

Allergens, IgE, mediators, inflammatory mechanisms

Increased levels of exhaled nitric oxide during nasal and oral breathing in subjects with seasonal rhinitis

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Background: Allergic rhinitis is associated with nasal mucosal inflammation. Exhaled nitric oxide may be a useful marker of inflammation and has recently been shown to be increased in patients with asthma.

Objective: The purpose of this study was to determine whether exhaled levels of nitric oxide are increased with nasal breathing in patients with seasonal allergic rhinitis compared with nonatopic individuals and whether there is an increase with oral breathing consistent with lower respiratory inflammation in the absence of clinical asthma.

Methods: Nitric oxide levels in exhaled air were measured by chemiluminescence in 18 nonatopic volunteers and 32 patients with seasonal rhinitis. Measurements were made with both nasal and oral exhalation and orally after 10 seconds and 60 seconds of breath-holding. The detection limit was 1 part per billion (ppb).

Results: In control subjects nasal levels of nitric oxide in exhaled air (mean $\pm$ SD, 24.7 ± 9.2 ppb) were higher than those after oral exhalation (11.1 ± 2.5 ppb, $p < 0.0001$). Breath-holding significantly increased levels of nitric oxide in exhaled air in a time-dependent manner. Levels of exhaled nitric oxide were significantly higher for all measurements in patients with seasonal rhinitis, with levels without breath-holding of 35.4 ± 11.3 ppb ($p < 0.001$) in nasally exhaled air and 16.3 ± 5.9 ppb ($p < 0.001$) in orally exhaled air. Nasal levels were significantly higher than oral levels in subjects with rhinitis ($p < 0.0001$).

Conclusions: The results indicate that exhaled nitric oxide may be a useful marker for nasal inflammation in patients with seasonal rhinitis and suggest that generalized airway inflammation may be present, even without clinical asthma, in such patients. (*J ALLERGY CLIN IMMUNOL* 1996;97:768-72.)

Key words: Nitric oxide, allergic rhinitis, inflammation

Allergic diseases of the respiratory tract are associated with mucosal inflammation. Within both the upper and lower respiratory tracts there is evidence of epithelial mast cell and eosinophil accumulation, tissue eosinophil accumulation, T-lymphocyte activation, and enhanced cytokine ex-

pression.¹ Mediators released from activated mast cells and eosinophils, through their interaction with neural and vascular elements within the nose, induce the characteristic rhinitic symptoms of nasal pruritus, sneezing, rhinorrhea, and obstruction.² In addition to these effects, there is evidence to suggest upregulation of the inducible form of the enzyme nitric oxide synthase within the lower airways in asthma.³

Nitric oxide synthase generates the gas nitric oxide from the amino acid L-arginine.⁴⁻⁶ Measurements of nitric oxide in exhaled air have identified elevated levels in asthma, and it has been proposed that this may serve as an indirect marker of airway inflammation.⁷⁻¹⁰ Consistent with this, inhaled corticosteroid therapy, which has been shown to re-

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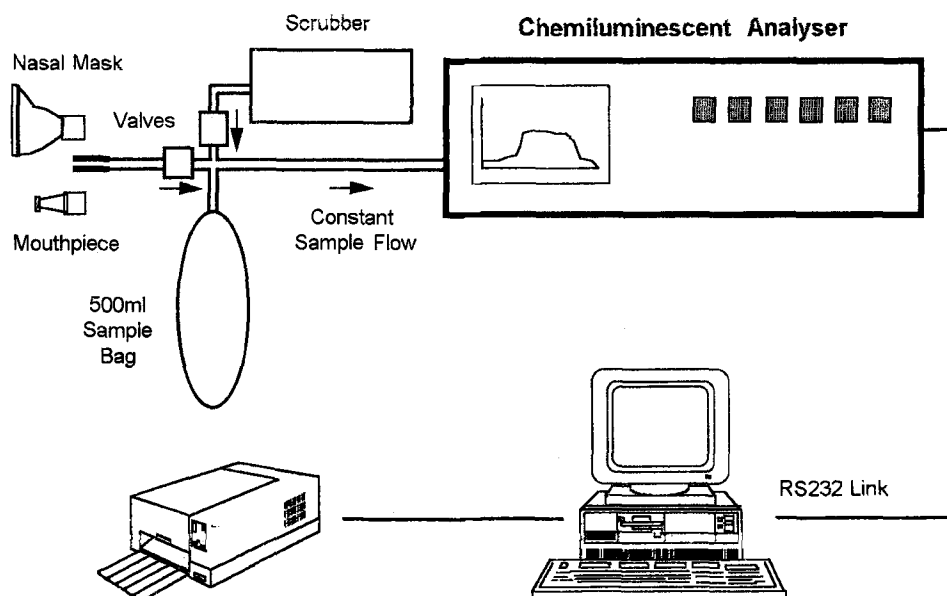


FIG. 1. Measurement of nitric oxide by chemiluminescence. Subjects breathed in, to residual functional residual capacity, and exhaled into the 500 ml reservoir bag, both orally and nasally and after 10 seconds and 60 seconds of breath-holding with oral exhalation. Once the reservoir was filled, the sample was continuously analyzed until the bag was empty. Nitric oxide was measured in parts per billion by chemiluminescence, which measures emitted light from the reaction $\text{NO} + \text{O}_3 = \text{NO}_2^* + \text{O}_2$ where * represents emitted light. The peak concentration of nitric oxide was recorded by an on-line microcomputer. When not in use, the system sampled charcoal-scrubbed air, which was free of nitric oxide.

duce airway inflammation, is associated with a reduction in exhaled levels of nitric oxide in asthma.⁸ No measures of nitric oxide in nasal exhaled air have been made in rhinitis, in which there is a common form of inflammation similar to that described in asthma in the lower airways.

Thus to determine whether levels of nitric oxide are increased in patients with seasonal allergic rhinitis, measurements of nitric oxide in exhaled air were made during nasal and oral breathing and with breath-holding in patients with seasonal rhinitis, and the results were compared with the same measurements made in nonatopic volunteers.

METHODS

Study subjects

The control subjects were 18 healthy, nonsmoking volunteers with a mean age of 30.2 years. They had no history of respiratory disease, were receiving no medication, and were all nonatopic as defined by a skin wheal response of less than 3 mm in the presence of negative saline and positive histamine controls on skin prick testing with the following common allergens: *Dermaphagoides pteronyssinus*, cat fur, mixed feathers, mixed grasses, and dog hair (Hollister Stier, Elkhart, Ind.). Thirty-two nonsmoking subjects with a mean age of 30.4 years with seasonal grass pollen-sensitive rhinitis were

also studied. All patients had a history of seasonal allergic rhinitis for at least 2 years, with upper airway but not lower airway symptoms and had positive skin test responses to mixed grass pollen extract. The study was performed in June during the grass pollen season in the United Kingdom. None of the patients were receiving topical or oral corticosteroids or topical cromoglycate preparations. The study was approved by the local ethics committee, and subjects gave written informed consent.

Study design

Both control subjects and patients with hay fever underwent measures of peak nitric oxide levels in exhaled air, with and without breath-holding. For each measure, subjects breathed in, to maximum inspiratory capacity, and then exhaled into a 500 ml reservoir bag (Fig. 1). Inhalation and exhalation were performed both orally and nasally and after 10 seconds and 60 seconds of breath-holding with oral exhalation. For oral exhalation a mouthpiece was used and a nose clip fitted, whereas for nasal measurements subjects exhaled through a tight-fitting face mask. Between measurements, subjects breathed quietly to tidal volume.

Protocol

Nitric oxide was measured with an NO/NO_x chemiluminescence analyzer (model 9841A; Siemens Plessey, Dorset, U.K.), which measures emitted light from the

TABLE I. Concentrations of nitric oxide (ppb) in exhaled air in normal volunteers and patients with seasonal rhinitis after a single oral breath without breath-holding, with 10 and 60 seconds of breath-holding, and after a single nasal breath with no breath-holding

	Oral breath			Nasal breath*
	0*	10 sec	60 sec	
Healthy volunteers	11.1 ± 2.5	15.6 ± 6.8	32.1 ± 15.0	24.7 ± 9.2
Patients with seasonal rhinitis	16.3 ± 5.9	34.0 ± 20.0	62.0 ± 53.1	35.4 ± 11.3

Results are expressed as means ± SD.

*No breath-holding.

reaction $\text{NO} + \text{O}_3 = \text{NO}_2^* + \text{O}_2$, where * represents emitted light. The system was modified to allow measurement of nitric oxide in exhaled air in parts per billion (ppb). When not in use, the system sampled charcoal-scrubbed air, which was free of nitric oxide, and the machine was not exposed to ambient air. The mouth-piece (or nasal mask) was connected with Teflon tubing and a nonrebreathable valve to a 500 ml rubber anesthetic rebreathing bag (East Healthcare, Oxford, U.K.). This reservoir, once filled, was continuously analyzed until the bag was empty, and the peak concentration (in parts per billion) was recorded by an on-line microcomputer. Little variation was observed between individual measurements because the sample was at equilibrium and a plateau was observed. The system was calibrated daily from a known standard and repeated measures of known quantities of nitric oxide showed no absorption by the sampling and reservoir apparatus. The detection limit for nitric oxide was 1 ppb.

Analysis

The measurements of nitric oxide were compared by using the Wilcoxon test for paired data and the Mann-Whitney test for unpaired data, respectively.

RESULTS

In all healthy control subjects nitric oxide was present in exhaled air after a single oral breath. The mean ± SD concentration was 11.1 ± 2.5 ppb, and this concentration increased significantly to 15.6 ± 6.8 ppb ($p = 0.01$) with 10 seconds of breath-holding (Table I). The concentrations were even higher after 60 seconds of breath-holding at 32.1 ± 15 ppb ($p < 0.001$, compared with 10 seconds of breath-holding). Similarly, nasal breathing produced significantly higher concentrations of nitric oxide compared with baseline oral breathing (24.7 ± 9.2 vs 11.1 ± 2.5 ppb, $p < 0.0001$).

In the subjects with seasonal rhinitis the baseline oral breath concentration of nitric oxide was significantly higher than that in the volunteers (16.3 ± 5.9 ppb, $p < 0.001$). Again, there was a marked progressive increase from baseline with breath-holding for 10 seconds (34 ± 20 , $p < 0.0001$

compared with baseline) and 1 minute (62 ± 53.1 , $p < 0.05$ compared with 10 seconds). Furthermore, the concentrations of exhaled nitric oxide with nasal breathing were twice as high as those with oral breathing (35.4 ± 11.3 ppb, $p < 0.0001$).

When the normal volunteers and patients were compared, it was found that the concentrations of exhaled nitric oxide were significantly higher in the patients with oral breathing at 10 seconds ($p = 0.0001$) and 1 minute ($p < 0.05$) (Fig. 2) and with nasal breathing ($p < 0.001$) (Fig. 3). The nasal levels of nitric oxide were uninfluenced by topical vasoconstrictor therapy.

DISCUSSION

We have observed that patients with seasonal allergic rhinitis have higher levels of exhaled nitric oxide than nonatopic volunteers when measurements are made with both nasal and oral exhalation. Although breath-holding increased the measured levels of orally exhaled nitric oxide in both groups, these levels were significantly greater in patients with rhinitis. These results suggest not only that nitric oxide may be a valuable marker of nasal inflammation in patients with rhinitis but that these individuals may also have asymptomatic lower airway inflammation, which is consistent with generalized inflammation of the respiratory tract.

Our findings of elevated nitric oxide levels in nasally exhaled air, in comparison with oral exhalation, are consistent with results of one previous study in which comparisons were made, as are the absolute measurements.⁷ We identified a level of 24.6 ppb in nasally exhaled air of nonatopic individuals in comparison with the 23 ppb recorded by Alving et al.⁷ Similarly, the levels of nitric oxide that we found with oral exhalation in healthy individuals in the absence of breath-holding, 11.0 ppb, are very similar to the results of other studies in which such measurements have been made.^{7, 9-11}

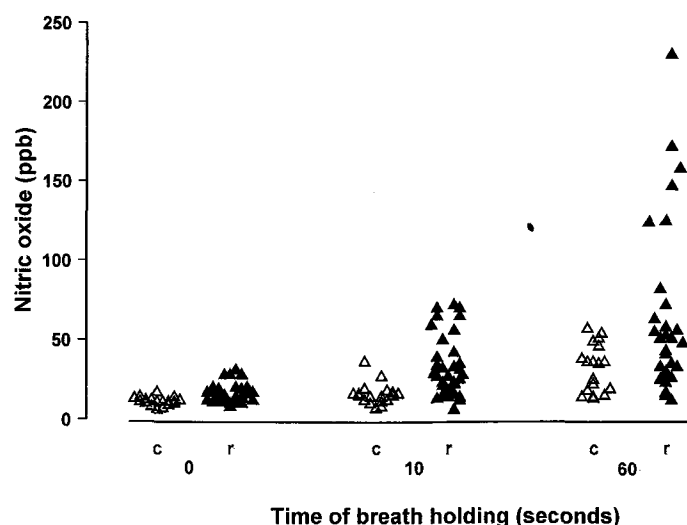


FIG. 2. Exhaled levels of nitric oxide in 18 nonatopic control subjects (*c*) and 32 patients with grass pollen seasonal rhinitis (*r*), measured during the pollen season with oral inhalation and exhalation and after 10 seconds and 60 seconds of breath-holding.

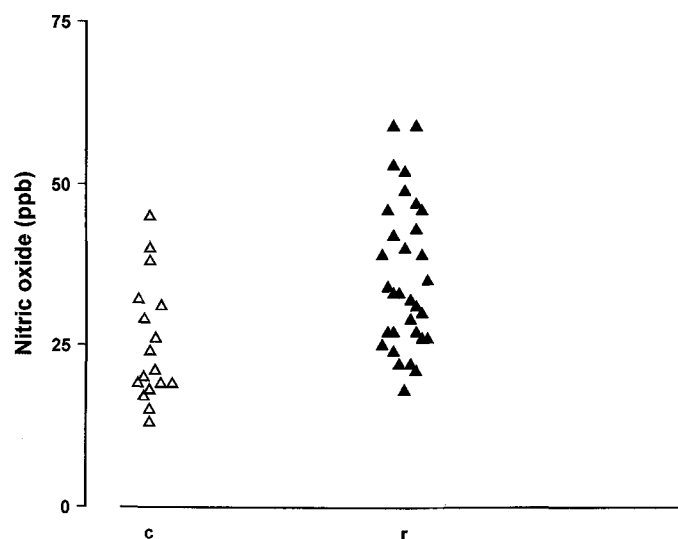


FIG. 3. Exhaled levels of nitric oxide in 18 nonatopic control subjects (*c*) and 32 patients with grass pollen seasonal rhinitis (*r*), measured during the pollen season after nasal inhalation and exhalation.

with normal subjects having a range of exhaled nitric oxide between 3 and 20 ppb. In agreement with the report by Persson et al.,¹¹ we found a time-dependent increase in exhaled nitric oxide with breath-holding, with orally exhaled levels being three times greater after 60 seconds of breath-holding than after spontaneous exhalation during quiet breathing. This finding is indicative of constant production of nitric oxide within the airways. A production rate of 2.7 nmol/min has previously been calculated within the lower airways,¹¹ with a higher rate suggested within the nose.^{12, 13} Mea-

surements of exhaled nitric oxide after inspiration and expiration involve dilution in the reservoir volume. Direct measures from the nose in the absence of breathing thus reveal higher levels of up to 1000 ppb.¹² Consistent with this finding, we have recorded very high levels of 178 to 812 ppb in air sampled directly from the nose.

In patients with allergic rhinitis we found elevated levels of nitric oxide in nasally exhaled air. There have been no previous reports of exhaled nitric oxide levels in active rhinitis. The role of nitric oxide in allergic rhinitis is undefined, but

because this gas can have vasodilatory properties, it is possible that the increased production within the nose could contribute both to nasal obstruction and to plasma protein extravasation. Further studies with nitrogen donors and L-arginine inhibitors will help elucidate the role of nitric oxide in nasal physiology.

The further increase in nasal nitric oxide levels above those found in nonatopic individuals would, however, be consistent with induction of inducible nitric oxide synthase in association with the mucosal mast cell, eosinophil, and T-lymphocyte activation, which characterize allergic rhinitis.² These inflammatory cell changes are very similar to those recorded within the lower airways in patients with asthma in which there is both upregulation of expression of inducible nitric oxide synthase³ and increased levels of nitric oxide in exhaled air.⁷⁻¹⁰ No studies of inducible nitric oxide synthase within the nasal mucosa in allergic rhinitis have been reported. The identification of elevated nasal nitric oxide in this study is consistent with such an occurrence in allergic rhinitis.

The additional finding of elevated levels of orally exhaled nitric oxide in patients with allergic rhinitis in comparison with those levels measured in nonatopic individuals is suggestive of inflammatory changes within the lower airways. In contrast to symptomatic rhinitis, lower airway symptoms were not present in any subject. Even in the absence of asthmatic symptoms, however, subclinical inflammation has been reported in patients with allergic rhinitis with evidence of eosinophil accumulation and enhanced collagen deposition.¹⁴ It is possible that in patients with seasonal allergic rhinitis, inflammatory cell accumulation occurs within the lower airways but does not reach a sufficient threshold to induce symptoms. The identification of enhanced nitric oxide levels in such patients suggests that nitric oxide measurement is a sensitive method of monitoring airway inflammation. However, in this study we cannot exclude the possibility that increased nasal production of nitric oxide accounts for some of the elevation in orally measured nitric oxide, but this is unlikely because the subjects were wearing nose clips.

Further studies are also required in patients with rhinitis to determine whether seasonal increments occur in association with the development of clinical disease expression and whether production is

diminished by disease-modifying therapies. The identification of elevated nitric oxide levels in nasally exhaled air does, however, suggest a potential role for this mode of measurement to monitor disease activity and airway inflammation in patients with allergic rhinitis.

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