

Short communication

The effect of platelet-activating factor (PAF) on nasal airway resistance in healthy subjects is not mediated by nitric oxide

Background: The nose is an important source of nitric oxide (NO) in man, but the relevance of NO production to the response to inflammatory mediators is not clear.

Methods: In this study, we evaluated the effect of NO inhibition on nasal airway resistance (NAR) at baseline, after an acute challenge with platelet-activating factor (PAF), a potent proinflammatory factor, and after an acute challenge with bradykinin (BK), both of which are mediators of allergic rhinitis in man. Eight healthy subjects were enrolled in the study. Nasal NO production was measured by the chemiluminescence method, and NAR was measured by active anterior rhinomanometry.

Results: Basal nasal NO concentration was 500.6 ± 115.6 ppb; it significantly decreased after topical administration of the NO-synthase inhibitor L-NAME, and the NO-synthase substrate L-arginine caused a recovery in NO production. The administration of L-NAME did not cause any change in basal NAR. In a double-blind fashion, we performed nasal challenge with PAF and BK after topical pretreatment with either placebo or L-NAME. After placebo pretreatment, both PAF and BK caused a significant increase in NAR (respectively, from 0.29 ± 0.11 Pa s cm⁻³ to 0.75 ± 0.21 Pa s cm⁻³, and from 0.36 ± 0.18 Pa s cm⁻³ to 0.71 ± 0.25 Pa s cm⁻³; $P < 0.001$, $n = 8$). Pretreatment with L-NAME did not prevent the PAF-induced increase in NAR (from 0.31 ± 0.10 Pa s cm⁻³ to 0.71 ± 0.27 Pa s cm⁻³), whereas it prevented the BK-induced increase in NAR (from 0.33 ± 0.15 Pa s cm⁻³ to 0.43 ± 0.16 Pa s cm⁻³).

Conclusions: Topical administration of the NO-synthase inhibitor L-NAME at doses sufficient to decrease NO nasal production does not prevent the PAF-induced increase in NAR, indicating that NO generation *in vivo* is not involved in the nasal response to PAF.

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Nitric oxide (NO), a potent vasodilator, is elaborated by several cells in the lungs and airways, including airway epithelial cells (1), nerve cells (2), and vascular endothelial cells (3). NO is involved in the control of vascular resistance, as changes in pulmonary vascular resistance follow changes in the exhaled concentrations of NO when the enzyme NO-synthase is stimulated and/or inhibited (3).

In man, several studies have recently demonstrated the major nasal contribution to NO production detected in exhaled air (4, 5). In the nose, most nasal airway resistance (NAR) to airflow is due to the state of the venous sinusoids (pseudoerectile tissue) of the turbinates and nasal septum (6) that have been shown to express NO-synthase (7). It has also been demonstrated that nasal vascular structures promptly respond with an increase of NAR to the local release and generation of mediators induced by the activation

of inflammatory cells (8). On the other hand, the human nasal airway response to bradykinin (BK) and histamine is altered by inhibitor of NO synthesis (9). Platelet-activating factor (PAF), a potent proinflammatory phospholipid, is one of the mediators implicated in the nasal response to allergens (10). PAF and its precursor are released in human nasal airways in response to nasal allergen challenge (9). In healthy subjects, PAF induces NAR increase and most symptoms of rhinitis (11). The mechanisms of these effects are not completely known, but in other vascular beds the effects of PAF are mediated by NO (12, 13).

Therefore, in the present study, we evaluated the effect of a NO-synthase inhibitor N^G-L-arginine methyl ester (L-NAME) on NAR at baseline and after an acute challenge with PAF and BK in healthy subjects.

Material and methods

Subjects

Eight normal volunteers recruited from hospital personnel were enrolled in the study. They had no history of allergy, nasal disease, asthma, or any other chronic airway disease. The subjects gave written informed consent, and the study was approved by our local hospital ethics committee.

Study design

The study was conducted in three phases.

Phase 1. For the subjects (five men and three women aged 25.7 ± 4.3 years), the concentration of NO was measured. The effect of L-NAME and subsequent administration of L-arginine on nasal expired NO levels was studied. Three measurements for each nostril were taken at intervals of 10 min to give a baseline estimate of NO production. Then, either a solution of L-NAME (Sigma, Poole, UK) (15 mg in 2.5 ml of normal saline) or vehicle diluent (placebo) was delivered in random order. After application of the solutions, measurements of NO were performed at three 10-min intervals. Thirty minutes after the administration of L-NAME, L-arginine (Sigma, UK) (50 mg in 2.5 ml of saline) was administered. A further three measurements of NO were then taken at 10-min intervals.

Furthermore, on different days, nasal NO levels were measured in four subjects at baseline and after nasal challenge with PAF (Fluka, Italy) (500 nmol) dissolved in 99.8% ethanol and diluted in 0.9% w/v NaCl solution, and BK (Fluka, Italy) (1000 μ g) in saline or placebo.

Phase 2. The subjects were randomly chosen to receive pretreatment with either L-NAME (15 mg in saline) or vehicle diluent (placebo) delivered in a double-blind fashion followed by nasal challenge with PAF. The administrations were performed at the same time on two separate days. The rhinomanometric examinations and noting of symptom scores were carried out at baseline and 10 min after treatment with either L-NAME or placebo. PAF was then administered, and rhinomanometric examinations were repeated after 20 min. Nasal symptom scores were also assessed 20 min after the application of PAF.

Phase 3. The subjects were also randomly chosen to receive pretreatment with either L-NAME or vehicle diluent (placebo) delivered in a double-blind fashion, followed by nasal challenge with BK. The rhinomanometric examinations and noting of symptom scores were carried out at baseline and 10 min after treatment with either L-NAME or placebo. BK in saline solution was then administered,

and rhinomanometric examinations were repeated after 4 min. Nasal symptom scores were also assessed 4 min after the application of BK.

Nasal challenge

L-NAME, L-arginine, and placebo were administered with a jet nebulizer (De Vilbiss, Somerset, PA, USA) for over 10 min during repeated breath-holds. PAF and BK were delivered bilaterally with a hand-held pump spray delivering 0.13 ml per activation with a coefficient of variation of 8%.

Measurement of nasal NO

The concentration of NO was measured by chemiluminescence (Model 42, Thermo Electron, Warrington, UK) with a detection limit of 0.5 ppb and response time of 40 s for a full-scale deflection of 0–95 ppb.

The air from one nostril was directly sampled, and the peak concentration of NO was recorded over a period of 60 s by a plastic cannula connected to the chemiluminescent analyzer. Another plastic cannula was placed in the other nostril, and this was directly connected to a mass spectrometer (MGA 200, Centronics, Gillingham, UK). This continuously sampled the nasal air for carbon dioxide (CO₂) as a check that the subject was not breathing. Subjects were asked to hold their breath for 1 min with the glottis closed. During the breath-hold, the nasal gas was sampled. The contralateral nostril was not obstructed.

Measurement of NAR

The method of active anterior rhinomanometry was applied to measure the resistance of each nasal cavity (15). The adhesive tape technique was employed. Total NAR was measured by a digital rhinomanometer (Rhinotest HP 500, Allergopharma, Germany). Rhinomanometric examinations were carried out at the same time of day and under similar environmental conditions. NAR was calculated as the average of five consecutive breaths at a fixed reference pressure pre-established at 150 Pa. Calculated values were expressed in Pa s cm⁻³. Intraindividual variability of NAR for repeated measurements was $10 \pm 5\%$.

Symptom score

The following symptoms were recorded: pain/discomfort, rhinorrhea, nasal obstruction, sneezing, and itching of the nose.

Statistics

The results are expressed as mean $\pm$ standard deviation (SD). Nasal NO levels were studied by analysis of variance (ANOVA) and Wilcoxon's matched pair test.

The paired *t*-test was used to analyze the effects of the treatments on NAR. A *P* value of less than 0.05 was taken to be significant.

Results

While nebulized normal saline produced no change in the NO concentration from the nose, L-NAME reduced the concentration from 500.6 ± 115.6 ppb to 356.6 ± 85.6 ppb ($P < 0.001$; $n = 8$). Subsequent addition of L-arginine restored the concentration to basal values (Fig. 1).

In two subjects, the duration of effect of L-NAME without L-arginine was measured. NO levels were reduced over 120 min.

The application of both PAF and BK did not cause any significant variation in NO levels in comparison to placebo.

The inhalation of L-NAME did not cause a significant change in NAR (from 0.33 ± 0.16 Pa s cm⁻³ to 0.35 ± 0.19 Pa s cm⁻³; $P = \text{NS}$, $n = 6$).

The applications of both PAF and BK after pretreatment with placebo caused a significant increase in NAR (respectively, from 0.29 ± 0.11 Pa s cm⁻³ to 0.75 ± 0.21 Pa s cm⁻³ and from 0.36 ± 0.18 Pa s cm⁻³ to 0.71 ± 0.25 Pa s cm⁻³; $P < 0.001$, $n = 8$). Pretreatment with L-NAME did not prevent the increase in NAR induced by PAF (from 0.31 ± 0.10 Pa s cm⁻³ to 0.71 ± 0.27 Pa s cm⁻³). In contrast, the application of BK after pretreatment with L-NAME significantly prevented the increase of NAR induced by BK (from 0.33 ± 0.15 Pa s cm⁻³ to 0.43 ± 0.16 Pa s cm⁻³) (Fig. 2).

No symptoms were present in any subject at baseline and after administration of L-NAME or placebo. PAF and BK caused an increase in nasal blockage, pain/discomfort, and rhinorrhea in all subjects. No differences in clinical score were appreciable after PAF and BK between L-NAME and placebo pretreatment.

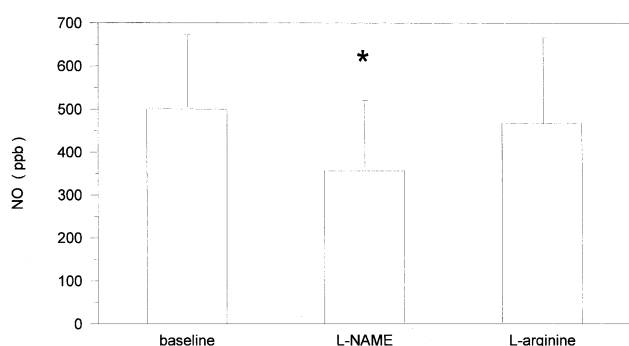


Figure 1. Mean values with standard deviation of nasal NO under basal conditions, after L-NAME (15 mg) and L-arginine (50 mg). ppb: parts per billion. * $P < 0.001$.

Discussion

In this study, we have shown that nasal administration of the NO-synthase inhibitor L-NAME, at doses capable of decreasing nasal NO levels, has no effect on NAR. Furthermore, it does not prevent the increase in NAR induced by an acute challenge with PAF, whereas it prevents the increase in NAR induced by topical application with BK. We obtained a percentage of reduction in NO levels after administration of L-NAME, like Silkoff et al. (16), who administered L-NAME to allergic rhinitics at doses 10-fold higher than those we used. Furthermore, in our study, the effect of L-NAME was reversed by L-arginine, a substrate of NO-synthase, confirming that nasal NO is formed by the L-arginine-citrulline pathway with NO-synthase.

We did not observe any effect of L-NAME on basal NAR in healthy subjects. Our data are consistent with other authors' findings that do not support a role of nasal NO in the control of resting NAR (17, 18). In addition, a similar fall in NO without affecting nasal minimal cross-sectional area has been reported (16). However, the limited effect of L-NAME on nasal NO production does not allow exclusion of a marginal influence on basal NAR. In fact, the inhibition of nasal NO production was not complete; this might be related to several factors. Firstly, it is likely that the major site of NO nasal production is paranasal sinuses, as high concentrations of NO and NO-synthase immunoreactivity have been mainly found here (19). These could not be reached by aerosolized L-NAME. Secondly, a part of nasal NO could be produced by denitrifying enzymes, which are found in bacteria commonly isolated from the nose and are able to reduce nitrites and nitrates to NO (20).

In this study, we have investigated whether NO may be involved in the acute nasal response to PAF. In healthy human subjects, topical administration of PAF causes an acute increase of NAR resulting from an increase in the volume of the capacitance vessels (21).

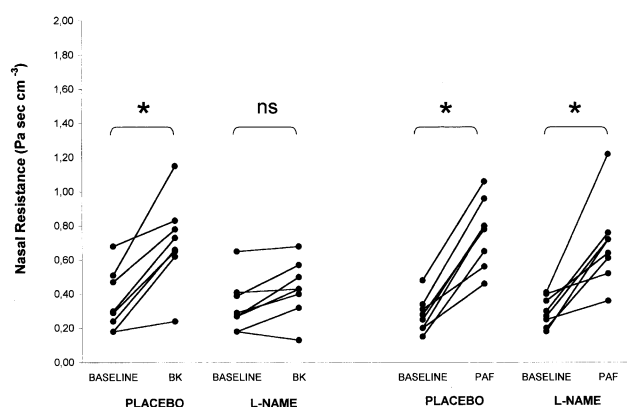


Figure 2. Individual values of NAR after nasal challenge with bradykinin (BK) and platelet-activating factor (PAF) in healthy subjects pretreated with placebo or L-NAME. * $P < 0.001$.

Furthermore, PAF increases albumin extravasation related to increase in vascular permeability (22). There is evidence that PAF modulates microvascular permeability through NO synthesis in several microvascular beds in animals (13, 14).

In our study, pretreatment with L-NAME did not cause any change in the increase in NAR induced by PAF. This result might be caused by the limited effect of L-NAME on nasal NO production. However, in the present study, a similar degree of nasal NO inhibition obtained by L-NAME was able to prevent the acute congestion following the topical administration of BK. Accordingly, Dear et al. have reported that nasal generation of NO may be involved in the nasal blockage caused by BK (9).

Alternatively, PAF might act through a NO-independent mechanism. In fact, there is evidence that PAF-induced constriction of rabbit glomerular afferent arterioles was mediated, at least in part, through cyclooxygenase products (14).

In our study, topical administration of PAF and

BK did not modify nasal NO. However, nasal NO or its metabolites have been reported to decrease or increase variably in patients with allergic rhinitis or healthy subjects after nasal challenges with different stimuli (23–25). Furthermore, it is possible that NO variations in the vascular endothelium, in close connection to the target site for BK and PAF (9, 22), were not detected by our method.

In conclusion, topical administration of the NO-synthase inhibitor L-NAME at doses able to decrease NO nasal production does not affect basal NAR. Furthermore, NO inhibition by L-NAME does not prevent the PAF-induced increase in NAR, suggesting a NO-independent mechanism in the nasal response to PAF.

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