British Journal of Pharmacology (1996) 118, 1177-1182

Attenuation of human nasal airway responses to bradykinin and histamine by inhibitors of nitric oxide synthase

J.W. Dear, S. Ghali & [1]J.C. Foreman

Department of Pharmacology, University College London, Gower Street, London WC1E 6BT

1 The effects of inhibitors of nitric oxide synthase and local anaesthetics were studied on changes in human nasal airway patency and albumin extravasation in response to bradykinin and histamine, *in vivo*.

2 Compared with the action of the vasoconstrictor, ephedrine, 2.5 μmol, N^G-nitro-L-arginine methyl ester (L-NAME), 1 μmol alone, did not change the resting value of the minimal cross-sectional area (A min) of the human nasal airway. L-NAME, 0.1 to 10 μmol, produced a dose-related inhibition of the reduction in A min caused by bradykinin, 300 μg. N^G-monomethyl-L-arginine (L-NMMA), 1 μmol, similarly reduced the effect of bradykinin, 300 μg, on A min, but N^G-nitro-D-arginine methyl ester (D-NAME), had no effect. L-NAME, 0.1 to 10 μmol, or L-NMMA, 10 μmol, failed to inhibit the effect of histamine, 300 μg on A min.

3 The inhibition by L-NAME, 1 μmol of the action of bradykinin, 300 μg on A min was maximal between 15 and 30 min after pretreatment with L-NAME.

4 L-NAME, 1 and 10 μmol, inhibited the extravasation of albumin into the nasal cavity induced by bradykinin, 300 μg, and also by histamine, 300 μg. D-NAME, 1 and 10 μmol had no effect on the extravasation of albumin in response to bradykinin or histamine.

5 L-Arginine, 30 μmol, reversed the effect of L-NAME, 1 μmol, on the bradykinin- and histamine-induced albumin extravasation into the nasal airway.

6 Local anaesthesia of the nasal airway with lignocaine, 10 mg, or benzocaine, 10 mg, failed to inhibit the reduction in A min or the albumin extravasation induced by either bradykinin, 300 μg, and histamine, 300 μg.

7 We conclude that the extravasation of plasma albumin caused by bradykinin and by histamine involves the generation of nitric oxide. The nasal blockage induced by bradykinin involves nitric oxide generation but the nasal blockage induced by histamine does not.

Keywords: Nitric oxide; nasal airway; bradykinin; histamine; L-NAME; local anaesthesia; human; plasma extravasation

Introduction

Bradykinin is a putative mediator of allergic rhinitis. Both bradykinin and kallidin have been detected in nasal washings following antigen challenge of subjects with allergic rhinitis caused by grass pollens (Proud *et al.*, 1983). Application of bradykinin to the nasal airway of normal subjects causes increased nasal airway resistance and a decrease in the nasal cross-sectional area, together with the release of albumin into nasal washings (Proud *et al.*, 1988; Rajakulasingham *et al.*, 1991; Austin & Foreman, 1994a). Pretreatment with the bradykinin B_2 receptor antagonist, icatibant (Hoe 140), of subjects with allergic rhinitis to house dust mite, reduces the nasal blockage which follows challenge with house dust mite antigen (Austin *et al.*, 1994). Bradykinin does not mimic all of the features of allergic rhinitis but appears to be responsible mainly for increased nasal airway resistance and an increase in nasal vascular permeability. Nasal challenge with bradykinin also causes a sore throat (Proud *et al.*, 1988; Doyle *et al.*, 1990).

Histamine is also considered to be a mediator of allergic rhinitis since its application to the nasal airway causes blockage (Britton *et al.*, 1978). Histamine is also detected in increased amounts in nasal lavage following the antigen-challenge of subjects with allergic rhinitis (Naclerio *et al.*, 1983), and pretreatment with a histamine H_1 receptor antagonist, of subjects with allergic rhinitis, reduces the effect of antigen-challenge (Rokenes *et al.*, 1988).

Both bradykinin and histamine are able to activate nociceptors (Belcher, 1979) and this raises the question of whether or not the actions of these compounds in the nose are neurally mediated. For example, bradykinin causes nasal discomfort and pain (Rajakulasingham *et al.*, 1991; Geppetti *et al.*, 1991), and histamine causes sneezing (Pipkorn, 1982). Furthermore, in certain tissues the vasodilator effects of bradykinin and histamine have been shown to be endothelium-dependent (Furchgott *et al.*, 1984; Toda & Okamura, 1989), and both substances are able to initiate the formation of the vasodilator, nitric oxide from endothelial cells (Palmer *et al.*, 1987; Furchgott & Vanhoutte, 1989).

The aim of this study was to determine whether the nasal actions of the putative mediators of allergic rhinitis, histamine and bradykinin, are caused by the liberation of the vasodilator, nitric oxide and whether the actions of these mediators are neurally-mediated. To achieve this aim, we have studied the action of local anaesthetics and of inhibitors of nitric oxide synthase on nasal responses to bradykinin and histamine.

Methods

Subjects

The study was approved by the local ethics committee of University College London. Healthy volunteeers comprised 16 males and 14 females with a mean age of 29 years (range 21-52) who were not atopic and had no history of airway disease or any other major illness. All subjects gave their informed consent and undertook to take no medication in the four weeks prior to, or during the study period.

[1] Author for correspondence.

Administration of drugs

Compounds were administered to the nasal cavity with a hand-held pump spray (Perfect-Vallois) that delivered a volume of 100 μl per actuation. The dose of each compound administered to a subject was controlled by varying the concentration of the compound in the pump spray. The vehicle was saline which also served as the control. In all experiments, compounds were delivered through both nostrils and the doses quoted in the results are the amounts given into each nostril.

Measurement of nasal patency

Nasal patency (cross-sectional area of the nasal cavity) was determined by the method of acoustic rhinometry as previously described (Austin & Foreman, 1994b). The parameter used to assess nasal patency was the minimal cross-sectional area (A min) of the nasal airway. For each determination of A min, triplicate measurements were made on each side of the nasal airway.

Nasal lavage and albumin assay

Nasal lavage was performed as previously described (Naclerio *et al.*, 1983) by the instillation of 5 ml of prewarmed (37°C) saline into each nostril for 10 s. Recovery was approximately 90%. The first two lavages at the beginning of each experiment were discarded and the third lavage was assayed for the baseline albumin release. The albumin content of the nasal lavage was determined with a commercially available single radial immunodiffusion assay. A protein standard serum was used to establish a calibration curve, and albumin from human sera served as a control to confirm the function of the immunodiffusion plates.

Experimental protocols

The basic protocol was a double-blind, cross-over experiment in which each subject received a control treatment on one occasion and an experimental treatment on a separate occasion. At least one week separated the two treatments in an individual subject.

For the study of nasal patency, an initial value of A min, was determined as described above. The value of A min determined at this point was used only to compare with the baseline value determined later in the protocol and to demonstrate no change induced by the treatment alone and no change with time. Subjects then received either the control or experimental treatment: which was given first was determined on a random basis. Twenty-five minutes after the treatment, A min was redetermined and this value served as the baseline against which changes induced by histamine or bradykinin were judged. Thirty minutes after the treatment, histamine, 300 μg or bradykinin, 300 μg, were administered into each nostril and A min was redetermined 10 min later. The doses of histamine and bradykinin were chosen, on the basis of pilot studies, to be approximately equipotent. The measurement of A min 10 min after bradykinin or histamine was chosen on the basis of prior studies showing that this was the time of maximum change in A min.

The protocol was followed to study the effect on histamine- or bradykinin-induced changes in A min of: L-NAME, 0.1, 1, 10 μmol; D-NAME, 0.1, 1, 10 μmol; L-NMMA, 10 μmol; lignocaine 10 mg; benzocaine 10 mg. The control for each of the treatments was saline. Each compound at each dose, or its appropriate control constituted a separate treatment in the above protocol.

The protocol was varied to study the effect of ephedrine, 2.5 μmol, L-NAME, 1 μmol, lignocaine, 10 mg or benzocaine, 10 mg, on the unstimulated nasal airway by substituting into the protocol described above, one of these treatments or the relevant control, and omitting the challenge with bradykinin or histamine. A further variation of the protocol was required to study the effect of the duration of pretreatment with L-NAME, 1 μmol, on the response to bradykinin. In this case, the interval between the L-NAME treatment and the bradykinin challenge was 0 (L-NAME and bradykinin together), 2, 15, 30 or 60 min and the baseline A min values were recorded 5 min prior to the bradykinin challenge.

The study of albumin release was conducted on separate occasions from the study of nasal patency. Again, a double-blind, cross-over design was used. Nasal lavage was performed, as described above, prior to any treatment, and the lavage was used for the measurement of baseline albumin release. Subjects then received either the control or experimental treatment: which was given first being randomly determined. Lavage was not performed after the treatment in order that the treatment was not washed from the nasal airway. Thirty minutes later, bradykinin, 300 μg, or histamine, 300 μg, was administered to each nostril and 10 min later another lavage was performed and the sample retained for albumin assay.

The protocol was undertaken for the following treatments: L-NAME, 0.1, 1, 10 μmol; D-NAME, 0.1, 1, 10 μmol; L-arginine, 30 μmol, plus L-NAME, 1 μmol; lignocaine, 10 mg; benzocaine 10 mg. In each case the control treatment was saline.

The protocol was varied to study the effect of L-NAME, 1 μmol, on the albumin release in the absence of histamine or bradykinin challenge, by omitting the challenge with these agents from the protocol.

Data analysis

The dimensions of the nasal airway vary between subjects and also within subjects from day to day and so the data have been normalized by expressing changes in A min as a percentage of the baseline control value. The absolute values for the control measurements have been given with each set of data. Means are given together with s.e.mean. The appropriate, non-parametric statistical test is given with each data set. A value of $P<0.05$ is taken as significant.

Materials

N^G-nitro-L-arginine methyl ester (L-NAME), L-arginine and histamine were obtained from Sigma, Poole, U.K.; N^G-monomethyl-L-arginine (L-NMMA) and bradykinin from Calbiochem, Nottingham, U.K.; ephedrine hydrochloride from Boots, Nottingham, U.K.; lignocaine from Phoenix Pharmaceuticals, Gloucester, U.K.; benzocaine from Astra, Kings Langley, U.K. Radial immunodiffusion assay plates for human serum albumin were obtained from Behring, Marburg, Germany.

Results

In order to demonstrate that L-NAME had no vasoconstrictor action of its own, which might affect nasal patency, the vasoconstricting sympathomimetic, ephedrine was used as a positive control. Ephedrine, 2.5 μmol per nostril, caused a significant increase compared with saline, in the minimal cross-sectional area (A min) of the unstimulated human nasal airway, whereas L-NAME, 1 μmol, alone, had no effect (Table 1).

Bradykinin and histamine both caused a decrease in the baseline (100%) A min in the absence of any other drug (Figure 1). Figure 1 shows the dose-response curve for the effect of L-NAME or D-NAME on the decrease in A min induced by administration of bradykinin, 300 μg, per nostril. Also shown is the dose-response curve for L-NAME on the decrease in A min caused by histamine, 300 μg. L-NAME or D-NAME were administered 30 min prior to challenge with bradykinin or histamine. L-NAME, 0.1 to 10 μmol, dose-dependently inhibited the decrease in A min caused by bradykinin (Friedman's test, $P<0.04$) whereas the same doses of L-NAME failed to inhibit the decrease in A min caused by his-

tamine, 300 μg (Friedman's test, $P>0.2$). D-NAME failed to inhibit the decrease in A min, caused by bradykinin (Friedman's test, $P>0.2$). Another inhibitor of nitric oxide synthase, N^G-monomethyl-L-arginine (L-NMMA), 1 μmol, also inhibited the bradykinin-induced reduction of A min. When saline was administered 30 min prior to bradykinin, 300 μg, the A min fell, after bradykinin challenge, to 74±3% (mean±s.e.mean; $n=7$) of the baseline value, whereas when L-NMMA, 1 μmol, was administered 30 min prior to bradykinin the A min fell to 83±3% of the baseline control: the difference being significant ($P<0.05$) when assessed by the Wilcoxon paired, sign-rank test. In contrast, L-NMMA, 10 μmol, failed to inhibit the fall in A min caused by histamine. When saline was administered 30 min prior to histamine, 300 μg, the A min fell to 80±4% (mean±s.e.mean, $n=5$) of control and when L-NMMA, 10 μmol, was administered 30 min prior to histamine, 300 μg, the A min fell to 79±5% of control. In the same subjects, this dose of L-NMMA did inhibit the histamine-induced albumin release into the nasal airway. Histamine-induced albumin release was 38±8 mg dl^{-1} after saline pretreatment and 20±9 mg dl^{-1} after L-NMMA, 10 μmol pretreatment: the difference being significant (Wilcoxon paired, sign-rank test, $P<0.05$).

Figure 2 shows the effect of the duration of pretreatment with L-NAME on the reduction in A min induced by bradykinin. The effect of L-NAME was significant when it was administered 15 and 30 min prior to bradykinin and the magnitude of the effect of L-NAME decreased with longer pretreatment time.

In addition to studying the action of L-NAME on nasal blockage induced by bradykinin and histamine, the effect of L-NAME on plasma extravasation induced by these substances was examined. Figure 3 shows that L-NAME, given 30 min prior to challenge with bradykinin, 300 μg, or histamine, 300 μg, reduced the extravasation of albumin into nasal lavage fluid, in a dose-dependent manner (Friedman's test, $P<0.05$). D-NAME, when administered in the same manner, did not reduce the albumin extravasation induced by either bradykinin or histamine (Friedman's test, $P>0.1$). Compared with saline, L-NAME, 10 μmol, alone did not cause an increase in the release of albumin into the nasal cavity. The albumin release after saline was 0.1±0.1 mg dl^{-1} (mean±s.e.mean, $n=5$) and after L-NAME, 10 μmol it was 0.1±0.1 mg dl^{-1}. This experiment would only detect an increase of albumin release by L-NAME, since the albumin release following saline is at the lower limit of detection of the assay used.

To test the specificity of L-NAME, we attempted to reverse its effect on albumin release into the nasal cavity by adding the nitric oxide synthase substrate, L-arginine. Addition of L-arginine, 30 μmol, together with L-NAME, 1 μmol, reversed the inhibitory action of L-NAME on the increased albumin ex-

Table 1 The effect of saline, L-NAME (1 μmol), ephedrine (2.5 μmol), lignocaine (10 mg) and benzocaine (10 mg) on the minimal cross-sectional area (A min) of the unstimulated human nasal airway

Treatment	*% of baseline A min*
Saline	102.9±3.2
L-NAME	96.4±3.0
Ephedrine	124.0±2.6*
Lignocaine	95.8±5.2
Benzocaine	101.9±4.9

The data are the means±s.e.mean from six subjects. The data have been normalized by expressing the A min values as a percentage of the baseline value for each subject. The mean±s.e.mean baseline value for A min was 0.55±0.04 cm^2. * Significant difference by the Wilcoxon paired, sign-rank test, between saline and ephedrine ($P<0.05$).

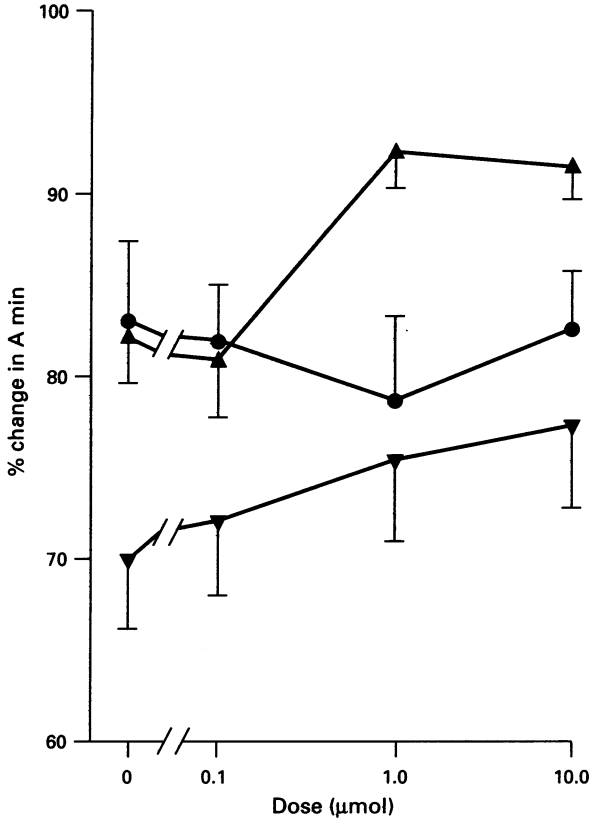

Figure 1 Dose-response curve for the effect of N^G-nitro-L-arginine methyl ester (L-NAME) on the reduction of minimal nasal cross-sectional area (A min) induced by bradykinin. L-NAME or D-NAME was administered 30 min prior to bradykinin or histamine and A min was measured 10 min after bradykinin or histamine administration. (▲) Bradykinin, 300 μg+L-NAME; (●) bradykinin, 300 μg+D-NAME; (▼) histamine, 300 μg+L-NAME. Data are the means± s.e.mean from 8 subjects. Changes in A min have been normalized by expressing them as a percentage of the baseline for each subject. The mean±s.e.mean baseline value of A min was 0.54±0.02 cm^2.

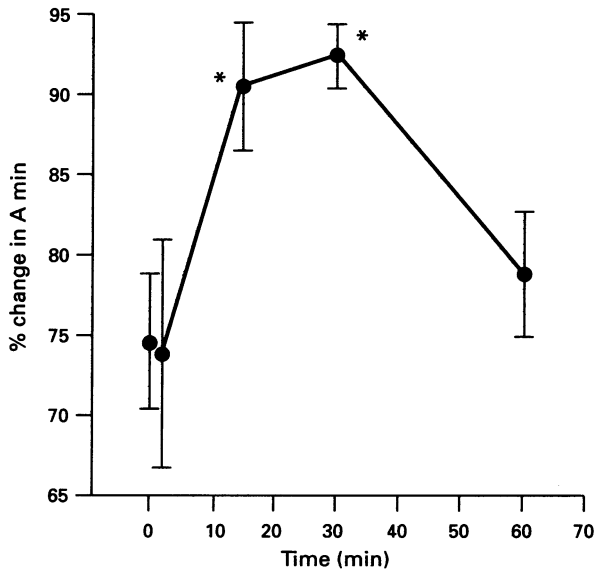

Figure 2 Effect of the duration of pretreatment with L-NAME on the reduction of A min caused by bradykinin, 300 μg. L-NAME, 1 μmol, was administered at the times shown prior to the challenge with bradykinin. The data are means±s.e.mean from 5 subjects. Changes in A min have been normalized by expressing them as a percentage of the baseline A min for each subject. The mean± s.e.mean baseline value of A min was 0.60±0.09 cm^2. *Significant difference by the Wilcoxon paired, sign-rank test, from the value at zero time when L-NAME and bradykinin were given simultaneously.

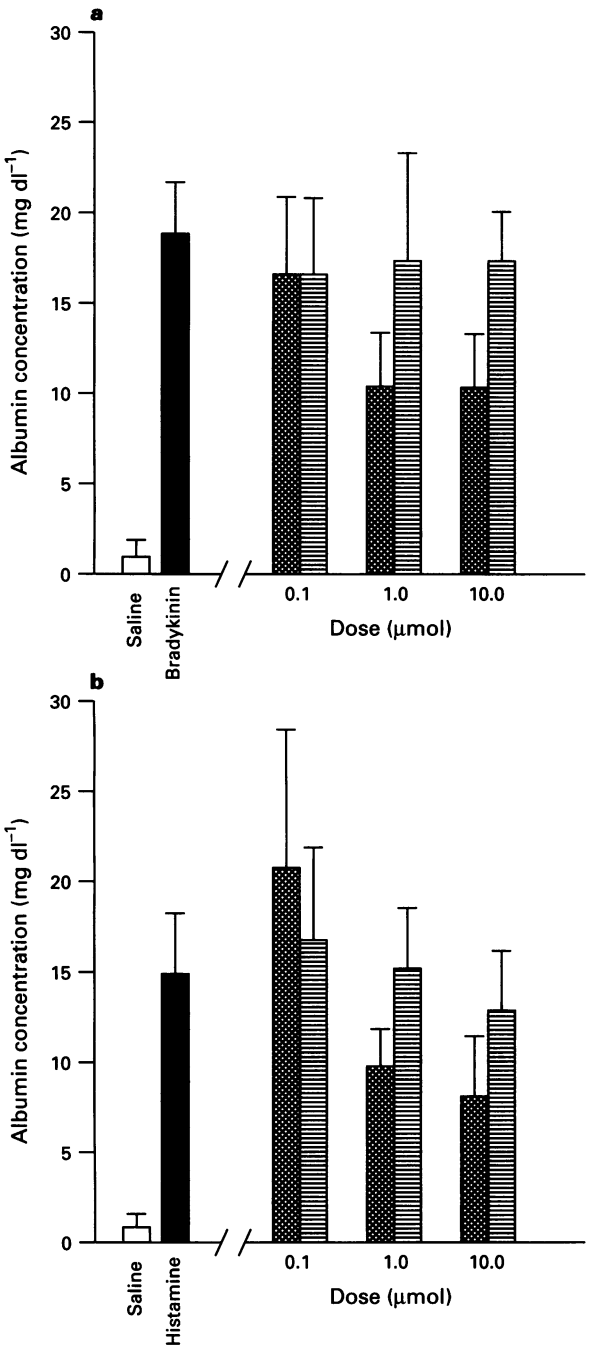

Figure 3 Dose-response relationship for the effect of L-NAME and D-NAME on (a) bradykinin- and (b) histamine-induced increases in the albumin concentration in nasal lavage. Open columns, saline; solid columns, bradykinin, 300 μg, or histamine, 300 μg; cross-hatched columns, bradykinin, 300 μg + L-NAME or histamine, 300 μg + L-NAME; hatched columns, bradykinin, 300 μg + D-NAME or histamine, 300 μg + D-NAME. The data are the means ± s.e.mean from 10 subjects. The effect of L-NAME was dose-related for both bradykinin and histamine (Friedman's test, $P<0.05$).

travasation induced by both bradykinin and histamine (Figure 4). L-Arginine alone was without effect on albumin extravasation (Figure 4).

To examine the possible involvement of nerves in the nasal responses to histamine and bradykinin, we studied the responses to these substances after local anaesthesia. The doses of lignocaine and benzocaine were selected on the basis of their relative potencies and the dose of lignocaine which is used clinically to achieve nasal anaesthesia. Both drugs abolished sensation to tactile stimuli applied inside the nostril. Application of lignocaine, 10 mg, or benzocaine, 10 mg, alone to the

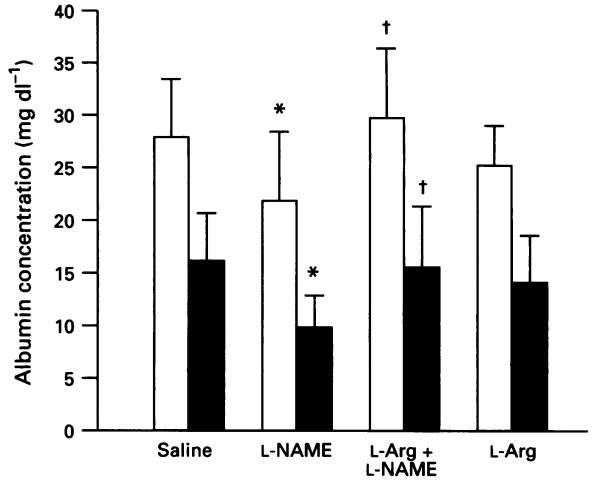

Figure 4 Reversal by L-arginine, 30 μmol, of the effect of L-NAME, 1 μmol, on bradykinin- or histamine-induced increases in albumin concentration in nasal lavage. Open columns, bradykinin (300 μg); solid columns, histamine (300 μg). The data are the means ± s.e.mean from 10 subjects. *Significant difference between the values for histamine or bradykinin alone and the values in the presence of L-NAME ($P<0.03$). †Significant difference between the values in the presence of L-NAME and the values in the presence of L-NAME + L-arginine ($P<0.03$). In both cases, the test of significance applied was the Wilcoxon paired, sign-rank test.

Table 2 The effect of lignocaine, (10 mg) or benzocaine (10 mg) on the histamine- and bradykinin-induced reduction of minimal nasal cross-sectional area (A min) and increased albumin concentration in nasal lavage

	% of baseline A min	*Albumin concentration (mg dl⁻¹)*
Bradykinin, 300 μg + lignocaine	79.2 ± 4.7	25.6 ± 5.4
Bradykinin, 300 μg + saline	78.5 ± 4.7	22.3 ± 6.1
Bradykinin, 300 μg + benzocaine	79.0 ± 6.1	14.8 ± 6.1
Bradykinin, 300 μg + saline	82.1 ± 5.8	16.4 ± 4.2
Histamine, 300 μg + lignocaine	80.1 ± 5.1	19.1 ± 5.8
Histamine, 300 μg + saline	75.1 ± 3.6	18.9 ± 4.7
Histamine, 300 μg + benzocaine	82.4 ± 5.2	19.2 ± 3.8
Histamine, 300 μg + saline	79.3 ± 4.9	21.0 ± 4.7

The data are the means ± s.e.mean from 5 subjects. To normalize the data, A min values are expressed as a percentage of the baseline value for each subject. The mean ± s.e.mean baseline value for A min was 0.48 ± 0.03 cm^2.

nasal airway did not change A min, although the vasoconstrictor, ephedrine, 2.5 μmol, significantly increased A min (Table 1). Pretreatment of the nasal airway with lignocaine, 10 mg, or benzocaine, 10 mg, failed to affect the reduction in A min or the albumin extravasation, induced by either bradykinin, 300 μg, or histamine 300 μg (Table 2).

Discussion

We have demonstrated that both bradykinin and histamine cause an increase in the extravasation of human serum albumin into nasal lavage fluid which we interpret as an increase in

vascular permeability of the nasal mucosal vessels induced by these agents. Extravasation of albumin into the nasal cavity requires an increase in vascular permeability but, in addition, an increase in blood flow into the permeabilized vascular bed will increase the extravasation. For both histamine and bradykinin, the increase in plasma extravasation that they induce was inhibited by the inhibitors of nitric oxide synthase, L-NAME or L-NMMA and this inhibition was not observed with D-NAME, suggesting that there was a selective effect of L-NAME. Furthermore, the inhibitory effect of L-NAME on plasma extravasation following histamine or bradykinin was reversed by adding the substrate for nitric oxide synthase, L-arginine. Together, these data lead to the conclusion that histamine- or bradykinin-induced increase in plasma extravasation into the nasal airway mucosa is secondary, at least in part, to the formation of nitric oxide.

In addition to their actions on nasal vascular permeability, both histamine and bradykinin cause a decrease in the minimal cross-sectional area (A min) of the nasal airway, which reflects a decrease in nasal patency, or, in other words, increased nasal blockage. It has been shown previously that a reduction in A min caused by histamine or bradykinin is accompanied by an increase in nasal airway resistance (Austin & Foreman, 1994b). In comparison with the vasoconstrictor ephedrine, L-NAME, at a concentration which did affect the responses to bradykinin, did not, by itself, cause an increase in A min which suggests that L-NAME is not acting as a vasoconstrictor in the unstimulated nasal airway. It also suggests that nitric oxide is not involved in the regulation of vascular tone in the unstimulated nasal airway. The nasal blockage induced by bradykinin was reversed by both L-NAME and by L-NMMA but not by D-NAME which we interpret as evidence for a role for nitric oxide formation, at least in part, in the nasal blockage caused by bradykinin. Interestingly, histamine-induced nasal blockage was not influenced by L-NAME, or L-NMMA, which implies that histamine induces nasal blockage by a mechanism different from that for bradykinin and in which nitric oxide is either not involved or is generated by an enzyme which is more resistant to the inhibitors we have used.

Changes in nasal airway patency result from the operation of several mechanisms. The nasal airway contains cavernous sinusoids analogous to the erectile tissue of the penis, and an increase in blood flow into these sinusoids produces a rapid reduction of the volume of the nasal cavity with resultant reduction in air flow. In addition, serous and mucous secretion into the nasal cavity reduce its patency. Both bradykinin and histamine induce this secretion (Baraniuk *et al.*, 1990; Raphael *et al.*, 1989) and differences in their potencies may reflect the inability of L-NAME to attenuate histamine-induced nasal blockage. The patency of the nasal cavity is also reduced by swelling of the nasal mucosa resulting from increased microvascular permeability and increased blood flow. Our data show that for both bradykinin and histamine the increased plasma extravasation in the nasal mucosa is nitric oxide-mediated. However, the actions on plasma extravasation are not a sole explanation for the increase in nasal blockage induced by these agents since nasal blockage induced by bradykinin involves nitric oxide formation while that induced by histamine does not.

Neuronal nitric oxide synthase is present in the nasal mucosa (Kulkarni *et al.*, 1994). However, we have shown that application of the local anaesthetics, lignocaine and benzocaine to the nasal mucosa fails to inhibit nasal blockage or albumin extravasation induced by either histamine or bradykinin. It appears, therefore, that nasal blockage and plasma extravasation in response to histamine and bradykinin does not involve activation of nerves.

We conclude that the reduction of human nasal patency and the extravasation of plasma albumin caused by bradykinin is mediated, in part, by the release of nitric oxide. Histamine-induced nasal blockage appears not to involve the generation of nitric oxide whereas its action on plasma extravasation does.

We would like to thank the subjects who agreed to participate in these studies. J.W.D. thanks the Medical Research Council for the provision of an award for Training in Research Methods. S.G. thanks the Medical Research Council for a grant to support the Intercalated B.Sc degree in pharmacology.

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(Received February 29, 1996
Accepted March 20, 1996)