

Nitric oxide-dependent neutrophil recruitment: role in nasal secretion

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Summary

Background Leukotriene B₄ (LTB₄), an inflammatory mediator, is a potent chemo-attractant for neutrophils that plays an important role in nasal secretion via release of elastase. Nitric oxide (NO) is an important modulator of leucocyte–endothelial cell interactions, endogenously produced in large quantities in the paranasal sinuses.

Objective To examine the role of NO in LTB₄-stimulated nasal secretion.

Methods A newly-developed method for isolating and superfusing a nasal segment in dogs was used.

Results Instillation of LTB₄ into the nasal segment caused a time-dependent increase in the volume of airway fluid and in the recruitment of neutrophils. N(G)-nitro-L-arginine-methylester (L-NAME), an inhibitor of NO synthase, prevented LTB₄-induced neutrophil recruitment and nasal secretion.

Conclusion These studies show that NO modulates LTB₄-induced neutrophil recruitment and subsequent fluid secretion in the nose, and they suggest a therapeutic role for NO inhibitors in modulating neutrophil-dependent nasal secretion.

Keywords: LTB₄, neutrophils, nitric oxide, L-NAME, nasal secretion

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Introduction

Allergic rhinitis is an inflammatory disease associated with local leukotriene release during periods of symptoms. Cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) stimulate bronchial secretion [1,2], whereas LTB₄ is known primarily for its effect as a chemoattractant for neutrophils [3] and to a lesser extent, for eosinophils and monocytes [4]. It has previously been demonstrated that LTB₄ recruits neutrophils into the nasal lumen and stimulates nasal secretion (an effect that was inhibited by pre-treatment with a neutrophil elastase inhibitor [5]). These results implicate the release of neutrophil elastase in LTB₄-induced nasal secretion.

The nasal production of NO is reported to increase during inflammation and allergic rhinitis due to increased activity of an inducible isoform of NO [6–8]. Exogenous NO has been shown to facilitate neutrophil chemotaxis

in vitro [9], whereas inhibition of NO prevents neutrophil recruitment [10,11]. Therefore, it was hypothesized that inhibition of NO in the nose, by preventing neutrophil recruitment, would prevent neutrophil-dependent nasal secretion. Therefore, a nasal segment in a dog was isolated and superfused [5], and the role of NO in LTB₄-induced nasal secretion was examined.

Methods

Animal preparation

Dogs (weight, 15–35 kg) were anaesthetized intravenously with Ketalar® (ketamine hydrochloride; 5 mg/kg bolus; Parke Davies, Walter Lambert Co., Morris Plains, NJ, USA) and Diazepam® (diazepam; 0.5 mg/kg, Elkins-Sinn Inc., NJ, USA), intubated with an endotracheal tube, and ventilated via a constant-volume respirator (Model 607 A, Harvard Apparatus, Dover, MA, USA) set at a tidal volume of 10 mL/kg and a frequency of 20 breaths/min.

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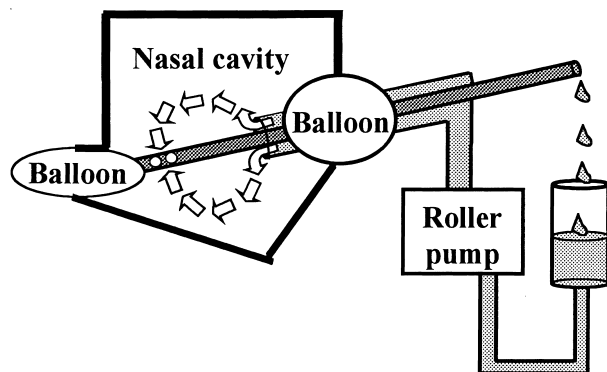


Fig. 1. An isolated segment of dog nasal cavity is created with a modified endotracheal tube equipped with a double-balloon system. One balloon is positioned in the vestibulum nasi and the other in the epipharynx. The isolated segment of the nasal cavity, in between the two balloons, is superfused continuously via a double-lumen catheter system. The buffer solution is pumped into the segment through one of the catheters (light grey). The buffer leaves the segment, drips into a reservoir through the other catheter (dark grey) and is re-circulated. An increase in the level of fluid in the reservoir is regarded as a reflection of the total secretion. The dripping fluid as well as the fluid in the container is sampled.

Anaesthesia was maintained with Halothane (1–1.5%; Halocarbon Laboratories, River Edge, NJ, USA). Intravenous fluid (Lactated Ringer's Injection USP, Baxter, Deerfield, IL, USA) was administered (50 mL/h) to prevent dehydration. The dogs were kept on a heating pad to maintain body temperature at 37 °C. Vital signs were monitored continuously during the study.

Continuous superfusion of the nose

A modified Sheridan endotracheal tube equipped with a latex double balloon system was used to create an isolated segment in the nasal cavity. The balloons were positioned in the vestibulum nasi and epipharynx, respectively. The two balloons were inflated, and the segment was superfused through an open system consisting of two small bore silastic [5]. Care was taken to ensure that the isolated nasal segment was patent and that no leaks occurred. The superfusate was allowed to drip into a polypropylene syringe barrel, which was used as a reservoir, and the fluid was pumped back into the nasal segment via a peristaltic roller pump (Buchler, Fort Lee, NJ, USA) at 14 mL/min (Fig. 1). The reservoir was placed next to the dog's nose, making the tubing length short, and the superfusate was re-circled every 2–3 min, all minimizing the risk of a fall in temperature. However, a slight shift in the superfusate

temperature cannot be excluded, contributing to the basal nasal secretion via activation of sensory nerves.

Experimental protocol

The isolated nasal segment was superfused with 20 mL of (a) sterile Hanks balanced salt solution supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin (HBSS) to prevent bacterial growth during the experiments, (b) LTB₄ diluted in HBSS or (c) LTB₄ diluted in HBSS with the addition of the NO synthase inhibitor L-NAME (final concentration, 10⁻⁵ M). After 5 h of superfusion with the LTB₄–L-NAME suspension, L-arginine or alternatively, D-arginine (10⁻³ final concentration) was added to the superfusate. LTB₄ was used in a final concentration (10⁻⁷) which induced roughly the same amount of neutrophil recruitment as ragweed in sensitized dogs using the same model.

Exposure to the different superfusates occurred at 3 weekly intervals. The amount of fluid in the reservoir was checked continuously, and an increase was regarded as a reflection of increased total secretion (Fig. 1). Samples from the superfusate (1 mL) were removed as it returned from the isolated segment. A specimen was taken at the beginning of the experiment and at 1 h intervals for 5 h. These samples were replaced with 1 mL each of the superfusate used in the ongoing experiment, and this dilution was taken into account when calculating results. A 10 µL sample of the superfusate was stained with methylene blue for cell counts on a haemocytometer. A Giemsa stain was performed on the cytospin of cytocentrifuge-concentrated samples. At least 200 cells per sample were examined for differential counts. If the superfusate after 10 min (the 'control' period) contained >50 cells/µL, the dog was considered to have an upper respiratory infection and was excluded from the study.

Chemical reagents and drugs

In addition to those mentioned above, sterile HBSS, TSBD, penicillin and streptomycin were obtained from the Tissue Culture Facility, UCSF, San Francisco, CA, USA; LTB₄ from Biomol Research Laboratories, Inc., Plymouth Meeting, PA, USA, and L-NAME, L-arginine and D-arginine from Sigma, St Louis, MO, USA.

Statistical analysis

All data are presented as means ± SEM and compared using Wilcoxon's test. Values of *P* < 0.05 were considered significantly different; ns = non-significant.

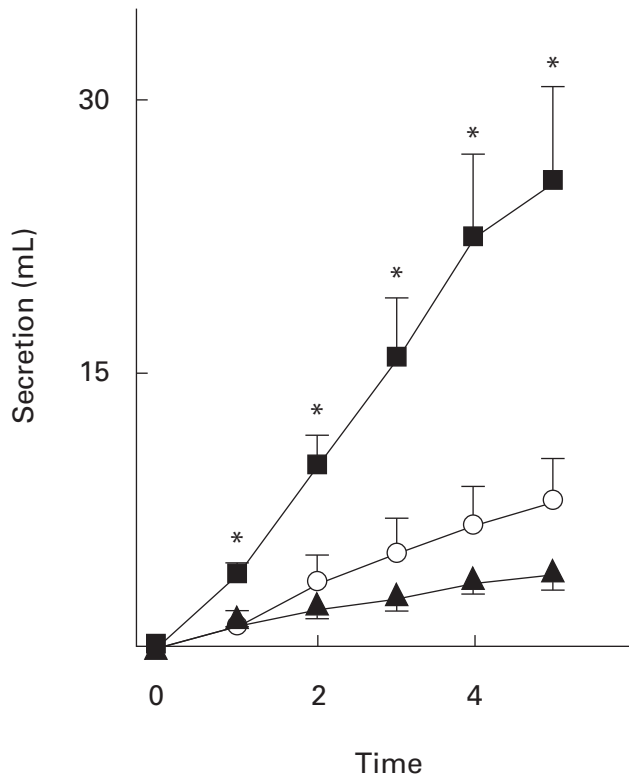


Fig. 2. Total secretion in the isolated nasal segment continuously superfused with Hanks balanced salt solution (HBSS) (○), LTB4 suspended in HBSS (10^{-7} M) (■) and LTB4 (10^{-7} M) + L-NAME (10^{-5} M) suspended in HBSS (▲). Data are mean $\pm$ SEM; $n = 5-6$ for all groups. * $P \leq 0.05$ for LTB4 compared with LTB4 + L-NAME.

Results

Before the start of the experiment, the nose was always rinsed with 20 mL HBSS buffer for 10 min. At the start of the experiment, this solution was replaced with the test solution. All dogs tested showed a low neutrophil count ($\leq 0.1 \times 10^6$ neutrophils) in the rinse solution. All cell populations counted during the experiment consisted of more than 95% neutrophils. The remaining cells were epithelial cells, macrophages and eosinophils.

During control conditions, an ongoing secretion was seen in all dogs (1.4 ± 0.5 mL/h, $n = 6$). In experiments where LTB4 was added to the superfusate, secretion was increased markedly (4.7 ± 0.9 mL/h, $P < 0.01$, $n = 6$), whereas in experiments using a combination of LTB4 and L-NAME, the secretory rate did not differ from that seen during control conditions (0.8 ± 0.2 mL/h, $P = \text{ns}$, $n = 5$). (Fig. 2).

Low numbers of neutrophils were detected in the nasal superfusate of the control dogs during the 5 h of the

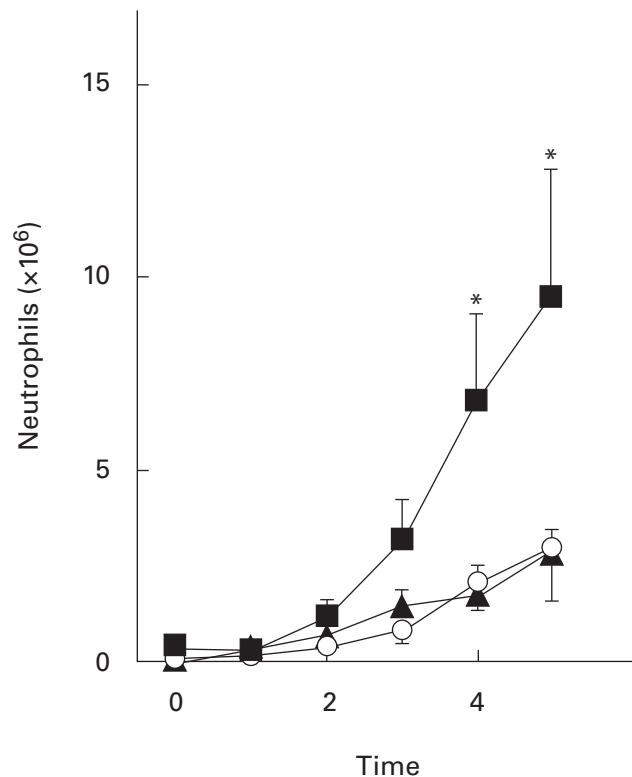


Fig. 3. Total number of neutrophils counted in the isolated nasal segment continuously superfused with Hanks balanced salt solution (HBSS) (○), LTB4 suspended in HBSS (10^{-7} M) (■) and LTB4 (10^{-7} M) + L-NAME (10^{-5} M) suspended in HBSS (▲). Data are mean $\pm$ SEM; $n = 5-6$ for all groups. * $P \leq 0.05$ for LTB4 compared with LTB4 + L-NAME.

experiment. In contrast, in experiments with LTB4 alone, there was an increase in neutrophils in the superfusate which followed a sigmoid curve, whereas experiments with LTB4 plus L-NAME showed almost the same neutrophil counts as during control conditions (Fig. 3). In four of the LTB4–L-NAME experiments, L-arginine was added to the superfusate at the end of the experiment (after 5 h), resulting in a marked increase in the neutrophil count within 5 min (from 2.9×10^6 to 6.7×10^6 neutrophils, $P \leq 0.05$). No such increase was seen in a corresponding experiment in which the dog was given D-arginine ($n = 1$).

Discussion

The present study shows that LTB4-induced neutrophil recruitment and fluid secretion in the nose were markedly reduced by L-NAME, an inhibitor of NO synthase. It has previously been reported that LTB4 recruits neutrophils into the nasal lumen and stimulates secretion from nasal

gland serous cells, an effect that was inhibited by pre-treatment with a selective neutrophil elastase inhibitor [5]. It has also been shown that chemoattractants in airways not only cause neutrophil recruitment but also, neutrophil-dependent goblet cell degranulation involving adhesive interaction between neutrophils and goblet cells, with elastase actively moving from cytoplasmic granules to the neutrophil surface where it is free to interact with adherent goblet cells [12]. The present results confirm the role of LTB₄ as a potent mediator of leucocyte-dependent nasal secretion.

The progression from a free-flowing neutrophil, via adhesion, migration and release of mediators stimulating secretion, to a neutrophil on the surface of the nasal epithelium is a co-ordinated process. One of the factors that could influence this process is NO. Recent studies suggest that NO derived from an inducible isoform of NO synthase may act as an endogenous modulator of the inflammatory response by inhibiting adhesion of leucocytes to endothelial cells [13–15]. It is also known that exogenous NO facilitates neutrophil chemotaxis *in vitro* [9], and that inhibition of NO synthase decreases neutrophil migration *in vitro* [10,11]. Here, it is shown that L-NAME markedly inhibits LTB₄-induced neutrophil-dependent nasal secretion *in vivo*. The distinct and immediate increase in neutrophils seen after the application of L-arginine to the superfusate, reversing the effects of L-NAME, supports the idea of a direct relation between neutrophils and NO. However, changes in mucosal blood flow and vascular leakage as the result of L-NAME application might also play a role in the changes in neutrophil influx and nasal secretion.

NO is known to be produced continuously in the nose and paranasal sinuses [16], and an ongoing NO production might be necessary for the continuous migration of neutrophils from the blood stream towards the surface of the nasal epithelium, with subsequent release of elastase. This process is seen during both control and inflammatory conditions. Reduction of NO production might therefore result in reduced nasal secretion via impaired elastase release.

In summary, NO modulates LTB₄-induced neutrophil recruitment and fluid secretion in the nose. Although hypersecretion of nasal fluids is one of the cardinal symptoms of allergic rhinitis, rhinorrhea should not be viewed solely as a pathological condition. Continuous nasal secretion plays an important role in the protection of the nasal and respiratory mucosa, and assists in the elimination of materials filtered from inhaled air. The role of neutrophils in chronic sinusitis is also well established, and further elucidation of the role of NO in relation to neutrophil-dependent secretion may lead to novel pharmacotherapeutic approaches to the treatment of both allergic rhinitis and chronic sinusitis.

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References

- 1 Johnson HG, McNee ML, Johnson MA, Miller MD. Leukotriene C₄ and dimethylphenylpiperazinium-induced responses in canine airway tracheal muscle contraction and fluid secretion. *Int Arch Allergy Appl Immunol* 1983; 71: 214–8.
- 2 Coles SJ, Neill KH, Reid LM *et al.* Effects of leukotrienes C₄ and D₄ on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 1983; 25:155–70.
- 3 Ford-Hutchinson AW, Bray MA, Smith MJ. Lipoxigenase products and the polymorphonuclear leucocyte. *Agents Actions* 1980; 10:548–50.
- 4 Bray MA. The pharmacology and pathophysiology of leukotriene B₄. *Br Med Bull* 1983; 39:249–54.
- 5 Cardell LO, Agustí C, Takeyama K, Stjarne P, Nadel JA. LTB₄ (4)-induced nasal gland serous cell secretion mediated by neutrophil elastase. *Am J Respir Crit Care Med* 1999; 160:411–4.
- 6 Arnal JF, Didier A, Rami J *et al.* Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy* 1997; 27:358–62.
- 7 Kharitonov SA, Rajakulasingam K, O'Connor B, Durham SR, Barnes PJ. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 1997; 99:58–64.
- 8 Kawamoto H, Takumida M, Takeno S, Watanabe H, Fukushima N, Yajin K. Localization of nitric oxide synthase in human nasal mucosa with nasal allergy. *Acta Otolaryngol (Suppl)* (Stockh) 1998; 539:65–70.
- 9 Beauvais F, Michel L, Dubertret L. Exogenous nitric oxide elicits chemotaxis of neutrophils *in vitro*. *J Cell Physiol* 1995; 165:610–4.
- 10 Kaplan SS, Billiar T, Curran RD, Zdzinski UE, Simmons RL, Basford RE. Inhibition of chemotaxis Ng-monomethyl-L-arginine: a role for cyclic GMP. *Blood* 1989; 74:1885–7.
- 11 Belenky SN, Robbins RA, Rennard SI, Gossman GL, Nelson KJ, Rubinstein I. Inhibitors of nitric oxide synthase attenuate human neutrophil chemotaxis *in vitro*. *J Lab Clin Med* 1993; 122:388–94.
- 12 Takeyama K, Agustí C, Ueki I, Lausier J, Cardell LO, Nadel JA. Neutrophil-dependent goblet cell degranulation: role of membrane-bound elastase and adhesion molecules. *Am J Physiol* 1998; 275:L294–302.
- 13 De Caterina R, Libby P, Peng HB *et al.* Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995; 96:60–8.
- 14 Khan BV, Harrison DG, Olbrych MT, Alexander RW,

- Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci USA* 1996; 93:9114–9.
- 15 Peng HB, Spiecker M, Liao JK. Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation. *J Immunol* 1998; 161:1970–6.
- 16 Lundberg JO, Farkas-Szallasi T, Weitzberg E *et al.* High nitric oxide production in human paranasal sinuses. *Nat Med* 1995; 1:370–3.