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Inhalation of nasally derived nitric oxide modulates pulmonary function in humans

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The vasodilator gas nitric oxide (NO) is produced in the paranasal sinuses and is excreted continuously into the nasal airways of humans. This NO will normally reach the lungs with inspiration, especially during nasal breathing. We wanted to investigate the possible effects of low-dose inhalation of NO from the nasal airways on pulmonary function. The effects of nasal and oral breathing on transcutaneous oxygen tension (tc$P$O$_2$) were studied in healthy subjects. Furthermore, we also investigated whether restoring low-dose NO inhalation would influence pulmonary vascular resistance index (PVRI) and arterial oxygenation (PaO$_2$) in intubated patients who are deprived of NO produced in the nasal airways. Thus, air derived from the patient's own nose was aspirated and led into the inhalation limb of the ventilator. In six out of eight healthy subjects tc$P$O$_2$ was 10% higher during periods of nasal breathing when compared with periods of oral breathing. In six out of six long-term intubated patients PaO$_2$ increased by 18% in response to the addition of nasal air samples. PVRI was reduced by 11% in four of 12 short-term intubated patients when nasal air was added to the inhaled air. The present study demonstrates that tc$P$O$_2$ increases during nasal breathing compared with oral breathing in healthy subjects. Furthermore, in intubated patients, who are deprived of self-inhalation of endogenous NO, PaO$_2$ increases and pulmonary vascular resistance may decrease by adding NO-containing air, derived from the patient's own nose, to the inspired air. The involvement of self-inhaled NO in the regulation of pulmonary function may represent a novel physiological principle, namely that of an enzymatically produced airborne messenger. Furthermore, our findings may help to explain one biological role of the human paranasal sinuses.

Keywords ARDS, oxygenation, paranasal sinuses, pulmonary vascular resistance

The lord God formed a human being from the dust of the ground and breathed into his nostrils the breath of life so that he became a living creature (Genesis, 2:7).

Healthy humans normally breathe through the nose where the nasal cavity serves to regulate the temperature and humidity of the inhaled air. However, the nasal airways may not only function as an ingenious heat exchanger and humidifier; recently it was shown that the potent vasodilator, nitric oxide (NO), is present in high concentrations in the nasal airways of healthy subjects (Lundberg *et al*. 1994b). NO in the upper airways is mostly produced by a constitutively expressed 'inducible-like' NO-synthase (NOS) in the epithelium of the paranasal sinuses (Lundberg *et al*. 1995a). Sinus NO enters the nasal cavity (Lundberg *et al*. 1994a) and will follow the airstream with every inspiration thereby flushing the lower airways with NO. During nasal breathing, substantially more NO is inhaled compared to when breathing through the mouth since the major NO excretion in the upper respiratory tract takes place in the nasal airways (Gerlach *et al*. 1994, Lundberg *et al*. 1994b). We (Lundberg *et al*. 1994b, Lundberg *et al*. 1995b) and others (Gerlach *et al*. 1994) have suggested earlier that there may be possible pulmonary effects of this

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continuous low-dose endogenous NO exposure (25–100 p.p.b.). Moreover, exogenous NO in doses as low as 100 p.p.b. has been shown to reduce pulmonary vascular resistance and improve arterial oxygenation in patients with severe pulmonary disease and pulmonary hypertension (Puybasset *et al.* 1994).

The purpose of this study was to investigate if endogenous NO from the nasal airways could affect pulmonary vasculature and improve arterial oxygenation. We have studied the effects of nasal and oral breathing on trancutaneus oxygen tension (tcPo_2) in healthy awake subjects. Furthermore, we investigated whether restoring low-dose NO inhalation would influence pulmonary vascular resistance index (PVRI) and Pao_2 in intubated patients who are deprived of endogenous NO inhalation. Thus, air derived from the patient's own nose was aspirated and led into the inhalation limb of the ventilator.

METHODS

Healthy spontaneously breathing subjects

Effects of nasal or oral breathing on tcPo_2 were studied in eight healthy non-smoking subjects (age 24–42 years, 7 male, 1 female). Two different modes of breathing were used designed so as to achieve maximal difference in inhaled endogenous NO concentrations. Oral breathing; the subjects were asked to inhale through the mouth while the nose was blocked and exhale through the nose with closed mouth. In this way a minimal inhalation of nasally derived NO is achieved, since the NO that is accumulated in the nasal airways during inspiration is washed out during the following nasal exhalation. Nasal breathing; the subject inhaled through the nose with the mouth closed and exhaled through the mouth with the nose blocked. Thereby, a maximum of NO from the nasal airways is inhaled while a minimum is lost during exhalation. Repeated 5-min periods of oral or nasal breathing were performed and tcPo_2 was calculated as the mean value during the last 3 min of the breathing periods. Ambient NO levels were < 4 p.p.b. during all measurements.

Nasal NO concentrations were measured in all subjects with a chemiluminescence NO analyser (CLD 700, Eco Physics, Dürnten, Switzerland) using an earlier described method (Lundberg *et al.* 1994b). The NO analyser was calibrated at known concentrations (100–1000 p.p.b.) of NO in N2 (AGA AB, Lidingö, Sweden), using an electromagnetic flow controller (Environics Inc, Middletown CT, USA).

A transcutaneous electrode (Radiometer, TCM 3, Copenhagen, Denmark) was used for measurements of tcPo_2. The electrode was calibrated with room air and placed on the test subjects chest wall. After a stabilizing period of 20 min, the experiment started with the subjects lying comfortably in a horizontal position and breathing normal tidal volumes.

In two subjects low-dose exogenous NO was inhaled from a reservoir (50 L) during a period of oral breathing. Exogenous NO (1000 p.p.m., AGA AB, Lidings, Sweden) was mixed with room air to a final concentration of 100 p.p.b. as measured by a chemiluminescence NO analyser. NO concentrations in the reservoir did not change during the course of the experiment.

In addition, in two subjects we compared tcPo_2 values during oral breathing with room air with those obtained during oral breathing with a moistering filter.

Ventilation was monitored by measuring end tidal Pco_2 (Datex, Stockholm, Sweden).

Intubated mechanically ventilated patients

Pao_2 were studied in six intubated patients (age 52–63 years) treated at a neurosurgical department. All patients had an arterial cannula and had been mechanically ventilated for 3–8 days at the time of the study. Ventilator settings including the inspired oxygen concentration were kept unchanged during the study period.

PVRI were studied in 12 coronary bypass grafting patients (age 52–70 years) in the immediate post-surgical period. Catheters had been inserted into the left atrium (LA) and pulmonary artery (PA, thermodilution). PVRI was measured in duplicate as the difference in mean pressure between PA and LA divided by cardiac index. The patients had no ongoing medication with NO-donating drugs like nitroglycerine or nitroprusside, although they had been treated with these drugs before and during the operation.

A nasal olive was connected to a pump-unit and air was aspirated (2 L min^{-1}) from the patient's nose in between two and four 10-min periods. Aspirated air was added to the inspiratory flow of the ventilator. Control periods (10 min) with ambient air sampling were performed between each nasal sampling period. In eight patients (four coronary by-pass patients and four long-term intubated patients) we used exogenous NO (100 p.p.b.) instead of nasal sampling during one treatment period. Pao_2 or PVRI were measured at the end of every 10-min period. A blood gas analyser (Radiometer ABL 300, Copenhagen, Denmark) was used for Pao_2 measurements.

Mean NO concentration measured with a chemiluminescence NO analyser, in the inspiratory limb of the ventilator was 19 ± 3 p.p.b. when air was aspirated from the patients nose, 24 ± 1 p.p.b. when exogenous

NO was used, and < 3 p.p.b. with ambient air. A subject was considered a responder when Pao_2 was higher or PVRI was lower than control levels before and after treatment periods with nasal air. Exogenous NO was only used in responders.

This study was approved by the local ethics committee.

RESULTS

Healthy spontaneously breathing subjects

In six subjects $tcPo_2$ was 10% higher during all nasal breathing periods (12.8 ± 0.9 kPa) compared to periods of oral breathing (11.6 ± 0.9 kPa, $P < 0.05$, Fig. 1). Addition of exogenous NO (100 p.p.b.) to orally inhaled air resulted in a 6 and 23% increase in $tcPo_2$ (Fig. 2). Humidification of orally inhaled air did not significantly change $tcPo_2$ as compared to when breathing dry room air.

Nasal NO concentration in the group of healthy subjects was 304 ± 19 p.p.b. In two subjects $tcPo_2$ did not increase during the periods of nasal breathing as compared to oral breathing. Nasal NO concentrations in these subjects were 410 and 295 p.p.b. End tidal Pco_2 did not differ significantly between oral and nasal breathing periods.

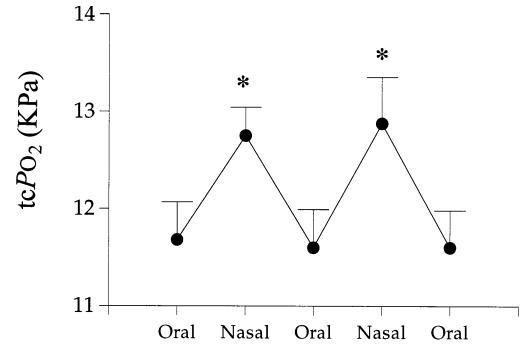

Figure 1 Transcutaneous oxygen tension (mean ± SEM) in six healthy subjects during repeated periods (5 min) of nasal or oral breathing. $*P < 0.05$ compared to previous period of oral breathing (Wilcoxon's signed rank test). Two subjects (non-responders) are not included in the figure.

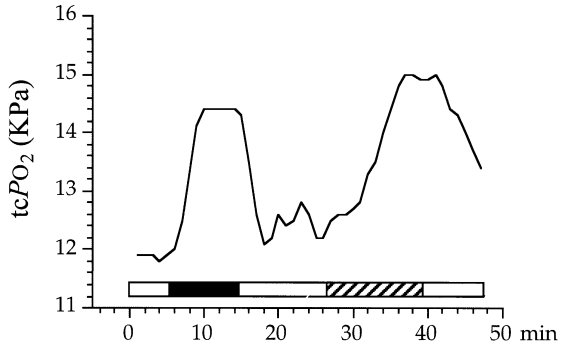

Figure 2 Representative tracing showing transcutaneous oxygen tension in a healthy subject during periods of oral breathing (empty bars), nasal breathing (filled bar) or oral breathing of air containing exogenous NO at a concentration of 100 p.p.b. (hatched bar).

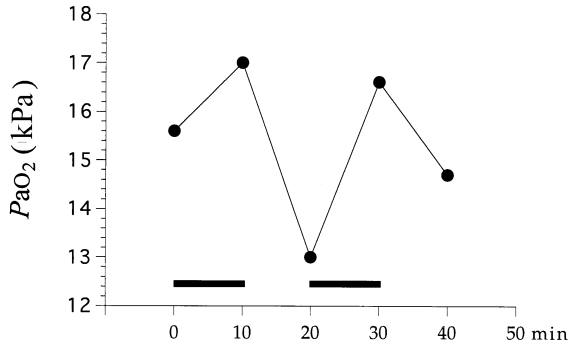

Figure 3 Arterial oxygen tension (Pao_2) in a long-term intubated subject after adding ambient air or nasal air at a flow of 2 L min^{-1} to the inspiratory limb of the ventilator. Bars indicate periods of administering nasal air.

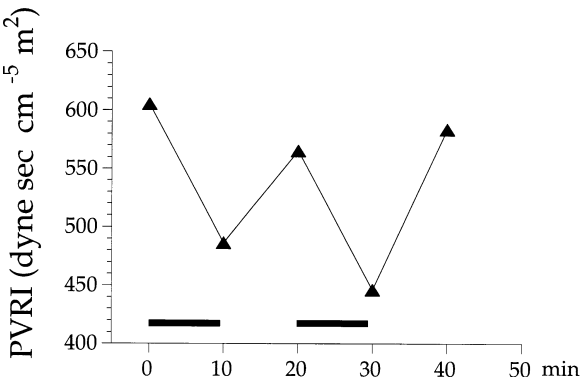

Figure 4 Pulmonary vascular resistance index (PVRI) in a short-term intubated coronary by-pass patient, after adding ambient air or nasal air at a flow of 2 L min^{-1} to the inspiratory limb of the ventilator. Bars indicate periods of administering nasal air.

Intubated mechanically ventilated patients

In six of six long-term ventilated patients Pao_2 increased (from 12.1 ± 1.3 kPa to 14.3 ± 2.4 kPa, mean 18 ± 3%) in response to addition of nasal air samples (Fig. 3). A 20 ± 3% rise in Pao_2 (from 12.4 ± 1.7–14.9 ± 2.1 kPa) was seen when exogenous NO was used.

In four of 12 coronary by-pass patients, PVRI was reduced (from 451 ± 28–400 ± 25 dyne s^{-1} cm^{-5}, mean 11 ± 2%) during nasal sampling periods compared with the period when ambient air was sampled (Fig. 4). PVRI was reduced by 17 ± 2% when exogenous NO was used in these responders. In eight coronary by-pass patients PVRI was unchanged during periods of nasal sampling.

DISCUSSION

We here show that arterial oxygenation is improved in healthy awake subjects during nasal breathing as compared with mouth breathing. We also show, in intubated patients, that pulmonary function may improve by adding air derived from the patient's own nose to the inspiratory flow of the ventilator.

The effects of nasal air on pulmonary function in healthy subjects and in the intubated patients were probably due to NO, since the effects of exogenous NO in the same concentration range did not differ significantly from those obtained with nasal air. Other possible explainations for increased oxygenation during nasal breathing include moistening of inhaled air, changes in airway mechanics or the presence of a naso-pulmonary reflex. However, administration of moistened air during oral breathing did not affect tcPO_2 in this study. Moreover, earlier studies have shown that humidification of inhaled air does not affect Pao_2 in intubated patients (Kuo *et al.* 1991). Airway mechanics were not altered in the intubated patients in this study and this explaination therefore seems unlikely. Furthermore, end tidal Pco_2 did not differ in the same subjects during periods of nasal and oral breathing. Also in the intubated patients Pco_2 was unchanged regardless of nasal or ambient air admixture. A naso-pulmonary reflex as a cause for increased Pao_2 is unlikely since exogenous NO in this study also increased Pao_2 in the intubated mechanically ventilated patients.

The major part of the NO that is normally inhaled, is produced in the paranasal sinuses as described recently (Lundberg *et al.* 1995a). We here postulate that one biologically significant role of the human paranasal sinuses is to produce NO. This NO may have a dual function. First, the very high local concentrations of NO in the sinuses may inhibit the growth of bacteria & virus thus contributing to the sterility of these cavities as suggested earlier (Lundberg *et al.* 1995a). Second, when diluted in the inhaled air, sinus derived NO may act as an 'aerocrine' messenger, produced and secreted in the upper airways and transported by the airstream to the lung. Since the NO source is proximal to the site of action this assures that the inhaled NO only reaches ventilated parts of the lungs. Thus, NO may selectively dilate vessels supplying well ventilated areas of the lung.

In all long-term intubated patients in this study, arterial oxygenation was increased following administration of endogenous NO while a third of the coronary by-pass patients responded with a reduction in PVR. This is in keeping with earlier studies in ARDS patients where low doses of inhaled NO showed more consistent effects on Pao_2 than on PVR (Bigatello *et al.* 1993). The factors that distinguish responders from non-responders concerning PVR changes are not clear, but non-responders are commonly seen also among patients with pulmonary hypertension who receive exogenous NO in much higher concentrations (Rossaint *et al.* 1995). Apparently, non-responders are found also in healthy individuals as shown in this study where Pao_2 did not increase in two subjects during nasal breathing despite normal nasal NO levels.

Since endotracheal intubation deprives patients of inhaling the NO produced in their own upper airways, it is tempting to speculate that a replacement of this loss would improve lung function in these patients. Beneficial effects of low-dose endogenous NO inhalation may extend beyond vasodilatation. Thus, NO is also known to upregulate ciliary motility (Jain *et al.* 1993) and to inhibit the growth of bacteria (Mancinelli & McKay 1983) & virus (Croen 1993).

We conclude that inhalation of endogenous NO, derived from the upper airways, may be involved in the regulation of pulmonary function in humans. This may represent a novel physiological principle, where NO acts as an 'aerocrine' messenger. Furthermore, it may also help to explain one biological role of the enigmatic human paranasal sinuses, the major sources of NO in the upper airways.

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