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RAPID COMMUNICATION

Increased amount of nitric oxide in exhaled air of asthmatics

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Increased amount of nitric oxide in exhaled air of asthmatics. K. Alving, E. Weitzberg, J.M. Lundberg. ©ERS Journals Ltd 1993.

ABSTRACT: The presence of nitric oxide (NO) in the exhaled air of humans has recently been described. We wanted to assess at what level exhaled NO originates in normal airways, and to determine whether airway inflammation induces changes in the levels of exhaled NO.

Exhaled NO was continuously measured by chemiluminescence technique during normal tidal breathing through the nose or mouth, with a detection limit of 1 part per billion (ppb). Twelve control subjects were compared to eight patients with mild atopic asthma and rhinitis caused by occupational allergen.

In control subjects, the major part of NO in exhaled air (up to 30 ppb) seemed to originate in the nasal airways, with only minor contribution from the lower airways and the oral cavity. However, in mild asthmatics, the level of exhaled NO during oral breathing, indicating the involvement of the lower airways, was increased 2–3 fold.

Since increased production of NO in the lower airways may involve activated macrophages or neutrophils, we suggest that exhaled NO may be used to instantly monitor ongoing bronchial inflammation, at least when involving inducible NO synthase. *Eur Respir J, 1993, 6, 1368–1370.*

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During the last decade, several studies of the biological role of nitric oxide (NO) have been made. The synthesis of NO, which is catalysed by specialized NO synthases using L-arginine as a substrate, has now been shown to take place in many cell types [1]. The NO synthase exists in several isotypes, that can be divided into two major classes: constitutive and inducible. The constitutive isotypes have been described in *e.g.* endothelial cells [2] and parasympathetic vasodilator nerves [3]. The inducible isotypes are found, after activation, in macrophages, neutrophils, endothelium and vascular smooth muscle. The production of NO has, so far, been difficult to measure directly *in vivo*, although increases in the end-products, nitrite and nitrate, in plasma or urine can be measured in some cases [4]. However, it was recently shown, that NO can be found in parts per billion (ppb) levels in exhaled air of experimental animals and humans [5]. The purpose of the present study was to examine the anatomical origin in the airways of exhaled NO, and to determine the possible influence of inflammatory diseases on these NO levels. Furthermore, the possible presence of nitrogen dioxide (NO_2) in exhaled air was examined.

Material and methods

Study subjects

The control subjects (n=12) were nonsmoking, healthy individuals, 27–52 years old, and the asthmatics (n=8) were nonsmoking, atopic individuals, 33–45 years old, with confirmed allergy towards at least rat allergen, and occupational symptoms of mild asthma and rhinitis. The asthmatics were tested during asymptomatic periods. Two of the asthmatics were inhaling a glucocorticoid (budesonide) regularly, two inhaled a β_2-agonist or cromoglycate when having symptoms, and four did not take any medication. All subjects were tested when they were subjectively free from respiratory infections, except in three cases of lower respiratory tract viral infections (which passed, without the use of antibiotics, within 2 weeks) in control subjects. Exhaled NO was also measured at an intensive care unit (ICU) in intubated and mechanically-ventilated patients without known asthma (n=5). The study was approved by the Local Ethics Committee.

Methods

A system was built to allow inhalation of NO- and NO_2-free air, delivered from a pressurized gas cylinder (AGA AB, Stockholm, Sweden), as well as simultaneous, continuous measurement of NO in the exhaled air (fig. 1). Briefly, the subjects inhaled air from an elastic rubber reservoir *via* a non-rebreathing valve leading to a face mask, during normal tidal breathing either through the nose with the mouth closed or through the mouth wearing a noseclip. To evaluate the contribution from the nasal airways, an NO-free airstream (2–5 $l \cdot min^{-1}$) was introduced into one nostril of control

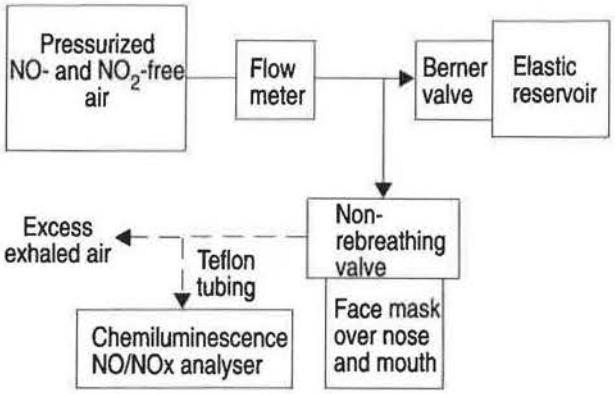

Fig. 1. – Principals of the experimental set-up. Pressurized NO- and NO_2-free air (≤1 and 2 ppb, respectively) was administered into an elastic rubber reservoir *via* a flowmeter and a Berner valve. The flow was adjusted to keep the reservoir inflated to approximately 75% (6–8 $l \cdot min^{-1}$). The outlet of the Berner valve was set to 2 cmH_2O to prevent the forcing of fresh air through the non-rebreathing valve. Teflon tubing was used after the non-rebreathing valve to avoid absorption of NO. The chemiluminescence NO/NOx analyser sampled air at a flow of 0.7 $l \cdot min^{-1}$ and excess exhaled air was led into the open, through tubing of sufficient length to prevent contamination with ambient air.

subjects, whilst breathing through the mouth or holding the breath, and outlet air was sampled from the contralateral side (n=5). Similar measurements were made in the oral cavity, whilst holding the breath, with the inlet and outlet in different corners of the mouth (n=5). The levels of NO and NO_2 on the outlet side were measured by continuous sampling at 0.7 $l \cdot min^{-1}$ *via* Teflon tubing into an NO/NOx chemiluminescence analyser (Eco Physics, Basel, Switzerland) which measures emitted light from the reaction: $NO + O_3 \rightarrow NO_2^* + O_2$ (where * symbolizes emitted light) by the use of a photomultiplicator tube [4, 6]. NO_2 was measured after reduction to NO, by the use of a thermal molybdenum converter and subtraction of the NO component (Eco Physics). The detection limit for NO and NO_2 was 1 and 2 ppb, respectively.

Results

In healthy control subjects, much higher plateau levels were noted during nasal breathing (23±2 ppb) compared to oral breathing (9±1 ppb) (fig. 2). Plateau levels of NO were reached within 4 min in this system, and no further changes were seen within a total of 10 min. Ventilation of the nasal airways with an NO-free airstream (2 $l \cdot min^{-1}$) resulted in very high levels of NO on the outlet side (fig. 2). These levels were further increased if the subjects were holding their breath with the mouth closed and, thus, forcing all air from one nasal cavity to the other *via* the nasopharynx. If the airflow was increased to 5 $l \cdot min^{-1}$, a slight increase (nonsignificant) in the levels of NO was noted (not shown). In contrast, similar measurements in the oral cavity resulted in low plateau levels of NO (≤4 ppb, n=5). Also, very low plateau levels of NO (≤3 ppb) were noted on the outlet side in intubated and mechanically-ventilated patients (n=5). Taken together, this suggests that the NO in exhaled air of normal subjects is generated mainly in the nasal mucosa. In some individuals, low levels of NO_2 (≤5 ppb) were seen in exhaled air at the beginning of the measurement period. However, the exhaled NO_2 concentration decreased during breathing of NO_2-free air, to reach levels below the dectection level (≤2 ppb) within 5 min.

In a group of non-symptomatic atopic subjects with mild asthma and rhinitis, the level of NO in exhaled air during oral breathing was 2–3 fold higher than levels in

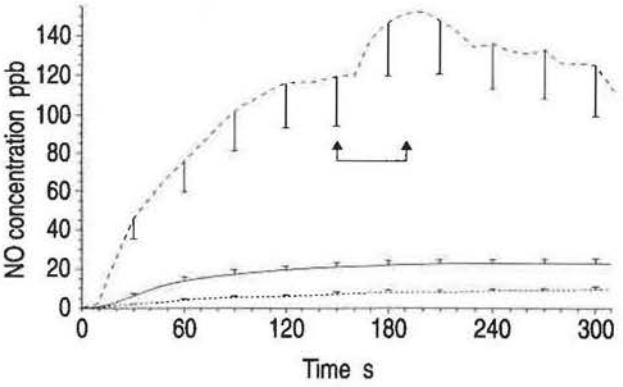

Fig. 2. – Detected levels of NO (ppb) by chemiluminescence technique in exhaled air of control subjects during the first 5 min of oral breathing (·····), nasal breathing (——), or nasal ventilation with an airstream (- - -). The arrows indicate a period of breatholding with the mouth closed. Data are given as mean±SEM.

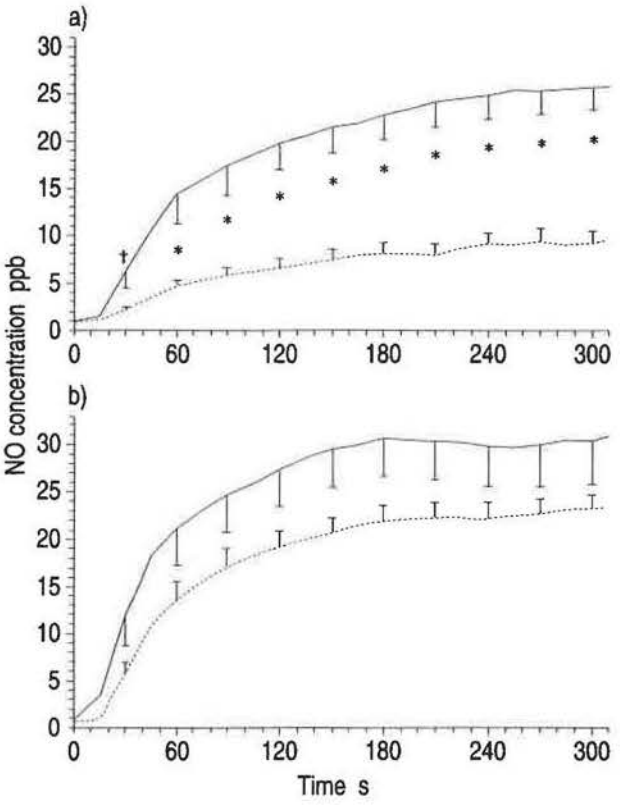

Fig. 3. – NO levels (ppb) in exhaled air of controls (·····) and asthmatics (——) during the first 5 min of: a) oral breathing; and b) nasal breathing. Data are given as mean±SEM. †: $p<0.01$, *: $p<0.001$ compared to controls (Mann-Whitney U-test).

control subjects (fig. 3a). When comparing plateau levels, there was no overlap between controls (range 5–16 ppb, n=12) and asthmatics (range 21–31 ppb, n=8). Also, during episodes of lower respiratory tract viral infections in control subjects, causing cough and tracheobronchial soreness, elevated levels of NO in exhaled air were noted during oral breathing (11±2 ppb before, 32±4 ppb during and 16±1 ppb after the symptomatic period, n=3). However, during nasal breathing, no significant elevation of NO levels in exhaled air was noted either in asthmatics (fig. 3b) or during lower respiratory tract infections (not shown), although a trend towards elevated levels was noted.

Discussion

Our study suggests that production of NO in normal human airways, as detected in exhaled air, is restricted to the nasal mucosa. The precise source of NO in normal nasal mucosa remains unclear, but could be endothelial cells [2], or parasympathetic nerves [3], containing constitutive NO synthases. This would fit with the apparently much lower basal levels of NO generated in the lower airways, since both vascularization and parasympathetic innervation [7] are less in the tracheobronchial mucosa, compared to the nasal mucosa. The higher levels of NO noted during oral breathing, compared to that detected in intubated subjects, may represent NO derived from the nasopharyngeal mucosa. The transient presence of NO_2 in exhaled air can be interpreted as clearance of NO_2 that had been absorbed from ambient air (NO_2 concentrations between 5–20 ppb) before the start of breathing NO_2-free air.

The increased amount of NO from the lower airways, but not the upper airways, as detected in exhaled air of subjects with atopic asthma and rhinitis during oral and nasal breathing, respectively, suggests the involvement of macrophages [8], which are the only cells producing high levels of NO found in much higher amounts in the bronchial, compared to the nasal, airways [9]. However, the involvement of neutrophils [10] or other cell types cannot be excluded at this stage.

The finding that the exhaled NO levels during nasal breathing in subjects with both allergic asthma and rhinitis were not significantly increased, may thus reflect lower levels of inducible NO synthase in luminal structures of the nasal airways. An alternative explanation could be that the permeability for NO in the inflamed nasal mucosa is reduced due to secretion, oedema and/or hyperaemia, resulting in decreased passage of NO from deeper structures, such as endothelium and parasympathetic nerves, out into the lumen. This could possibly mask an increased production of NO in luminal structures of the nasal mucosa when measured in exhaled air.

The finding that the NO levels in exhaled air of two subjects on regular glucocorticoid inhalation treatment did not differ from the other asthmatic subjects included in the study was unexpected, because of the described effects of glucocorticoids on the expression of inducible NO synthase, at least when stimulated by endotoxin [11]. However, we do not know what the basal NO levels in these subjects were, before introducing the steroid, and, therefore, cannot say anything about the effect of steroid treatment. Further studies are required to determine the effect of glucocorticoid treatment on NO production in the asthmatic airways.

We suggest that the level of NO in exhaled air, as detected by chemiluminescence technique, may be used to monitor ongoing inflammation, involving the formation of inducible NO synthase, in the lower airways.

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