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SPECIAL REPORT

Reduction by N^G-nitro-L-arginine methyl ester (L-NAME) of antigen-induced nasal airway plasma extravasation in human subjects *in vivo*

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In non-allergic subjects, histamine induced a reduction of minimal nasal cross-sectional area (Amin) and an increase in albumin release into nasal lavage. The effect of histamine on albumin release was inhibited by pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME), 1 μmol but not by D-NAME, 1 μmol. L-NAME, 1 and 10 μmol, did not inhibit the histamine-induced reduction of Amin. In subjects allergic to grass pollen, antigen challenge induced a reduction in Amin that was not changed by pretreatment with L-NAME, and an increase in albumin release that was inhibited by L-NAME, 1 μmol. The data support a role for nitric oxide in mediating plasma extravasation in the nose induced by antigen challenge or histamine.

Keywords: Allergic rhinitis; histamine; nitric oxide; L-NAME; allergen challenge

Introduction Chemical mediators including histamine (Naclerio & Togias, 1991), bradykinin (Proud *et al.*, 1983) and leukotrienes (Knapp, 1990) are implicated in the pathophysiology of allergic rhinitis. These mediators are believed to be released, or synthesized, following the interaction between specific antigen and cell-bound IgE antibody. Some inflammatory mediators and neurotransmitters act on the vasculature to cause increased blood flow by stimulating the production of nitric oxide (Vallance & Collier, 1994). Histamine is a putative mediator of seasonal allergic rhinitis because histamine H_1 receptor antagonists reduce some of the symptoms (Rokenes *et al.*, 1988). Moreover, in certain tissues, histamine induces vasodilatation by an endothelial-dependent mechanism which involves nitric oxide (NO) generation (Kelm *et al.*, 1993). Nitric oxide synthase is present in the human nasal mucosa (Kulkarni *et al.*, 1994; Lundberg *et al.*, 1995).

Our aim was to determine the role of NO in changes in human nasal airway patency and plasma extravasation, induced by histamine or antigen.

Methods The study was approved by the local Ethics Committee and subjects gave informed consent. Ten non-allergic, healthy volunteers were used for the study of histamine provocation (20 to 52 years old) and 10 subjects with positive skin prick tests and nasal provocation tests to grass pollen were used for the study of antigen provocation (21 to 52 years old).

Minimal cross-sectional area of the nasal cavity (Amin) was determined by acoustic rhinometry as previously described (Austin & Foreman, 1994). Plasma extravasation in the nasal airway was assessed by measuring the albumin content of nasal lavage (Naclerio *et al.*, 1983), with a radial immunodiffusion assay (Behring Diagnostics, Milton Keynes, U.K.).

Compounds were delivered into the nasal airway in 100 μl, by means of a nasal pump spray (Perfect-Valois, U.K., Ltd). Histamine (Sigma Chemicals, Poole, Dorset, U.K.), N^G-nitro-L-arginine methyl ester (Sigma Chemicals, Poole, Dorset, U.K.) and mixed grass pollen antigen (Allerayde, Nottingham, U.K.) were dissolved in sterile saline at concentrations of 3 mg ml^{-1}, 10 mmol l^{-1} or 100 mmol l^{-1} and 10,000 u ml^{-1} respectively.

For the histamine provocation study, a double-blind, cross-over design was employed: the treatments received by each subject being selected randomly. A baseline Amin value was determined (Austin & Foreman, 1994). Subjects were then given a nasal aerosol of either saline or L-NAME, 1 μmol to each nostril and 30 min later this was followed by provocation with histamine, 300 μg per nostril. Amin was redetermined 5 min after the histamine provocation. One week later, the cross-over study was performed. The protocol was repeated on a separate occasion with L-NAME, 10 μmol. For determination of albumin release into the nasal airway, the protocols were repeated on separate occasions: nasal lavage being substituted for acoustic rhinometry. In the nasal lavage experiments, a separate study of D-NAME, 1 μmol, was included. At least one week separated the different studies in the same subjects. The doses used and the timing of the measurements was based on pilot experiments.

For the antigen provocation study, a single-blind cross-over design was employed and the first treatment for each subject was allocated randomly. The protocol was similar to that for the histamine study except that mixed grass pollen antigen, 1000 u per nostril replaced the histamine for provocation. In addition, nasal lavage and acoustic rhinometry were determined in the same study; nasal lavage following each Amin determination.

Results Table 1 shows that histamine, 300 μg per nostril caused a reduction in the minimal cross-sectional area of the nasal airway (Amin) and an increased leakage into the nasal airway of serum albumin. The nitric oxide synthase inhibitor, L-NAME, 1 μmol per nostril, given 30 min prior to histamine, inhibited the histamine-induced albumin leakage into the nasal airway but failed to inhibit the histamine-induced reduction of Amin. Raising the dose of L-NAME to 10 μmol failed to inhibit the histamine-induced reduction of Amin, or cause any greater inhibition of albumin release than L-NAME (1 μmol). D-NAME (1 μmol) did not inhibit the histamine-induced leakage of albumin into the nasal airway.

Figure 1 shows that mixed grass pollen antigen, in allergic subjects, caused a reduction of nasal airway patency (Amin) and an increase in the leakage of serum albumin into the nasal

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Table 1 The effect, in non-allergic subjects of N^G-nitro-L-arginine methyl ester (L-NAME) on histamine-induced reduction in nasal patency (Amin) and of L-NAME and D-NAME on histamine-induced albumin release into nasal lavage

Acoustic rhinometry		*Albumin release*	
Treatment	*Amin value* (cm^2)	*Treatment*	*Albumin release* ($mg\,dl^{-1}$)
Baseline (a)	0.49 ± 0.02	Baseline	0.34 ± 0.26
Histamine	0.31 ± 0.03**	Histamine	14.86 ± 3.41**
Baseline (b)	0.54 ± 0.02	Histamine + L-NAME, 1 μmol	9.9 ± 2.03*
Histamine + L-NAME, 1 μmol	0.37 ± 0.03	Histamine + L-NAME, 10 μmol	8.1 ± 3.40*
Baseline (c)	0.60 ± 0.02	Histamine + D-NAME, 1 μmol	15.3 ± 3.30
Histamine + L-NAME, 10 μmol	0.48 ± 0.03		

Each datum point is the mean ± s.e.mean from 10 subjects. For Amin measurements, baselines (a), (b) and (c) refer to the baseline Amin in the separate parts of the cross-over study. For albumin determinations all the baseline values have been pooled. Two-tailed, non-parametric, paired comparison gave the following P values: **$P < 0.01$ for baseline (a) compared with histamine (Amin) and baseline compared with histamine (albumin); *$P < 0.03$ for histamine compared with histamine + L-NAME, 1 μmol (albumin) and histamine compared with histamine + L-NAME, 10 μmol (albumin).

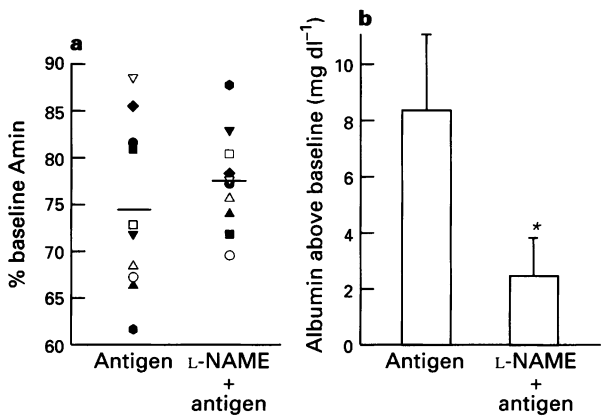

Figure 1 The effect of either saline or N^G-nitro-L-arginine methyl ester (L-NAME) 1 μmol per nostril, given 30 min prior to antigen challenge (1000 u mixed grass pollens per nostril) on (a) the change in nasal patency (Amin) and (b) the change in albumin release into the nasal airway. In (a) each symbol represents a separate subject and the horizontal bars indicate the mean values which were not significantly different. Data are presented as percentage change from the baseline Amin which was 0.59 ± 0.02 cm^2 for antigen and 0.52 ± 0.04 cm^2 for antigen + L-NAME. In (b) the data are given as mean ± s.e.mean albumin concentrations in nasal lavage from the same 10 subjects. *Indicates a significant difference compared with antigen ($P < 0.02$) using a two-tailed, paired, non-parametric test.

airway. L-NAME, 1 μmol per nostril, given 30 min prior to antigen, inhibited the antigen-induced leakage of albumin into the nasal airway but failed to affect the antigen-induced reduction of Amin.

Discussion We have used L-NAME as an inhibitor of nitric oxide synthesis to evaluate the role of nitric oxide in the nasal airway responses to either histamine in non-allergic subjects or specific antigen in allergic subjects. Our data confirm that both stimuli cause nasal blockage and plasma extravasation into the nasal airway. The increase in plasma extravasation caused by histamine in non-allergic subjects or antigen in allergic subjects was inhibited by L-NAME, and in the case of histamine provocation, D-NAME was not effective. Thus, NO is involved in the pathway by which histamine or antigen challenge cause plasma extravasation. L-NAME failed to block completely both histamine- and antigen-induced plasma extravasation which may imply an NO-independent mechanism for part of this response. Nitric oxide synthase in the human nasal airway has been localized in neurones (Kulkarni *et al.*, 1994) but other locations cannot be precluded.

Plasma extravasation may involve both an increase in vascular permeability and an increase in blood flow; therefore, NO could be involved at either site. In other tissues (Kelm *et al.*, 1993), histamine-induced vasodilatation is mediated by endothelial generation of NO, so the effect of L-NAME that we observed on nasal airway plasma extravasation may result from a reduction of NO-mediated vasodilatation.

Blockage of the nasal airway can result from a number of processes including increased blood flow into cavernous venous sinusoids, increased secretion into the nasal cavity, and swelling of the mucosa resulting from plasma extravasation. We suggest that nasal blockage and increased plasma extravasation following the stimuli we employed, occur by different mechanisms: the former being independent of NO generation and the latter involving NO generation.

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