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Involvement of nitric oxide in pollen-induced biphasic nasal blockage in sensitised guinea pigs

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Abstract

We have developed a reproducible allergic rhinitis model showing biphasic nasal blockage on repetitive inhalation challenge with Japanese cedar pollen in sensitised guinea pigs. The role of nitric oxide (NO) in inducing nasal blockage was evaluated with this model. N^{ω}-nitro-L-arginine methyl ester (L-NAME), a non-selective NO synthase (NOS) inhibitor, intravenously administered before the challenge, significantly inhibited both early and late nasal blockage by approximately 80% and 50%, respectively. When L-NAME treatment was performed after the challenge, the late response was inhibited by approximately 70%. This inhibition was completely reversed by co-administration of L-arginine. However, aminoguanidine and L-N^6-(1-iminoethyl)lysine, selective inhibitors of inducible NOS, negligibly influenced the degree of nasal blockage. Meanwhile, the α-adrenergic agonist, naphazoline, strongly suppressed both early and late nasal blockage. These results indicate that NO, likely produced by constitutive rather than inducible NOS, plays a major role in the occurrence of biphasic nasal blockage, primarily by inducing vasodilatation.

Keywords: Allergic rhinitis; Cedar pollen; Nasal blockage; Nitric oxide (NO); Sneeze

1. Introduction

Allergic rhinitis is a typical atopic disease, which is characterised by nasal blockage, sneezing and rhinorrhea. When the specific allergen was applied to the nasal cavities of patients suffering from allergic rhinitis, over 90% showed an immediate response of sneezing, rhinorrhea and nasal blockage. In addition, approximately 50% further developed a late-phase reaction, with the predominant symptom being nasal blockage (Iliopoulos et al., 1990). Nasal blockage is considered to be the most serious problem for patients with these symptoms, because most drugs other than corticoteroids and decongestants are largely ineffective against nasal blockage (Naclerio, 1991). In contrast, antihistaminics are useful for the treatment of sneezing and watery rhinorrhea (Simons, 1989). Nasal airways contain venous sinusoids, and an increase in blood flow into the sinusoids produces a rapid reduction in the volume of the nasal airway cavity. Thus, nasal blockage has been strongly suggested to be induced mainly by dilatation of the blood vessels in the sinusoids (Eccles, 1995).

Nitric oxide (NO) is a powerful vasodilator that modulates systemic vascular tone (Rees et al., 1989). In addition, NO can cause tissue injury by contributing to the generation of highly reactive oxygen radicals (Beckman et al., 1991). One study showed that a large amount of NO originating from the paranasal sinuses (Lundberg et al., 1995) was continuously produced in the nasal cavities of healthy subjects. Furthermore, Arnal et al. (1997) and Kharitonov et al. (1997) reported that the NO concentration in exhaled air was elevated in patients with allergic rhinitis compared to that in normal subjects. Constitutive NO synthase (cNOS) has two isoforms, neural NOS (nNOS, NOS-1) and endothelial NOS (eNOS, NOS-3), which have been reported to be expressed in nerve cells and endothelial cells in arterioles, sinusoid vessels and capillary bed, respectively (Hanazawa et al., 1993, 1994; Kawamoto et al., 1998). Another form of NOS is inducible

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NOS (iNOS, NOS-2), the expression of which in epithelial cells, submucosal glands and inflammatory cells in the nasal mucosa of allergic rhinitis patients is more marked than that of subjects without nasal allergy (Kawamoto et al., 1998). However, due to the limitations of clinical research, the pathophysiological role of NO in the occurrence of allergen provocation-induced nasal blockage remains to be clarified.

We recently developed an allergic rhinitis model based on repetitive inhalation challenge with Japanese cedar pollen as an antigen in the guinea pig, which was sensitised by repeated intranasal application of pollen extracts adsorbed on $Al(OH)_3$ (Nabe et al., 1998). In the experimental allergic rhinitis, both frequent sneezing and marked biphasic nasal blockage were induced following pollen challenge.

In the present study, the role of vasodilatation and the type of NOS that contributes to biphasic nasal blockage were investigated using an α-adrenoceptor agonist, naphazoline, a non-selective inhibitor of both cNOS and iNOS, N^{ω}-nitro-L-arginine methyl ester (L-NAME), and selective iNOS inhibitors, aminoguanidine (Corbett et al., 1992; Griffiths et al., 1993; Misko et al., 1993; Mehta et al., 1998) and L-N^6-(1-iminoethyl)lysine (L-NIL) (Connor et al., 1995; Stenger et al., 1995; Bryk and Wolff, 1998; Chlopicki et al., 1999).

2. Materials and methods

2.1. Animals

Male 4-week-old Hartley guinea pigs weighing 300–350 g (Japan SLC, Hamamatsu, Japan) were sensitised and challenged with Japanese cedar pollens. The animals were housed in an air-conditioned room at a temperature of 23 ± 1°C and 60 ± 10% humidity with lights on from 8:00 a.m. to 8:00 p.m.; they were fed a standard laboratory diet and given water ad libitum. This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.2. Reagents

Japanese cedar (*Cryptomeria japonica*) pollen samples were harvested in Gifu and Shiga prefectures (Japan). N^{ω}-nitro-L-arginine methyl ester (L-NAME) and N^{ω}-nitro-D-arginine methyl ester (D-NAME) hydrochloride were purchased from Wako (Osaka, Japan). Naphazoline hydrochloride, L-arginine hydrochloride and aminoguanidine dihydrochloride were from Sigma (St. Louis, MO, USA). L-N^6-(1-iminoethyl)lysine (L-NIL) was from Cayman Chem. (Ann Arbor, MI, USA).

$Al(OH)_3$ gels were prepared with 0.5 N NaOH and 0.5 N $Al(SO_4)_3$ as previously described (Nabe et al., 1997a).

2.3. Sensitisation and challenge, and administration of drugs

Guinea pigs were sensitised by intranasal instillation of cedar pollen extracts adsorbed onto $Al(OH)_3$ at a dose of 0.3 μg pollen protein/0.3 mg $Al(OH)_3$/3 μl/nostril twice a day for 7 days (Nabe et al., 1998; Mizutani et al., 1999). The sensitised animal was then intranasally challenged once every week, for a total of 15 times, by inhalation of the cedar pollen using an inhalation apparatus, which allowed quantitative inhalation of pollen (1.8 mg/each nostril) (Nabe et al., 1997b). A non-sensitised, non-challenged group was prepared as negative control.

Naphazoline (0.1 mg/kg) was intravenously administered 55 or 235 min after the 7th pollen inhalation challenge, followed by measurement of specific airway resistance 5 min after the administration. L-NAME (10 mg/kg, i.v.) and its inactive enantiomer, D-NAME (10 mg/kg, i.v.), were administered 15 min before or 3 h after, and 15 min before the 7th challenge, respectively. Furthermore, in order to investigate the reversing effect of L-arginine on L-NAME-induced suppression of nasal blockage, L-arginine (300 and 600 mg/kg, i.v.) was co-administered with L-NAME 15 min before or 3 h after the 15th antigen challenge. To evaluate whether intranasal application of L-NAME alters the baseline specific airway resistance, L-NAME (1 and 1000 μg/10 μl/nostril) was instilled bilaterally into the nasal cavities of the non-sensitised, non-challenged and the sensitised, non-challenged animals at the time corresponding to the 7th challenge. Aminoguanidine (3, 10 and 30 mg/kg) was administered intravenously 15 min before or 3 h after the 14th or 15th challenge, respectively. Intravenous administration of L-NIL (10 mg/kg) was performed 30 min before the 11th challenge (Fig. 1).

2.4. Measurement of specific airway resistance

As an indicator of respiratory function (nasal blockage), specific airway resistance before and 10 min–10 h after antigen challenge in conscious guinea pigs was measured using a two-chambered, double-flow plethysmograph system according to the method of Pennock et al. (1979). In brief, an animal was placed with its neck extending through the partition of a two-chambered box, and specific airway resistance was measured using a Pulmos-I data analyser (MIPS, Osaka, Japan) and a PC 9801 FA computer (NEC, Tokyo, Japan) after the airflow was monitored via sensors attached to both the front and rear chambers. Because this technique is non-invasive, the changes in airway mechanics could be monitored throughout the experiment (approximately 4 months).

Because the guinea pig functionally respires through the nose and not through the mouth, specific airway resistance

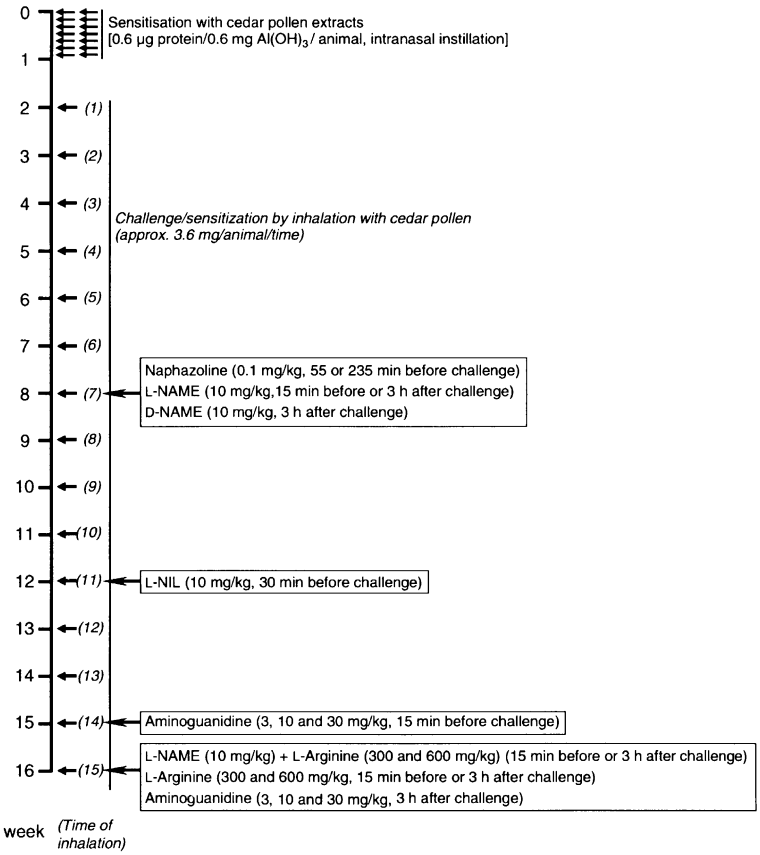

Fig. 1. Schedules for sensitisation with cedar pollen extracts and subsequent nasal challenge/sensitisation by inhalation with cedar pollens in the guinea pig, and for intravenous administrations of naphazoline, N^{ω}-nitro-L-arginine methyl ester (L-NAME), N^{ω}-nitro-D-arginine methyl ester (D-NAME), L-arginine, aminoguanidine and L-N^{6}-(1-iminoethyl) lysine (L-NIL).

can be taken as the total resistance of the upper and lower airways. However, when spontaneously breathing conscious guinea pigs were forced to inhale the pollen as described above, almost all of the inhaled pollen was trapped in the upper airways, and less than 0.001% reached the lower airways (Nabe et al., 1997b). Furthermore, although the antigen inhalation-induced early bronchoconstrictor response was reflected by rapid and shallow breathing in a guinea-pig model of asthma (Iijima et al., 1987), the pollen-inhalation challenge-induced elevation of specific airway resistance correlated well with the decrease in respiratory frequency in the present experimental allergic rhinitis model (Nabe et al., 1998). These findings indicate that the change in specific airway resistance induced by the pollen challenge (specific airway resistance value measured at a certain period after the challenge-specific airway resistance value measured before the challenge) can be considered to reflect upper airway obstruction in our model.

2.5. Sneezing frequency

Sneezing frequency was determined at 0–10 min and 10 min–1 h after the pollen inhalation challenge.

2.6. Statistical analysis

Data are presented as the means ± S.E.M. Statistical analyses were performed by one-way analysis of variance. If a significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. A probability value (P) of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of naphazoline on the occurrence of nasal blockage

Baseline specific airway resistance did not differ between the non-sensitised, non-challenged and the sensitised–challenged groups [1.19 ± 0.07 and 1.05 ± 0.08 cm $H_2O \times$ ml (ml/s); $n = 9$, respectively]. Administration of naphazoline (0.1 mg/kg, i.v.) to the non-sensitised, non-challenged animals lowered their baseline specific airway resistance. The antigen-induced increase in specific airway resistance at the early phase (1 h) was strongly suppressed to near baseline specific airway resistance by naphazoline,

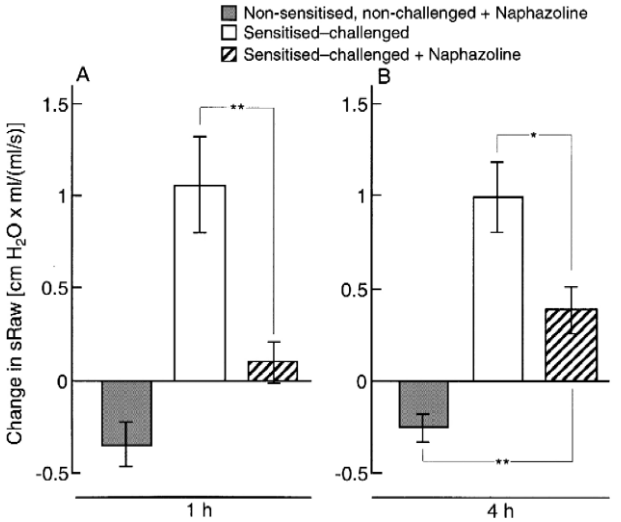

Fig. 2. Effect of naphazoline on early (1 h after challenge; A)- and late (4 h after challenge; B)-phase nasal blockage induced by inhalation challenge with Japanese cedar pollen in the sensitised guinea pig. Naphazoline (0.1 mg/kg) was i.v. administered 55 (A) and 235 (B) min after the 7th challenge. Specific airway resistance was measured 5 min after each administration of naphazoline. Each point represents the mean ± S.E.M. for 9 or 10 animals. $^{*}P < 0.05$, $^{**}P < 0.01$.

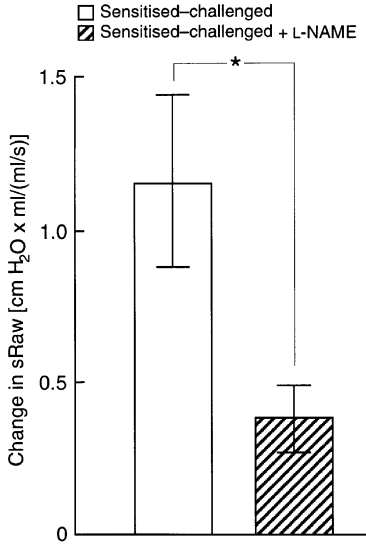

Fig. 4. Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME) on late-phase nasal blockade induced by inhalation challenge with Japanese cedar pollen in the sensitised guinea pig. L-NAME (10 mg/kg) was i.v. administered 3 h after the 7th challenge. Each column represents the mean ± S.E.M. for 12 animals. $^{*}P < 0.05$.

yet the lowered specific airway resistance did not reach the level observed in the non-sensitised, naphazoline-treated group. When naphazoline was administered 5 min before the measurement of specific airway resistance at the 4th hour, the late-phase specific airway resistance elevation was also significantly suppressed by naphazoline. However, the suppression was less prominent than that at the early phase (Fig. 2).

3.2. Effect of L-NAME on the occurrence of nasal blockage

L-NAME (10 mg/kg) administered intravenously 15 min before the challenge significantly inhibited both early (1–2 h) and late (4–6 h) phase of nasal blockage by approximately 80% and 40%, respectively (Fig. 3A). In addition, when L-NAME was administered 3 h after the challenge, a stronger inhibition of the specific airway resistance elevation at the 4th hour was seen (Fig. 4). On the other hand, intravenous administration of L-NAME (10 mg/kg) did not alter specific airway resistance in the non-sensitised, non-challenged guinea pigs until the 10th hour (Fig. 3A). The effect of D-NAME (10 mg/kg, i.v.) on both types of nasal blockage was negligible (Fig. 3B).

When L-NAME (1 and 1000 μg/nostril) was intranasally instilled into the nasal cavities of non-sensitised, non-challenged and sensitised, non-challenged guinea pigs,

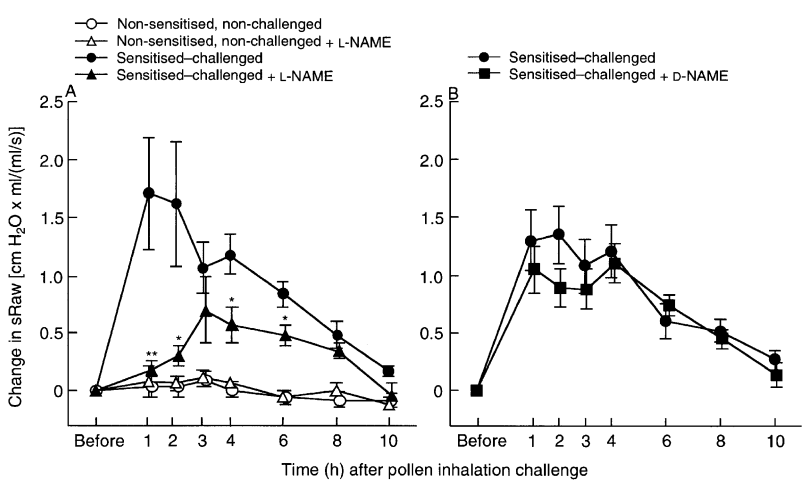

Fig. 3. Effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME; A) and N^{ω}-nitro-D-arginine methyl ester (D-NAME; B) on early- and late-phase nasal blockade induced by inhalation challenge with Japanese cedar pollen in the sensitised guinea pig. L-NAME and D-NAME (both 10 mg/kg) were i.v. administered 15 min before the 7th challenge. Each point represents the mean ± S.E.M. for 10–13 animals. Significantly different from the sensitised–challenged animals treated with saline ($^{*}P < 0.05$ and $^{**}P < 0.01$).

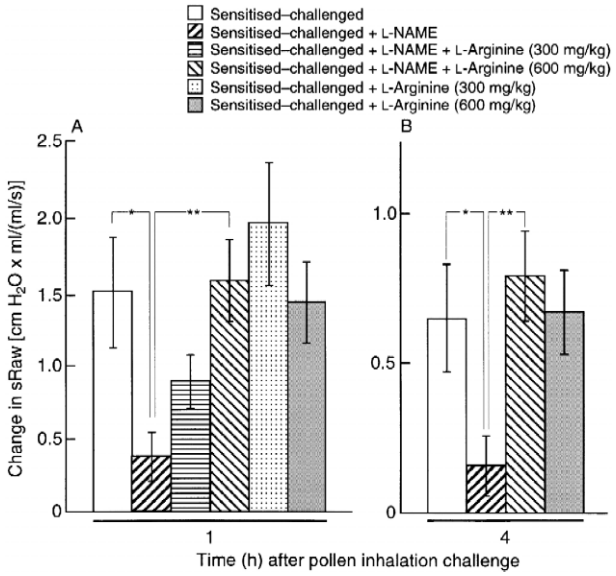

Fig. 5. Reversal by L-arginine of the N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced suppression of early (1 h after challenge; A)- and late (4 h after challenge; B)-phase nasal blockage following inhalation challenge with Japanese cedar pollen in the sensitised guinea pig. L-NAME (10 mg/kg) and/or L-arginine (300 and 600 mg/kg) were i.v. administered 15 min before (A), and 3 h after (B) the 15th challenge. Each column represents the mean ± S.E.M h. for 12 or 13 animals. $^{*}P < 0.05$, $^{**}P < 0.01$.

no significant alteration in baseline specific airway resistance was observed within 60 min of instillation (data not shown).

3.3. Effect of L-arginine on the L-NAME-induced suppression of the nasal blockage

When L-arginine (300 and 600 mg/kg) was intravenously co-administered with L-NAME (10 mg/kg, i.v.) 15 min before the challenge, the L-NAME-induced inhibition of the early specific airway resistance elevation at the 1st hour was dose-dependently reversed. Complete restoration was induced by 600 mg/kg L-arginine (Fig. 5A). Co-administration of L-arginine (600 mg/kg) with L-NAME 3 h after the challenge also completely reversed the suppressive effect of the NOS inhibitor on the late-phase (the 4th hour) nasal blockage (Fig. 5B). L-Arginine treatment alone, before and after the challenge, exerted no effect on the pollen-induced early and late specific airway resistance elevations (Fig. 5A,B).

Baseline specific airway resistance in the sensitised guinea pig was negligibly influenced by L-NAME (10 mg/kg) and L-arginine (600 mg/kg) (data not shown).

3.4. Effect of L-NAME on the occurrence of sneezing

Approximately 10 episodes of sneezing were observed at 0–10 min and 10 min–1 h after the challenge. Neither L-NAME (10 mg/kg, i.v.) nor D-NAME (10 mg/kg, i.v.) influenced this symptom (data not shown).

3.5. Effects of selective iNOS inhibitors on the occurrence of nasal blockage

Aminoguanidine (3 and 10 mg/kg, i.v.) administered 15 min before the challenge did not affect either the early- or late-phase specific airway resistance elevation. Although 30 mg/kg of aminoguanidine slightly suppressed the late-, but not the early-phase specific airway resistance elevation, the reduction was not statistically significant (Fig. 6A). When aminoguanidine (3, 10 and 30 mg/kg) was administered 3 h after the challenge, the late-phase specific airway resistance elevation at the 4th hour was not significantly affected (data not shown).

Fig. 6B shows the effect of L-NIL on early- and late-phase nasal blockage. L-NIL treatment (10 mg/kg, i.v.) 30

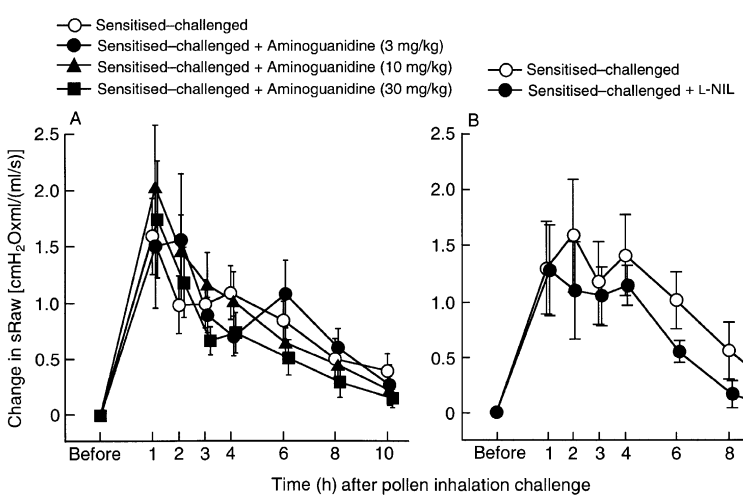

Fig. 6. Effect of aminoguanidine (A) and L-N^6-(1-iminoethyl) lysine (L-NIL; B) on early- and late-phase nasal blockage induced by inhalation challenge with Japanese cedar pollen in the sensitised guinea pig. Aminoguanidine (3, 10 and 30 mg/kg) and L-NIL (10 mg/kg) were i.v. administered 15 and 30 min before the 14th and the 11th challenges, respectively. Each point represents the mean ± S.E.M h. for 12 and 13 animals, respectively.

min before the challenge produced no significant effect on either the early- or late-phase specific airway resistance elevation.

4. Discussion

It has been suggested that 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). In the present study, we first evaluated the effect of a potent vasoconstricting α-adrenoceptor agonist, naphazoline, which is known to be very effective to prevent nasal blockage in patients with allergic rhinitis, on the antigen-induced early and late increase in specific airway resistance. Although intranasal application of the drug may produce a clearer result than systemic administration by excluding the influence of changes in blood pressure on nasal patency, we found that the topically applied drug was drained from the nasal cavity with rhinorrhea during the occurrence of nasal allergy. Therefore, we decided to administer naphazoline intravenously. The drug clearly inhibited the increases in specific airway resistance at 1 (early phase) and 4 (late phase) h after the challenge by approximately 90% and 60%, respectively. On the other hand, naphazoline lowered the baseline specific airway resistance of the sensitised, but not of the challenged animals. Thus, the inhibition by naphazoline of the specific airway resistance increase was concluded to be approximately 70% (early phase) and 50% (late phase). Naphazoline secondarily suppresses increased vascular permeability as a consequence of vasoconstriction. Given that specific airway resistance was measured 5 min after the administration of naphazoline, it can be concluded that the drug-induced suppression of the specific airway resistance increase was entirely due to the inhibition of vasodilatation, and not to plasma extravasation.

NO is known to cause marked vasodilatation by producing an increase in the intracellular cyclic GMP level, and to control systemic vascular tone. Therefore, the contribution of NO to the antigen-induced increase in specific airway resistance was evaluated using L-NAME, which was also administered intravenously, not intranasally, for the reason noted above. In anaesthetised rabbits, blockage of NO production by intravenous administration of L-NAME reportedly caused a significant and sustained increase in arterial blood pressure (Ward and Angus, 1993). In the present study, systemic administration of L-NAME induced a sustained elevation of blood pressure (data not shown), a finding which is consistent with the above report. Despite this blood pressure elevation, the baseline specific airway resistance was not altered in response to treatment. In addition, intranasal application of L-NAME to the sensitised, non-challenged animals and the non-sensitised, non-challenged animals, in which the topically administered drug solution was not drained from the nasal cavity because of the absence of rhinorrhea, did not cause a significant change in baseline specific airway resistance. These results, which are in agreement with those of a clinical report (Silkoff et al., 1999), suggest that NO may not participate in control of the resting tonus of nasal vascular smooth muscles.

The specific airway resistance elevations at the early and late phases induced by antigen challenge were suppressed by L-NAME, which was intravenously administered 75 or 60 min before the specific airway resistance measurement. Suppression was greater than 90% and approximately 65%, respectively. In contrast, D-NAME, an inactive enantiomer of L-NAME, did not prevent this increase. The L-NAME-induced suppression in both phases was completely reversed by co-administration with a NOS substrate, L-arginine. These results indicate that NO plays an important role in the occurrence of biphasic specific airway resistance elevation.

The inhibitory action of L-NAME on both the early and late specific airway resistance increases, but in particular, the early-phase increase is assumed to be mainly due to the direct suppression of nasal vasodilatation. One study reported that NO induces microvascular leakage as a result of indirect vasodilatation with direct endothelial contraction at the site of leakage (Kageyama et al., 1997). In the present experiments, the inhibition by L-NAME of the specific airway resistance increase was slightly stronger than that by naphazoline in both the early and late phases. Thus, it appears that L-NAME inhibits the specific airway resistance increase by attenuating NO-induced plasma extravasation, in addition to suppressing vasodilatation.

Although increased rhinorrhea does participate in allergic nasal blockage, the resulting suppressed nasal secretion might not be involved in the prevention by L-NAME of the specific airway resistance elevation observed in the present study, given that one previous study showed that NO did not mediate secretion (Lane et al., 1997).

Whether the NO responsible for the specific airway resistance elevation is formed by iNOS was tested using two selective iNOS inhibitors, aminoguanidine and L-NIL. Prior to the assessment with aminoguanidine, appropriate doses were carefully determined in view of the fact that, at high doses, the drug has been shown to inhibit not only iNOS but also cNOS activity (Laszlo et al., 1995). As the inhibition of cNOS activity elevates arterial blood pressure (Laszlo et al., 1994), we compared the effect of aminoguanidine on the blood pressure of guinea pigs with the effect of L-NAME. The results were consistent with those in another report (Mehta et al., 1998) that aminoguanidine (3, 10 and 30 mg/kg, i.v.) had a negligible effect on blood pressure (data not shown). Another iNOS inhibitor, L-NIL, was previously reported to be 20–30-fold more effective than aminoguanidine to sup-

press iNOS activity in vitro (Stenger et al., 1995). Thus, L-NIL at 10 mg/kg (i.v.), with which no significant increase in blood pressure was observed, (data not shown), was used for the evaluation. We subsequently found no evidence for the involvement of iNOS activation in nasal blockage, with neither aminoguanidine nor L-NIL significantly inhibiting the antigen-induced increase in specific airway resistance. These results suggest that local production of NO is induced by activation of cNOS, an isoform (nNOS or eNOS) we could not identify based on the present results, and not by iNOS, during the early and late phases of nasal blockage.

The mediators responsible for activation of cNOS have not been identified. However, late, but not early, nasal blockage was significantly attenuated by pranlukast, a specific cysteinyl leukotriene receptor (CysLT1 receptor) antagonist, by more than 50% (Mizutani et al., 2001). In addition, an increase in the amount of cysteinyl leukotrienes in nasal cavity lavage fluid could be detected biphasically concomitantly with the early and late specific airway resistance elevations after the challenge (Yamasaki et al., unpublished data). Furthermore, we found that marked nasal hyperresponsiveness to intranasal instillation not only of histamine (Mizutani et al., 1999), but also of leukotriene D_4 (Mizutani et al., 2001), was reproducibly observed 10 h and 2 days, but not 7 days, after the inhalation challenge in the sensitised guinea pig, and that the leukotriene D_4-induced nasal blockage was strongly inhibited by both pranlukast and L-NAME (Mizutani et al., 2001). Interestingly, nasal hyperresponsiveness to histamine was observed as early as 4 h after the challenge (Mizutani et al., unpublished data), suggesting that nasal hyperresponsiveness to leukotriene D_4 was also acquired during the late phase of nasal blockage. Based on these findings, we concluded that the late-phase nasal blockage is at least in part due to vasodilatation, likely by NO production by cNOS via CysLT1 receptor activation in the endothelial cells of the nasal mucosal vessel. On the other hand, it has been shown that intranasal application of histamine causes nasal blockage in both healthy and allergic rhinitis individuals (Britton et al., 1978; Austin and Foreman, 1994), and that histamine-induced vasodilation is mediated by NO produced from endothelial cells (Furchgott et al., 1984; Palmer et al., 1987; Toda and Okamura, 1989; Furchgott and Vanhoutte, 1989). However, mepyramine, a classic antihistaminic, had no effect on the early and late phases of nasal blockage in our model (Nabe et al., 2001), suggesting that histamine does not play a significant role in the occurrence of nasal blockage. Further study is needed to identify mediators other than cysteinyl leukotrienes that may activate cNOS to produce NO.

Given that the early and late specific airway resistance elevations were not completely prevented by either naphazoline or L-NAME, and in particular, that inhibition of the late-phase elevation was incomplete, mechanisms other than vasodilatation by NO production and the resultant increase in cyclic GMP level must be involved in inducing this response. Mediators that accelerate nasal (viscous) secretion should be considered.

We previously found that mepyramine strongly inhibited the occurrence of sneezing, but not that of nasal blockage (Nabe et al., 2001). Therefore, histamine derived from the activated mast cells plays a major role in sneezing. Mast cell functions have been suggested to be regulated by NO because an NOS inhibitor, N^G-monomethyl-L-arginine, enhanced the lipopolysaccaride-induced histamine release from rat peritoneal mast cells (Masini et al., 1991), and an NO donor, sodium nitroprusside, inhibits the immunological and non-immunological release of histamine from the rat mast cell (Masini et al., 1994; Iikura et al., 1998). However, in the present study, L-NAME did not significantly affect the frequency of sneezing, suggesting that NO does not modulate mast cell function in vivo.

In conclusion, our results strongly indicate that NO produced by activation of cNOS, rather than of iNOS, is involved in pollen-induced early- and late-phase nasal blockage.

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