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The Paranasal Sinuses as Reservoirs for Nitric Oxide

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Objective—Nitric oxide (NO) is an important mediator and inflammatory marker in human upper airways. Enzymes responsible for NO production have been demonstrated both in the nose and in the paranasal sinuses, but NO levels in the sinuses are reported to be several times higher than those in the nose. It has been postulated that the paranasal sinuses may be the primary sites for NO production in the upper respiratory tract. The present study was designed to compare the NO levels sampled from the nose with those found in the paranasal sinuses.

Material and methods—NO levels in the maxillary sinus and nose were determined using a continuous chemiluminescence measuring technique in seven healthy volunteers.

Results—When NO was sampled, via a drainage tube inserted into the maxillary sinus, a transient peak in NO level was recorded. The maximal NO level ($5{,}761 \pm 1{,}513$ ppb; $n = 7$) was reached within 10 s and was followed by the establishment of a lower steady-state level (304 ± 51 ppb). When NO was continuously sampled from the nose a steady-state level, similar to that found in the sinus, was immediately established (313 ± 52 ppb).

Conclusion—The data presented confirm previous findings of extremely high NO levels in the paranasal sinuses and suggest that these cavities may also function as reservoirs for NO. *Key words*: *humans*, *maxillary sinus*, *nose*.

INTRODUCTION

The sinuses are air-filled outgrowths from the nose, which are connected to the nasal airways via an ostium which opens into the nose. Nitric oxide (NO) levels in the paranasal sinuses have been demonstrated to be several times higher than those in the nose. This, together with the finding of inducible NO-synthase (iNOS), the rate-limiting enzyme for NO production, in the sinus mucosa, has led to the conclusion that the paranasal sinuses may be the primary source of NO in the upper respiratory tract (1, 2).

NO is known to exhibit bactericidal, fungicidal and antiviral effects, thereby helping the immune system to fend off invading microorganisms (3, 4). The mucociliary system is also dependent on NO for its function, in that NO stimulates ciliary activity and low levels of nasal NO correlate with impaired mucociliary function in the upper airways (5, 6). Furthermore, NO derived from the upper airways has been suggested to play a role in maintaining a good ventilation:perfusion ratio in the lungs (7, 8). The presence or absence of NO throughout the respiratory tract has been studied in connection with a number of conditions and increased concentrations have been reported in conditions such as asthma, rhinitis and upper respiratory tract infection. Decreased NO levels have been found in patients with acute and chronic sinusitis and nasal polyposis (9–14). Based on these results, NO has been implied to serve both as a marker and mediator of airway inflammation and infection. In this study, we examined the role of the maxillary sinuses in upper airway NO production and their possible function as reservoirs for locally produced NO.

MATERIAL AND METHODS

Patients

Seven healthy volunteers (5 women; mean age 35 ± 8 years) participated in the study. Before inclusion an experienced ENT consultant examined all volunteers. Smokers, those on medication and those with signs of infection or allergy were excluded. None of the subjects had any history of allergic disorders or recurrent airway problems. Approval for this study was obtained from the local Ethics Committee of the University of Lund and informed written consent to participate in the study was obtained from all subjects.

Experimental protocol

A drainage tube (plasticized PVC with a polyetherimide tip; total volume ≈ 0.23 ml) was inserted into the right maxillary sinus, as previously described, using a SinoJect™ puncture set (Atos Medical AB, Hörby, Sweden) (15). The inserted tube was connected to a NO analyser, and remained closed until the measurements started. Air was then sampled at a constant flow from the maxillary sinus into the analyser. After 20 min the NO probe was attached to a nasal "olive" that was introduced into the contralateral vestibulum nasi. NO recording from the nose continued for another 20 min.

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At the end of the measurements, the drainage tube was sealed but left in place. The following morning the whole set of measurements was repeated. The drainage tube functioned without problems in five of the subjects. In three of these subjects the tube was sealed again and a third set of measurements was obtained after another 5 h.

NO measurements

The concentration of NO was measured by chemiluminescence using a CLD 700 AL NO analyser (ECO PHYSICS AG; Dürnten, Switzerland) (6, 12). The amount of NO in sampled air was continuously analysed and the values were stored for later analysis. The upper detection limit of the setup was 10,000 ppb NO and was reached in 3 subjects. The maximum measured NO value was 9,720 ppb (Fig. 1). The background levels of NO never exceeded 10 ppb.

During the experiment, air was continuously drawn into the analyser at a constant sampling flow rate of 0.7 l/min. This flow rate has previously been used for continuous sampling from the nasal cavity in conjunction with comparative measurements of nasal and sinusal NO production (2). This is below the recommended flow rate for sampling of nasal NO as recommended in the American Thoracic Society guidelines (16). However, a lower flow rate is better suited to the present type of continuous intrasinusal measurements, as it allows "replacement air" to enter via the sinus ostia without causing discomfort. As the measurements lasted >40 min, all subjects were instructed to breathe normally through the mouth, thus leaving their velum open during the whole procedure. This procedure was chosen as few subjects are able to close the velum voluntarily for more than a couple of minutes. All measurements were performed in a seated position.

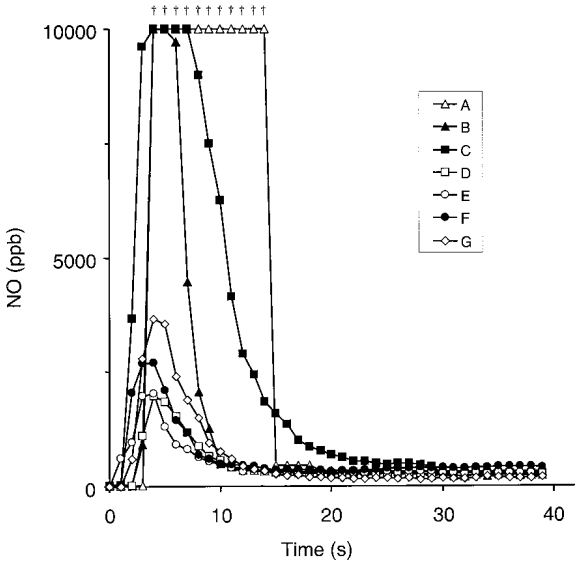

Fig. 1. NO levels during the first 40 s when sampled from the maxillary sinus. Curves are presented for each of seven subjects (A–G). † Data points where NO levels were higher than the measuring range of the analyser, i.e. $>10{,}000$ ppb NO.

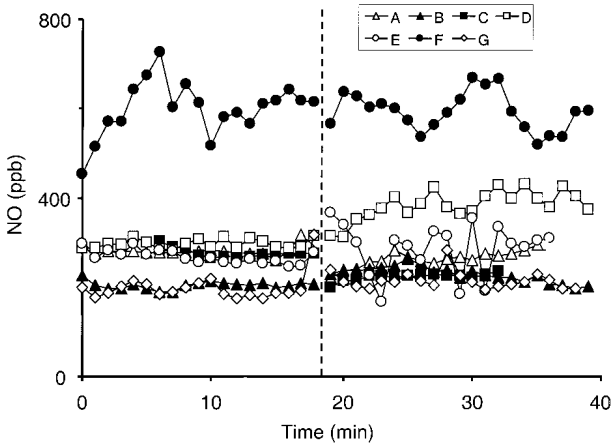

Fig. 2. Steady-state NO levels in the maxillary sinus and nose during continuous sampling. NO levels were first sampled continuously from a drainage tube inserted into the right maxillary sinus (left-hand side; initial peaks not shown) and thereafter from the contralateral nostril (right-hand side). Curves are presented for each of seven subjects (A–G).

Statistics

Results are expressed as mean $\pm$ SEM unless stated otherwise. Statistical comparisons were made using Wilcoxon's signed ranks test and $p < 0.05$ was considered statistically significant.

RESULTS

When NO was continuously sampled via a drainage tube inserted into the maxillary sinus all subjects tested displayed a transient peak in the NO levels recorded. The maximal NO level ($5{,}761 \pm 1{,}513$ ppb; median 3,650 ppb; range 1,970– $>10{,}000$ ppb; $n = 7$) was always reached within 10 s. After 20 s of continuous sampling the peak had passed and a steady-state level was established (Fig. 1). Peak levels in the maxillary sinus varied among the subjects tested, being 4–49 times higher than the subsequent steady-state levels.

After 20 min of continuous sampling from the maxillary sinus, the NO probe was switched to the nasal cavity and the recording proceeded for another 20 min. As a result of this switch, a new steady-state level was immediately established. There was no difference between the steady-state levels obtained from sampling in the maxillary sinus and the levels found

in the nasal cavity (304 ± 51 ppb and 313 ± 52 ppb, respectively; $n = 7$; $p = 0.6$; Fig. 2).

In five out of seven subjects, the drainage tube was sealed and left in the maxillary sinus. The comparative measurements were then repeated, according to the same procedure, ≈ 17 h later. Peak values for this subgroup of 5 subjects were as follows: Day 1, $4{,}066 \pm 1{,}514$ ppb (median 2,690 ppb; range 1,970–$> 10{,}000$ ppb); and Day 2, $1{,}608 \pm 305$ ppb (median 1,160 ppb; range 1,100–2,590 ppb). In three of these subjects, the drainage tube was then sealed again and a third set of measurements was performed a further 5 h later. Peak values for this subgroup of 3 subjects were as follows: Day 2 (morning), $1{,}937 \pm 417$ ppb (median 2,060 ppb; range 1,160–2,590 ppb); Day 2 (afternoon), $1{,}567 \pm 107$ ppb (median 1,480 ppb; range 1,440–1,780 ppb). The same pattern of a transient peak followed by a much lower steady-state level was seen in all these measurements and the steady-state levels for NO derived from the maxillary sinus and nasal cavity were always of the same magnitude (data not shown).

DISCUSSION

This study demonstrates the presence of very high concentrations of NO in air "trapped" in the human maxillary sinuses. The NO levels in air that has been contained in the sinuses are up to 49 times higher than those recorded in the nasal cavity. When air is continuously evacuated from the maxillary sinuses a much lower steady state-level of NO is quickly established and this level seems to be more or less equivalent to the levels of NO found in air derived directly from the nasal cavity. The mean concentration of NO in air "trapped" in the maxillary sinuses reported in the present paper ($5{,}761 \pm 1{,}513$ ppb; median 3,650 ppb; range 1,970–$> 10{,}000$ ppb) is in line with that reported by Lundberg *et al.* (2).

The maxillary sinuses are air-filled outgrowths from the nose which are completely encased in bone except for a single small orifice, the ostium, which opens into the nose. In a normal adult the volume of each maxillary sinus is ≈ 15 ml. When air was continuously sampled from the maxillary sinus, via a drainage tube, its entire volume could be expected to be washed out within 2 s and replaced by air entering from the nasal cavity via the ostium. Owing to the properties of the NO analyser and the unavoidable dead space in the tubing the lag-time for measurements greatly exceeded 2 s. Peaks lasted, with one exception, for only a few seconds and the symmetrical form of the peaks, steeply up and steeply down, indicated that what is measured initially must be NO in gas "trapped" in the maxillary sinus.

It is not known for certain whether the majority of NO released from the adult nose is derived from the epithelium lining of the nasal cavity or whether it comes from the paranasal sinuses. It has been suggested that the major proportion of NO measured in the nose derives from the paranasal sinuses (1, 2). In contrast, the findings of a recent case report challenged this view, suggesting that nearly 90% of nasal NO is derived from the nose itself (17). During acute sinusitis, with radiographic signs of filled maxillary sinuses, the level of nasal NO is reduced and returns to normal after successful antibiotic treatment (13). In allergic rhinitis (where nasal inflammation is present), nasal NO levels have been reported to be either unaltered or elevated (18–21). These findings support a maxillary sinus origin of nasal NO. Furthermore, it has been shown that severe polyposis, which causes blockage of the sinus ostia, reduces nasal NO levels (20, 22). In one of those studies an inverse correlation was found between nasal NO levels and tomodensitometric alteration of the paranasal sinuses (22). The high NO peak found during the initial sampling from inside the paranasal sinuses in the present study also favours the idea of a high contribution of NO from the paranasal sinuses.

To further corroborate our results the present results were compared with data from a recent study of the upper airway origin of carbon monoxide (15). Using the same technique and sampling protocol it was demonstrated that carbon monoxide could be derived from both the nose and the paranasal sinuses. However, in contrast to what is presently reported for NO, no peaks in the carbon monoxide levels could be recorded when sampling directly from the paranasal sinuses. This supports the idea of a special role for the sinus epithelium in the upper airway production of NO. Previous findings of large differences between the nasal and sinus responses to local administration of the NOS-inhibitor, L-N^G-nitroarginine methylester, further augment the present finding (2).

The present data also emphasize the role of the sinuses as reservoirs enabling NO levels to reach extremely high levels. It is worth noting that both the present findings and the previous report by Lundberg *et al.* (1) demonstrated large variations in the maximal NO levels between different subjects tested. Furthermore, the present study, by performing repeated measurements over about a 24-h period, also reveals interpersonal variations. Some of these variations are reflected in subject F, whose NO levels were nearly twice as high as those found in the other subjects. Subject F also displayed an initial peak, although it was relatively small compared to that obtained in some of the other subjects tested. The low peak found

in subject F may be related to a large ostial opening into the tested maxillary sinus, preventing NO from accumulating to extremely high levels inside the sinus. However, as neither ostial diameter nor functionality were measured this is merely speculation at this point. The nasal cycle is known to affect the patency of the ostium (23) and it is therefore intriguing to speculate on a role for the ostium in regulating local NO production. The oxygen content in the sinus is related to the patency of the ostium and during periods of occlusion a relative hypoxia develops inside the paranasal sinuses (24). As NOS expression is inversely correlated with oxygen levels (25–27) this may be a way of regulating the NO production and thereby the NO content in the sinus reservoirs. Furthermore, burst release of NO from these reservoirs into the nasal cavity during different conditions may be of importance, for instance in the defence against invading microorganisms or in the regulation of nasal patency. Thus, further research will be needed in order to determine if cyclic variations in the ostia are linked to the great variations in NO levels seen between different individuals and in the same individual on different occasions. To summarize, the data presented support the idea of a special role for the paranasal sinuses in upper airway production of NO and suggest a role for the sinuses as reservoirs allowing accumulation of extremely high concentrations of NO.

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