B_1 and B_2 receptors in allergen-induced bronchial hyperresponsiveness. Am. J. Respir. Crit. Care Med. 160, 1717–1723.

Iijima, H., Ishii, M., Yamauchi, K., Chao, C.-L., Kimura, K., Shimura, S., Shindoh, Y., Inoue, H., Mue, S., Takishima, T., 1987. Bronchoalveolar lavage and histologic characterization of late asthmatic response in guinea pigs. Am. Rev. Respir. Dis. 136, 922–929.

Iliopoulos, O., Proud, D., Adkinson Jr., N.F., Norman, P.S., Kagey-Sobotka, A., Lichtenstein, L.M., Naclerio, R.M., 1990. Relationship between the early, late, and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. J. Allergy Clin. Immunol. 86, 851–861.

Imai, A., Nabe, T., Mizutani, N., Sakurai, H., Takenaka, H., Kohno, S., 2001. Involvement of nitric oxide in pollen-induced biphasic nasal blockage in sensitised guinea pigs. Eur. J. Pharmacol. 423, 63–70.

Imamura, T., Dubin, A., Moore, W., Tanaka, R., Travis, J., 1996. Induction of vascular permeability enhancement by human tryptase: dependence on activation of prekallikrein and direct release of bradykinin from kininogens. Lab. Invest. 74, 861–870.

Kamijo, Y., Hayashi, I., Soma, K., Ohwada, T., Majima, M., 2001. Effect of neutral endopeptidase inhibitor on bradykinin-induced bronchoconstriction. Life Sci. 70, 1–15.

Kanthawatana, S., Maturim, W., Fooanant, S., Manorot, M., Trakultivakorn, M., 1997. Evaluation of threshold criteria for the nasal histamine challenge test in perennial allergic rhinitis. Asian Pac. J. Allergy Immunol. 15, 65–69.

Lung, C.C., Jagels, M.A., Daffern, P.J., Tan, E.M., Zuraw, B.L., 1998. Induction of human B_2 bradykinin receptor mRNA and membrane receptors by IFNγ. Immunopharmacology 39, 243–253.

Marceau, F., Hess, J.F., Bachvarov, D.R., 1998. The B_1 receptors for kinins. Pharmacol. Rev. 50, 357–386.

Mizutani, N., 2003. Studies on the experimental allergic rhinitis induced by Japanese cedar pollen—role of cysteinyl leukotrienes in nasal allergic symptoms. Yakugaku Zasshi 123, 1–8 (Abstract in English).

Mizutani, N., Nabe, T., Sasaki, K., Takenaka, H., Kohno, S., 1999. Nasal hyperresponsiveness to histamine induced by repetitive exposures to cedar pollen in guinea pigs. Eur. Respir. J. 14, 1368–1375.

Mizutani, N., Nabe, T., Imai, A., Sakurai, S., Takenaka, H., Kohno, S., 2001. Markedly increased nasal blockage by intranasal leukotriene D_4 in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. Jpn. J. Pharmacol. 86, 170–182.

Nabe, T., Shimizu, K., Mizutani, N., Saeki, Y., Yamamura, H., Takenaka, H., Kohno, S., 1997a. A new model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs. Jpn. J. Pharmacol. 75, 243–251.

Nabe, T., Shinoda, N., Yamashita, K., Yamada, M., Yamamura, H., Kohno, S., 1997b. Comparative studies on nebulizers for antigen inhalation in experimental asthma. Allergol. Intern. 46, 261–267.

Nabe, T., Mizutani, N., Shimizu, K., Takenaka, H., Kohno, S., 1998. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. Inflamm. Res. 47, 369–374.

Nabe, T., Mizutani, N., Osaki, S., Sugahara, S., Takenaka, H., Kohno, S., 2001. Comparison of cedar pollen-induced allergic rhinitis in passively and actively sensitised guinea pigs. Jpn. J. Pharmacol. 85, 409–415.

Naclerio, R.M., 1991. Allergic rhinitis. N. Engl. J. Med. 325, 860–869.

Narita, S., Asakura, K., Shirasaki, H., Kataura, A., 1997. Effects of a cysteinyl leukotriene antagonist, ONO-1078 (pranlukast), on total airway resistance after antigen challenge in sensitized guinea pigs. Inflamm. Res. 46, 143–146.

Pelikan, Z., 1978. Late and delayed response of the nasal mucosa to allergen challenge. Ann. Allergy 41, 37–47.

Pennock, B.E., Cox, C.P., Rogers, R.M., Cain, W.A., Wells, J.H., 1979. A noninvasive technique for measurement of changes in specific airway resistance. J. Appl. Physiol. 46, 399–406.

Proud, D., Togias, A., Naclerio, R.M., Cruch, S.A., Norman, P.S., Lichtenstein, L.M., 1983. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. J. Clin. Invest. 72, 1678–1685.

Proud, D., Siekierski, E.S., Bailey, G.S., 1988. Identification of human lung mast cell kininogenase as tryptase and relevance of tryptase kininogenase activity. Biochem. Pharmacol. 37, 1473–1480.

Rajakulasingam, K., Polosa, R., Holgate, S.T., Howarth, P.H., 1991. Comparative nasal effects of bradykinin, kallidin and [Des-Arg^9]-bradykinin in atopic rhinitis and normal volunteers. J. Physiol. 437, 577–587.

Sedgwick, J.B., Calhoun, W.J., Gleich, G.J., Kita, H., Abrams, J.S., Schwartz, L.B., Volovitz, B., Ben-Yaakov, M., Busse, W.W., 1991. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. Characterization of eosinophil and mast cell mediators. Am. Rev. Respir. Dis. 144, 1274–1281.

Turner, P., Dear, J., Scadding, G., Foreman, J.C., 2001. Role of kinins in seasonal allergic rhinitis: icatibant, a bradykinin B_2 receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen. J. Allergy Clin. Immunol. 107, 105–113.

Walden, S.M., Proud, D., Lichtenstein, L.M., Kagey-Sobotka, A., Naclerio, R.M., 1991. Antigen-provoked increase in histamine reactivity. Observations on mechanisms. Am. Rev. Respir. Dis. 144, 642–648.

Walls, A.F., 2000. The roles of neutral proteases in asthma and rhinitis. In: Busse, W.W., Holagate, S.T. (Eds.), Asthma and Rhinitis, 2nd ed. Blackwell, Oxford, pp. 968–998.

Walls, A.F., Bennett, A.R., Suieras-Diaz, J., Olsson, H., 1992. The kininogenase activity of human mast cell tryptase. Biochem. Soc. Trans. 20, 260S.

Wenzel, S.E., Fowler, A.A., Schwartz, L.B., 1988. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am. Rev. Respir. Dis. 137, 1002–1008.

Wirth, K., Hock, F.J., Albus, U., Linz, W., Alpermann, H.G., Anagnostopoulos, H., Henk, S., Breipohl, G., Konig, W., Knolle, J., 1991. Hoe 140 a new potent and long acting bradykinin-antagonist: in vivo studies. Br. J. Pharmacol. 102, 774–777.

Yamasaki, M., Mizutani, N., Sasaki, K., Nabe, T., Matsumoto, T., Ashida, Y., Kohno, S., 2001a. Involvement of thromboxane A_2 and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. Inflamm. Res. 50, 466–473.

Yamasaki, M., Sasaki, K., Mizutani, N., Nabe, T., Sakura, T., Matsumoto, T., Ashida, Y., Kohno, S., 2001b. Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. Inflamm. Res. 50, 474–482.