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Induction by inhibitors of nitric oxide synthase of hyperresponsiveness in the human nasal airway

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1 The effects of inhibitors of nitric oxide synthase (NOS) on the responsiveness of the human nasal airway were investigated, by measuring the nasal response to histamine and bradykinin.

2 Repeated intranasal administration of N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA), 1 μmol per nostril every 30 min for 6 h, increased the nasal obstruction induced by histamine, 50–500 μg, and bradykinin, 200 μg per nostril. A single administration of L-NAME, 1 μmol per nostril did not induce hyperresponsiveness to histamine.

3 Pretreatment with L-arginine, 30 μmol, abolished the hyperresponsiveness to histamine caused by L-NAME, 1 μmol. Pretreatment with N^G-nitro-D-arginine methyl ester (D-NAME), 1 μmol, did not induce hyperresponsiveness to histamine.

4 Repeated administration of L-NAME, 1 μmol, caused a significant reduction in the amount of nitric oxide measured in the nasal cavity.

5 Neither L-NMMA, 1 μmol, nor L-arginine, 30 μmol, altered the nasal hyperresponsiveness induced by platelet activating factor (PAF), 60 μg. PAF did not alter the levels of nitric oxide in the nasal cavity.

6 The results suggest that inhibition of nitric oxide synthase induces a hyperresponsiveness in the human nasal airway, and that this occurs by a mechanism different from that involved in PAF-induced hyperresponsiveness.

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Abbreviations: Amin, Minimal cross-sectional area of the nasal airway; L-arg, L-arginine; AUC, area under curve; D-NAME, N^G-nitro-D-arginine methyl ester; L-NAME, N^G-nitro-L-arginine methyl ester; L-NMMA, N^G-monomethyl-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PAF, platelet activating factor

Introduction

Subjects with allergic rhinitis have an increased response of the nasal airway to a range of stimuli, including histamine and bradykinin (Mullins *et al*., 1989), a phenomenon known as airway hyperresponsiveness. In normal, non-atopic subjects, platelet activating factor (PAF) induces a nasal hyperresponsiveness similar to that seen in allergic rhinitis (Austin & Foreman, 1993), *via* a process which is dependent upon the activation of bradykinin B_2 receptors (Turner *et al*., 2000a). The action of bradykinin in the human nasal airway is mediated, at least in part, by the formation of nitric oxide (Dear *et al*., 1996).

In the lower airways of the guinea-pig, inhibitors of nitric oxide synthase (NOS) induce an airway hyperresponsiveness to histamine (Nijkamp *et al*., 1993; Schuiling *et al*., 1998), bradykinin (Ricciardolo *et al*., 1994) and antigen in ovalbumin-sensitized animals (Persson *et al*., 1993). Furthermore, inhibition of NOS potentiates bradykinin-induced bronchoconstriction in asthma (Ricciardolo *et al*., 1996). However, no similar studies have yet been carried out in the human nasal airway.

There is evidence of increased nitric oxide (NO) production in both perennial (Garrelds *et al*., 1995) and seasonal allergic rhinitis (Kharitonov *et al*., 1997b), and this may contribute to the hyperresponsiveness observed in allergic rhinitis. For example, inducing NO production in the mouse airway potentiates the airway response to allergen (Takano *et al*., 1998).

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All three isoforms of NOS are present in the human nasal mucosa (Kulkarni *et al*., 1994; Furukawa *et al*., 1996), and the plasma extravasation induced by histamine, bradykinin or antigen challenge in the human nasal airway is reduced by a single pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) (Dear *et al*., 1995; 1996). Our aim was to determine the role of NOS in the development of hyperresponsiveness in the normal, human nasal airway.

Methods

Subjects

The study was approved by the local ethics committee of University College London, and all subjects gave their informed consent. In all experiments, normal, non-atopic, healthy volunteers with an age range of 19–52 years were used. Subjects with symptoms of nasal infection, or who were taking medication at the time of the study or within the previous 4 weeks, were excluded. Experiments were performed in a laboratory with a controlled temperature (21°C) and humidity.

Administration of drugs

Compounds were administered to the nasal cavity using a hand-held pump spray (Perfect-Vallois U.K. Ltd.), which

delivered a volume of 100 μl per actuation. The dose administered was controlled by varying the concentration of the compound in the pump spray. Compounds were prepared in sterile saline (NaCl 154 mmol l^{-1}), which also served as the control. In all experiments, compounds were delivered to both nostrils, and the doses stated are the amounts delivered into each nostril.

Fresh solutions were made each day from stock solutions stored at $-20°C$, and were allowed to equilibrate with room temperature prior to administration. PAF was prepared in sterile saline at a concentration of 600 μg ml^{-1}. Other compounds were dissolved in saline at the following concentrations: histamine, 0.5–5 mg ml^{-1}; bradykinin, 2 mg ml^{-1}; icatibant, 2 mg ml^{-1}; L-NAME, 1–100 μmol ml^{-1}; N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-D-arginine methyl ester (D-NAME), 10 μmol ml^{-1}; L-arginine, 300 μmol ml^{-1}. The doses used were based on previous studies (Dear *et al*., 1996) and pilot experiments.

Measurement of nasal patency

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

Study design

In all experiments, a double-blind, balanced randomised-block, cross-over design was used. Subjects were randomly assigned to the treatment protocols. Subjects received all treatments on separate occasions, at least 72 h apart.

Effect of NOS inhibitors on nasal airway hyperresponsiveness

The resting patency of the nasal airway was determined as described above, following which the subjects received one of the following combinations of nasal aerosol: saline (control), L-NAME (1 μmol), L-NMMA (1 μmol), D-NAME (1 μmol) or L-NAME (1 μmol) plus L-arginine (30 μmol) combined. The action of L-NAME and L-NMMA in the human nasal airway, at the concentrations used in this study, is maximal about 30 min after administration (Dear *et al*., 1996). Therefore, this pretreatment was repeated every 30 min for 5.5 h. The development of PAF-induced hyperresponsiveness in the human nasal airway is present 6 h after administration (Austin & Foreman, 1993). Therefore, we investigated the presence of hyperresponsiveness 6 h after the initial dose of NOS inhibitor. After the pretreatment, a baseline Amin was determined, followed by challenge with either histamine or bradykinin, 200 μg per nostril. Amin was then redetermined 2, 5 and 10 min after the challenge. The measurement of Amin immediately prior to challenge with histamine or bradykinin formed the baseline value, and subsequent values of Amin were expressed as a percentage decrease in Amin from this baseline.

In a separate experiment utilising a similar protocol, subjects received a pretreatment with saline or L-NAME at the following concentrations: 0.1, 1 and 10 μmol, every 30 min for 5.5 h. Subsequent histamine challenges were performed at 2, 6, 24 and 48 h after the initial pretreatment, and the response monitored by acoustic rhinometry. The doses of histamine used were 50, 200 and 500 μg, and only one dose was used on each occasion. All subjects therefore received all combinations of pretreatment and histamine challenge on separate occasions, at least 72 h apart. A further experiment was carried out in which subjects received a single dose of either saline or L-NAME, 1 μmol, with a histamine challenge (200 μg) 6 h later.

Effect of icatibant, a bradykinin B_2 receptor antagonist, on L-NAME-induced hyperresponsiveness in the human nasal airway

In order to investigate whether kinins are involved in the nasal hyperresponsiveness induced by L-NAME, the following study was carried out. The administration of icatibant, 200 μg, prevents PAF-induced nasal hyperresponsiveness (Turner *et al*., 2000a). Therefore, subjects were given a nasal spray of either icatibant, 200 μg, or vehicle (saline). Two min later, a further spray was administered, containing either L-NAME (1 μmol) or a saline control. This second spray was then administered every 30 min, for 5.5 h, as before. The duration of action of icatibant in the nasal airways is about 2 h (Dear & Foreman, unpublished), so subjects received either saline or icatibant again 2 and 4 h after the start. Finally, at 6 h, a baseline measurement of Amin was taken, following by challenge with histamine, 200 μg. Amin was then redetermined 2, 5 and 10 min after the challenge, as before.

Role of NOS in PAF-induced nasal airway hyperresponsiveness

In this study, L-NMMA (1 μmol), L-arginine (30 μmol) or a saline control was administered into the nasal cavity. After 2 min, a second aerosol of PAF (60 μg) or saline (control) was given, L-NMMA, L-arginine or saline was then administered every 30 min for 5.5 h as before, 6 h after the start, a histamine challenge was carried out and the nasal response monitored as before. Each subject received the following four combinations of treatment, on separate occasions: Saline/Saline, Saline/PAF, L-NMMA/PAF and L-arginine/PAF.

Effect of L-NAME and PAF on nitric oxide production in the human nasal airway

In both the following experiments, the amount of NO in nasal air was measured by direct chemiluminescence, according to the method of Kharitonov *et al*. (1997a). Initially nasal NO was measured, following which subjects received a nasal spray of either L-NAME, 1 μmol, or saline, every 30 min for 5.5 h. At 2 and 6 h after the treatment, nasal NO was reassessed. In a separate experiment, nasal NO was measured, following which subjects received a single administration of PAF, 60 μg, or saline. Nasal NO was monitored at 2 and 6 h later.

Data analysis

The dimensions of the nasal airway vary between subjects, and also within subjects from day to day, so the data have been normalized by expressing changes in Amin as the percentage decrease in Amin from the baseline control value. The absolute values for the control measurements have been given, together with s.e.means, in each experiment. For each determination of nasal patency in a subject, following nasal challenge, a response-time curve was constructed and the area under curve (AUC) calculated. Data are expressed as mean AUC $\pm$ s.e.mean. Analysis of baseline values was used to control for

variation between experiments. The data were first analysed using non-parametric analysis-of-variance tests, followed by an appropriate, *post-hoc* test. In all cases, the comparisons were made between active treatment and saline control. The non-parametric statistical test is given with each data set. A value of $P<0.05$ is taken as significant.

Materials

L-NAME, L-NMMA, D-NAME, L-arginine and histamine were purchased from Sigma (Poole, U.K.). Bradykinin and platelet activating factor (C_{16}) were obtained from Calbiochem (Nottingham, U.K.). Icatibant was a gift of Dr K. Wirth, Hoechst AG (Frankfurt, Germany). All other substances used were of Analar or similar quality.

Results

Effect of NOS inhibitors on nasal airway hyperresponsiveness

Figure 1 shows that the NOS inhibitors L-NAME and L-NMMA, at a concentration of 1 μmol per nostril and given every 30 min, increased the nasal response to histamine, 200 μg, 6 h later, compared to pretreatment with saline control (average increase 35.1 and 48.6% respectively; $P<0.05$, Friedman's test; $P<0.05$, Wilcoxon sign-rank test). L-NAME, at the same dose, also induced a hyperresponsiveness to bradykinin, 200 μg ($P<0.05$, Wilcoxon sign-rank test). To test the specificity of L-NAME, we attempted to prevent the hyperresponsiveness, induced by L-NAME, using L-arginine. Co-administration of L-arginine (30 μmol per nostril) abolished the ability of L-NAME to induce hyperresponsiveness to histamine ($P>0.05$, Wilcoxon sign-rank test). Furthermore, D-NAME (1 μmol/nostril) did not induce hyperresponsiveness to histamine ($P>0.05$, Wilcoxon sign-rank test). None of the pretreatments caused a significant change in the resting Amin ($P>0.05$, Friedman's test, data not shown).

Pretreatment of the nasal airway with L-NAME, at a concentration of 0.1 μmol, failed to induce a nasal hyperresponsiveness ($P>0.05$, Wilcoxon sign-rank test). At higher concentrations (1 and 10 μmol), a nasal hyperresponsiveness was induced ($P<0.05$, Wilcoxon sign-rank test), though no significant dose-response relationship was observed ($P>0.05$, Friedman's test) (Figures 2 and 3). When the nasal airway was treated with a single dose of L-NAME, 1 μmol, no hyperresponsiveness to histamine was observed ($P>0.05$, Wilcoxon sign-rank test) (Figure 4). Furthermore, a nasal hyperresponsiveness was only present after 6 h of repeated L-NAME administration, and not after 2 h of treatment, as shown in Figure 5. The hyperresponsiveness was not present 24 h after initial pretreatment ($P<0.05$, Wilcoxon sign-rank test). However, when the nasal airway was pretreated with a higher dose of L-NAME (10 μmol) the duration of the hyperresponsiveness was prolonged ($P<0.05$, Friedman's test).

Effect of icatibant, a bradykinin B_2 receptor antagonist, on L-NAME-induced hyperresponsiveness in the human nasal airway

Pretreatment of the nasal airway with icatibant, 200 μg, failed to prevent the hyperresponsiveness induced by L-NAME ($P>0.05$, Friedman's test), as shown in Figure 6.

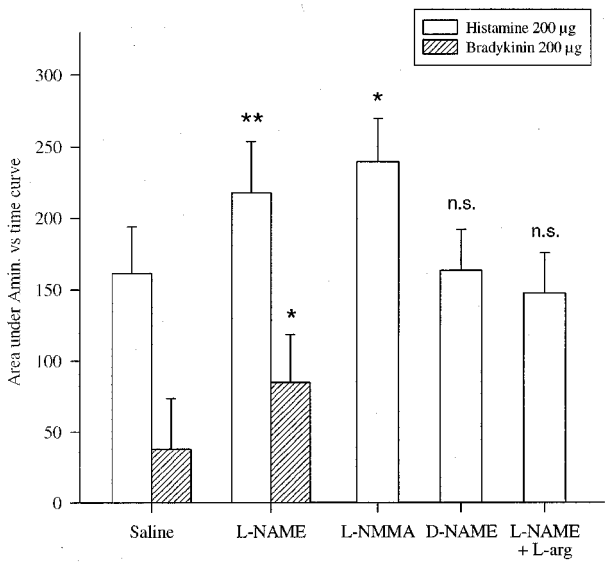

Figure 1 The effect of inhibitors of nitric oxide synthase on the response to nasal challenge with histamine or bradykinin, 200 μg. The nasal cavity was repeatedly pretreated with one of the following: saline, L-NAME, 1 μmol; L-NMMA, 1 μmol; D-NAME, 1 μmol, L-NAME, 1 μmol, plus L-arginine (L-arg), 30 μmol; as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from 10 subjects. Vertical bars represent s.e.mean. The means$\pm$s.e.mean baseline value for Amin was 0.60 ± 0.02 cm^2. */**Significant difference in AUC following histamine challenge after pretreatment with L-NAME compared to saline control (*$P<0.05$, **$P<0.01$, Wilcoxon sign-rank test), n.s. = no significant difference in AUC between saline and the treatments shown ($P>0.05$, Wilcoxon sign-rank test).

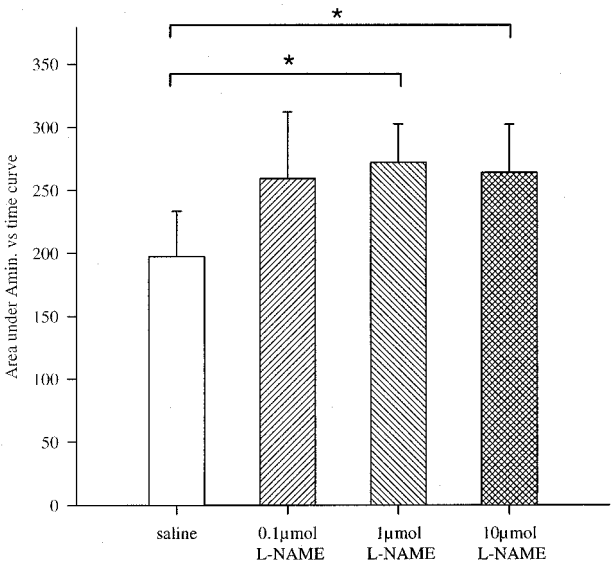

Figure 2 The effect of different concentrations of L-NAME on the response to nasal challenge with histamine, 200 μg. The nasal cavity was pretreated with repeated administrations of L-NAME, at the doses shown, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from six subjects. Vertical bars represent s.e.mean. The mean$\pm$s.e.mean baseline value for Amin was 0.56 ± 0.04 cm^2. *Significant difference in AUC following histamine challenge after pretreatment with L-NAME, 1 and 10 μmol, compared to saline control ($P<0.05$, Wilcoxon sign-rank test).

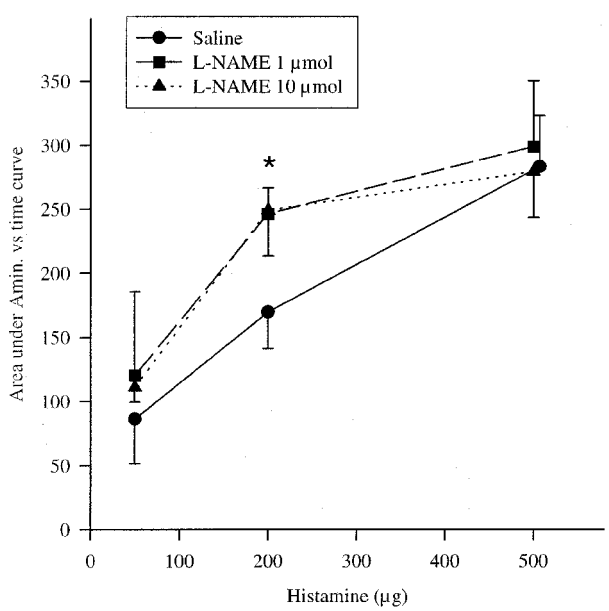

Figure 3 Dose-response curve for histamine, following repeated pretreatment of the nasal cavity with L-NAME, 1 μmol, or a saline control, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from five subjects. Vertical bars represent s.e.mean. The mean$\pm$s.e.mean baseline value for Amin was 0.58 ± 0.03 cm^2. *Significant difference in AUC following histamine challenge after pretreatment with L-NAME, compared to saline control ($P<0.05$, Wilcoxon sign-rank test).

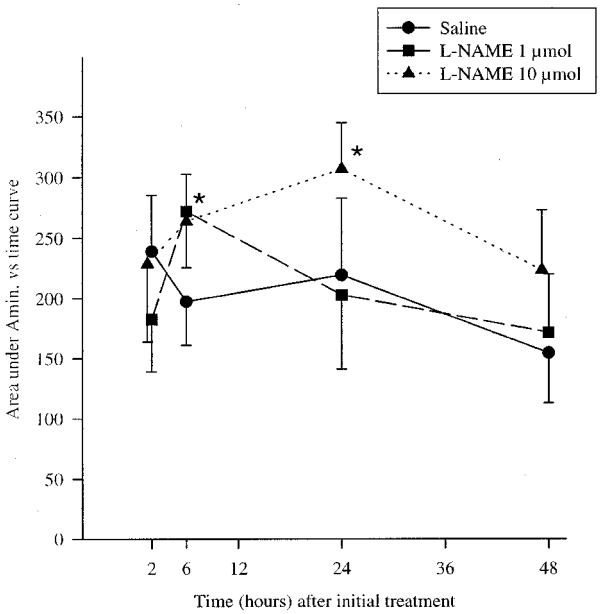

Figure 5 Time course of the hyperresponsiveness, induced by L-NAME, to histamine, 200 μg. The nasal cavity was pretreated with L-NAME, at the doses shown, every 30 min, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from six subjects. Vertical bars represent s.e.mean. The mean$\pm$s.e.mean baseline value for Amin was 0.56 ± 0.03 cm^2. *Significant difference in AUC following histamine challenge after repeated pretreatment with L-NAME compared to saline control ($P<0.05$, Wilcoxon sign-rank test).

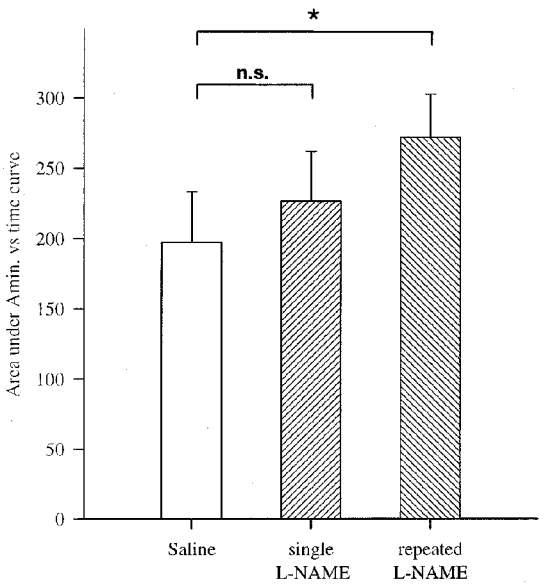

Figure 4 Effect of changes in the dosing regimen of L-NAME on the response to nasal challenge with histamine, 200 μg. The nasal cavity was pretreated with either saline, a single dose of L-NAME, 1 μmol, or repeated administrations of L-NAME, 1 μmol, every 30 min, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from six subjects. Vertical bars represent s.e.mean. The mean$\pm$s.e.mean baseline value for Amin was 0.57 ± 0.04 cm^2. *Significant difference in AUC following histamine challenge after repeated pretreatment with L-NAME, compared to saline control ($P<0.05$, Wilcoxon sign-rank test). n.s. = no significant difference in AUC between treatments shown ($P>0.05$, Wilcoxon sign-rank test).

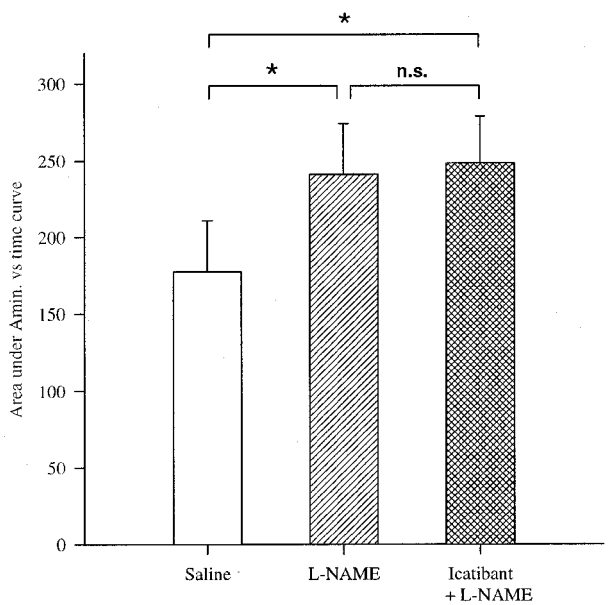

Figure 6 Effect of pretreatment with icatibant, a bradykinin B_2 receptor antagonist, on L-NAME-induced hyperresponsiveness to histamine, 200 μg. The nasal cavity was pretreated with saline, icatibant, 200 μg, and/or L-NAME, 1 μmol, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from eight subjects. Vertical bars represent s.e.mean. The mean$\pm$s.e.mean baseline value for Amin was 0.58 ± 0.04 cm^2. *Significant difference in AUC following histamine challenge after pretreatment shown, compared to saline control ($P<0.05$, Wilcoxon sign-rank test), n.s. = no significant difference in AUC between treatments shown ($P>0.05$, Wilcoxon sign-rank test).

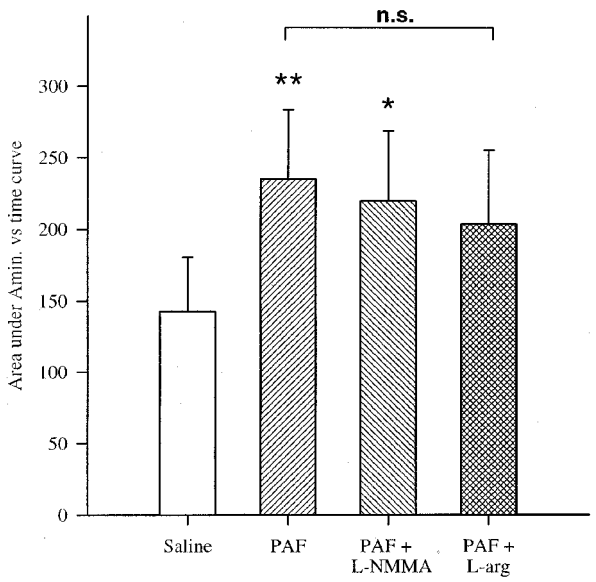

Figure 7 Effect of L-NMMA and L-arginine (L-arg) on PAF-induced hyperresponsiveness to histamine, 200 μg. The nasal cavity was pretreated with one of the following: saline; PAF, 60 μg; PAF, 60 μg, plus L-NMMA, 1 μmol; PAF, 60 μg, plus L-arginine (L-arg), 30 μmol, as described in the text. The response to histamine was measured as the area under Amin vs time curves (AUC) following nasal challenge. Data are means from 10 subjects. Vertical bars represent s.e.mean. The mean $\pm$ s.e.mean baseline value for Amin was 0.63 ± 0.02 cm^2. */**Significant difference in AUC following histamine challenge after pretreatment shown, compared to saline control (*$P<0.05$, **$P<0.01$, Wilcoxon sign-rank test), n.s. = no significant difference in AUC between treatments shown ($P>0.05$, Friedman's test).

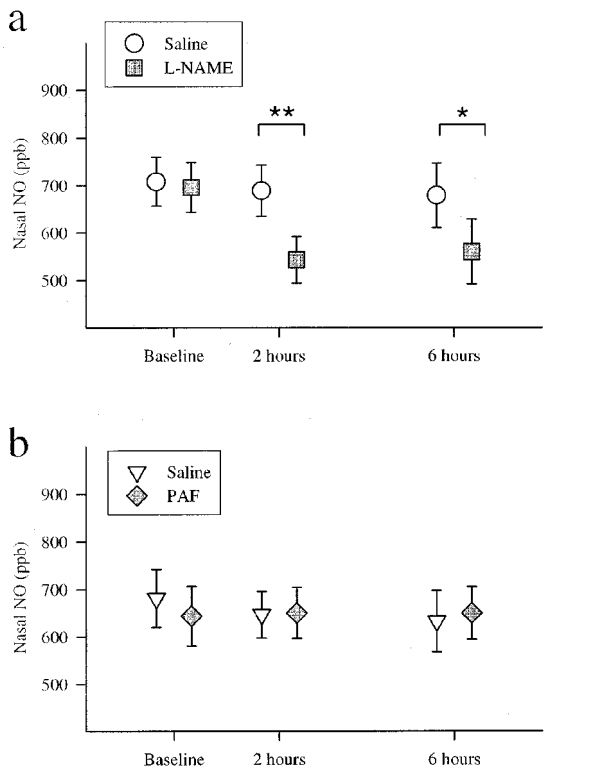

Figure 8 Generation of nitric oxide (NO) in the human nasal airway following treatment with L-NAME, 1 μmol, or saline control, every 30 min for 5.5 h (graph a) or with PAF, 60 μg, or saline control (graph b). Nasal NO was determined by direct chemiluminescence, and the data is presented as means $\pm$ s.e.mean from eight subjects. */**Significant difference in nasal NO following treatment with L-NAME compared to saline control (*$P<0.05$, **$P<0.01$, Wilcoxon sign-rank test).

Role of NOS in PAF-induced nasal airway hyperresponsiveness

PAF, 60 μg, caused a nasal hyperresponsiveness to histamine 6 h later, compared to pretreatment with saline control ($P<0.01$, Wilcoxon sign-rank test), as shown in Figure 7. This hyperresponsiveness was also present following pretreatment with L-NMMA (1 μmol every 30 min) ($P<0.05$, Wilcoxon sign-rank test). Pretreatment with L-arginine (30 μmol every 30 min) after PAF did not induce a significant degree of airway hyperresponsiveness; however, there were no differences in the nasal response to histamine challenge with either L-NMMA or L-arginine, implying that PAF-induced nasal hyperresponsiveness is not modulated by L-NMMA or L-arginine at the doses used ($P>0.05$, Friedman's test).

Effect of L-NAME and PAF on nasal NO production

Treatment of the nasal cavity with L-NAME, 1 μmol every 30 min, caused a significant reduction in the amount of NO in the nasal airway 2 and 6 h later, compared to the saline control ($P<0.01$ and $P<0.05$ respectively, Wilcoxon sign-rank test) (Figure 8a). PAF, 60 μg, did not alter nasal NO levels, compared to the saline control ($P<0.05$, Wilcoxon sign-rank test) (Figure 8b). There were no significant differences in the baseline levels of NO between treatments, in either experiment ($P>0.05$, Friedman's test).

Discussion

In this paper, we have demonstrated that the NOS inhibitors, L-NAME and L-NMMA, can induce a hyperresponsiveness to histamine and bradykinin in the human nasal airway. This effect was stereo-selective and abolished by the administration of L-arginine, suggesting that decreased production of basal levels of nitric oxide may induce hyperresponsiveness to histamine and bradykinin in the non-allergic human nasal airway. This hypothesis is further supported by the observation that L-NAME decreased the amount of NO detected in the human nasal airway. While this effect was present 2 and 6 h after initial administration, the hyperresponsiveness was only detected after 6 h. This suggests that the inhibition of basal NO production may be an early event in the process by which NOS inhibitors induce nasal airway hyperresponsiveness. The ability of L-NAME to induce a nasal hyperresponsiveness may have been the result of its action as a weak antagonist at muscarinic receptors (Buxton *et al*., 1993). However, this is unlikely, because the L-NAME-induced nasal hyperresponsiveness was prevented by concomitant administration of L-arginine, implying a reversal of NOS inhibition. Furthermore, L-NMMA, a NOS inhibitor with no affinity for the muscarinic receptor, also induced a hyperresponsiveness to histamine.

The assessment of nasal airway hyperresponsiveness in human subjects is difficult, partly due to the variability in response to nasal challenge between individuals (N. Mygind, personal communication). In this study, the variation in nasal responses are probably a result of this factor. The degree of hyperresponsiveness demonstrated in this study is equivalent to that induced following nasal administration of PAF (Austin & Foreman, 1993) or antigen in subjects with seasonal allergic rhinitis (Turner *et al*., 2000b). Walden *et al*. (1991) found that there is a limit to the degree of inducible hyperresponsiveness,

implying that an increase in nasal response would only be observed with the use of sub-maximal doses of histamine. This would explain the observation in this study that no hyperresponsiveness was seen using histamine, 500 μg, for nasal challenge. The apparent absence of a significant increase in response with low doses of histamine (e.g. 50 μg) has been demonstrated elsewhere (Walden *et al*., 1991; Austin & Foreman, 1993).

Previous studies in our laboratory have indicated that a single administration of L-NAME, at the same dose used in this study, had no effect on histamine-induced reduction in Amin but did inhibit the reduction in Amin induced by bradykinin (Dear *et al*., 1996). Therefore, NOS inhibition might be expected to inhibit the nasal response to histamine and bradykinin. In the current study, we have demonstrated that repeated administration of NOS inhibitors increased both the histamine- and bradykinin-induced increase in nasal obstruction. These contrasting results may be due to an inhibition of a different isoform of NOS, or the different dosing regimen of inhibitor used. The expression of inducible NOS (iNOS) in the nasal airway is strongly linked to the degree of inflammation present (Furukawa *et al*., 1996), so it is probable that the activity of iNOS in the normal, non-inflamed airway is negligible. Therefore, the hyperresponsiveness may be the result of the prolonged inhibition of one of the constitutive isoforms of NOS present in the nasal airway.

The nasal hyperresponsiveness induced by L-NAME was not dose-dependent, unlike that induced by PAF (Austin & Foreman, 1993). Dear *et al*. (1996) demonstrated that the action of L-NAME in opposing albumin extravasation following exposure to histamine and bradykinin was not dose-dependent, and only occurred at a dose of 1 μmol L-NAME, or greater. Furthermore, 10 μmol L-NAME did not cause a greater inhibition compared to 1 μmol. This suggests that in the human nasal airway, a minimal dose of 1 μmol L-NAME is required to inhibit NOS, and higher doses do not cause further inhibition, a finding which is consistent with the current data. Interestingly, in this study, the hyperresponsiveness induced by L-NAME was prolonged when a dose of 10 μmol was used. The initial inhibition of NOS by L-NAME is rapidly reversible, while increased exposure to L-NAME causes a different type of inhibition, which is only slowly reversible (Griffith & Gross, 1996). Therefore, the prolonged hyperresponsiveness observed following pretreatment with L-NAME, 10 μmol, may be a result of this phenomenon.

Nitric oxide can act as a scavenger of oxidative free radicals, including superoxide (Muijsers *et al*., 1997). Superoxide and other reactive oxygen species have been implicated in the development of hyperresponsiveness in the lower airways of sheep (Lansing *et al*., 1991) and guinea-pigs (Ikuta *et al*., 1992), and in both the lower and nasal airways in man (Hilterman *et al*., 1998; Michelson *et al*., 1999). The majority of NOS in the human nasal airway is associated with the nasal epithelium (Furukawa *et al*., 1996). Oxidative free radicals can induce airway hyperresponsiveness by causing epithelial damage (Hulsmann *et al*., 1994). Therefore, the production of basal levels of NO, by the epithelium, could represent a defensive mechanism, so inhibition of epithelium-associated NOS might increase the susceptibility of the epithelium to oxidative damage, resulting in hyperresponsiveness (Nijkamp *et al*., 1993). Alternatively, NO may regulate the responsiveness of the airways by its action as an inhibitory neurotransmitter of non-adrenergic non-cholinergic (NANC) transmission (Li & Rand, 1991). Neuronal NOS has been identified in the nerves of the human nasal mucosa (Kulkarni *et al*., 1994). A reduction in NO production by this enzyme may reverse NO-mediated inhibition of the NANC system, thus increasing the response of the nasal mucosa to stimuli such as histamine and bradykinin. In allergic rhinitis, the increased response to bradykinin is partly mediated by neuronal reflexes (Riccio and Proud, 1996). It has also been proposed that nitric oxide can stimulate the production of prostaglandins *via* cyclo-oxygenase, therefore inhibitors of NOS may induce hyperresponsiveness by causing a shift in the metabolism of arachidonic acid, from products of cyclo-oxygenase to 5-lipoxygenase, generating leukotrienes (Folkerts *et al*., 1995).

Paradoxically, an increase in NO production has been associated with airway hyperresponsiveness in both asthmatics and subjects with allergic rhinitis (Kharitonov & Barnes, 1996). The production of NO by the airways is raised in both asthma and allergic rhinitis (Garrelds *et al*., 1995; Kharitonov *et al*., 1997b), and this increase is probably caused by an increase in the expression of iNOS (Kharitonov & Barnes, 1996). NO readily reacts with the superoxide anion to form peroxynitrite; this induces hyperresponsiveness in the guinea-pig lower airway both *in vivo* and *in vitro* (Sadeghi-Hashjin *et al*., 1996), and its formation in the asthmatic airway is strongly associated with a hyperresponsiveness to methacholine (Saleh *et al*., 1998). Therefore, while a basal level of NO production may be protective against airway hyperresponsiveness, excessive NO release, possibly mediated by an upregulation of iNOS, may be destructive and cause airway hyperresponsiveness.

PAF may induce a hyperresponsiveness in the human nasal airway by a mechanism dependent on the generation of free radicals (Austin & Foreman, 1993). Therefore, a NOS inhibitor could, theoretically, potentiate PAF-induced airway hyperresponsiveness. Alternatively, PAF may upregulate the expression of iNOS, generating higher levels of NO which can combine with superoxide and free radicals to form peroxynitrite, causing hyperresponsiveness (Sadeghi-Hashjin *et al*., 1996; Saleh *et al*., 1998). However, in this study, modulating NOS activity had no effect on the hyperresponsiveness induced by PAF, nor was an increase in nasal NO observed after PAF administration. This suggests that PAF causes hyperresponsiveness *via* a mechanism independent of NOS activity in the human nasal airway. This is further supported by the finding that icatibant, a bradykinin B_2 receptor antagonist, abolishes the nasal airway hyperresponsiveness induced by PAF (Turner *et al*., 2000a), but not by NOS inhibitors.

We therefore conclude that inhibition of NOS can cause hyperresponsiveness in the human nasal airways, and propose that this may occur by a mechanism different to that of PAF-induced hyperresponsiveness.

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