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Nitric Oxide and the Paranasal Sinuses

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ABSTRACT

The discovery within the paranasal sinuses for the production of nitric oxide (NO) has altered the traditional explanations of sinus physiology. This review article reports the ongoing investigation of sinus physiology beginning with the discovery of NO gas production in the paranasal sinuses that occurred in 1995, and the impact that finding has had both in the basic science and clinical arenas. It was shown that healthy paranasal sinus epithelium expresses an inducible NO synthase that continuously generates large amounts of NO, a pluripotent gaseous messenger with potent vasodilating, and antimicrobial activity. This NO can be measured noninvasively in nasally exhaled breath. The role of NO in the sinuses is likely to enhance local host defense mechanisms via direct inhibition of pathogen growth and stimulation of mucociliary activity. The NO concentration in a healthy sinus exceeds those that are needed for antibacterial effects *in vitro*. In patients with primary ciliary dyskinesia (PCD) and in cystic fibrosis, nasal NO is extremely low. This defect NO generation likely contributes to the great susceptibility to chronic sinusitis in these patients. In addition, the low-nasal NO is of diagnostic value especially in PCD, where nasal NO is very low or absent. Intriguingly, NO gas from the nose and sinuses is inhaled with every breath and reaches the lungs in a more diluted form to enhance pulmonary oxygen uptake via local vasodilation. In this sense NO may be regarded as an "aerocrine" hormone that is produced in the nose and sinuses and transported to a distal site of action with every inhalation. Anat Rec, 291:1479–1484, 2008.

Key words: paranasal sinuses; nitric oxide; respiratory physiology

Why are humans equipped with paranasal sinuses? This question has occupied researchers in the area for hundreds of years (Blanton and Biggs, 1969) but still today, there is no clear answer for the physiological significance of these enigmatic cavities. What is painfully clear, however, is that the sinuses are very vulnerable structures, and this is reflected in the very high prevalence of sinus related disorders. As an example, sinusitis affects about 16% of the US population annually, and the cost for this is gigantic, approaching as much as half of the total cost for asthma management (Kaliner et al., 1997; Anand, 2004). In general, the sinuses have an anatomically unfavorable position where they lie in close connection to the nasal cavity which is heavily colonized by myriads of potentially pathogenic bacteria. Although the nose can clear freely in both directions, the sinuses are left with a single tiny ostium through which mucus and invading bacteria and viruses are drained. Even worse is the position of the ostium in the maxillary sinus at the top of the cavity, which forces the drainage system to work against the laws of gravity (Drettner and Aust, 1977; Aust et al., 1994). So, with this in mind, one could just as well view things the other way around; it is remarkable that so many of us after all do not develop sinus disease.

Here, I discuss some novel theories regarding mechanisms of sinus host defense that have evolved over the years in our lab and in other labs. In addition, a provocative alternative physiological role of the paranasal sinuses in physiological regulation of pulmonary func-

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tion is discussed. The article is based around the discovery that the sinuses produce great quantities of the gas nitric oxide (NO), a highly bioactive signaling molecule.

GENERATION AND PHYSIOLOGICAL EFFECTS OF NO

The free-radical gas NO is produced by various cells throughout the body and serves to regulate a vast number of physiological processes including blood flow, neurotransmission, and host defense (Moncada and Higgs, 1993). Endothelial cells generate small amounts of NO in response to agonists or shear stress, and this NO diffuses to the underlying smooth muscle cells to cause vasorelaxation (Furchgott and Zawadzki, 1980; Ignarro, 2002). In the nervous system, NO is also released in small controlled quantities to modulate, for example parasympathetic signaling in the airways and in the gastrointestinal tract. NO is synthesized by specific enzymes; the NO synthases which utilize L-arginine, and molecular oxygen to form NO and L-citrulline (Nathan and Xie, 1994). Two distinct types of constitutive NO synthases, the endothelial NOS and the neuronal NOS control the functions described earlier. A third isoform, the inducible NOS (iNOS) is expressed in white blood cells, epithelial, and other cells in response to proinflammatory cytokines or bacterial components. This isoform can generate large amounts of NO for extended periods and at high concentrations NO and its reaction products are cytotoxic to viruses, bacteria, tumor cells, and possibly even to host cells (Nathan, 1997). In this case, the amounts of NO generated are orders of magnitude higher than in blood vessels and in nerves. During inflammation, iNOS is upregulated, and there has been great debate as to whether this is of benefit or harm to the host. Still today, the role of NO in inflammation has not been settled as both pro- and antiinflammatory effects of NO and its reaction products have been described (Lundberg et al., 1997).

NO IN THE AIRWAYS

In 1991, Gustafsson et al. (1991) showed that NO gas was present in exhaled breath of experimental animals and humans. It was clear that this was endogenously produced since treatment with a NO synthase inhibitor abolished the exhaled NO signal. Initially, it was believed that exhaled NO originated from the alveolar region (Borland et al., 1993) as for other exhaled gases including oxygen and carbon dioxide. However, a study by Alving et al., in 1993 suggested that this was not the case (Alving et al., 1993). They found that NO levels were higher during nasal breathing compared with oral exhalations, which suggested a contribution from the upper airways. The same group went on to study this in more detail (Lundberg et al., 1994b). In subjects with a permanent tracheostomy exhaled NO was measured from three different levels of the respiratory tract; the subjects were asked to exhale either through the tracheostomy, the mouth, or via the nose. Single-breathe measurements showed low-NO levels when exhaling via the tracheostomy, intermediate levels from the mouth and high levels in nasal exhalation (Lundberg et al., 1994b). This clearly proved that in resting healthy adults the major part of exhaled NO is originating from the upper airways with less contribution from the lower airways and the lungs.

IDENTIFYING THE PARANASAL SINUSES AS GREAT NO PRODUCERS

Although it was now obvious that the nasal passages was the main source of exhaled NO in healthy people, it was still not clear exactly where this NO was coming from and by which cells it was produced. Experiments with topical NO synthase inhibitors in the nose gave the first clue. Surprisingly, these agents did not affect nasal NO levels to greater extent. This led us to speculate that a major NO source must be situated somewhere in the nasal region but out of reach for NOS inhibitors administrated via the inhalation route. The paranasal sinuses seemed like plausible candidates; these cavities lie adjacent to the nose, but they would not be readily accessible to topical drug administration. Yet, a gas like NO, if produced inside the sinuses, could easily pass into the nasal cavity through the communicating ostia. We went on to puncture our own maxillary sinuses, leaving a catheter in place allowing for aspiration of gas directly from the sinus cavity. Remarkably, the levels of NO in aspirated sinus gas were orders of magnitude higher than what we had found in exhaled breath before (Lundberg et al., 1994a, 1995a). In fact, in some subjects, the levels approached the maximum allowed environmental pollution levels for this gas, which is 25 ppm. Also, repeated aspiration of the entire sinus volume showed rapid accumulation of new NO gas to the same high levels, suggesting a continuous production. Local instillation of a NOS inhibitor decreased sinus NO levels by 80%, proving that the production was of enzymatic origin (Lundberg et al., 1995a). Finally, in biopsies from healthy subjects undergoing reconstructive facial surgery we could demonstrate that the enzyme responsible for this NO was a calcium-independent inducible NOS in the epithelial cells lining the sinuses (Lundberg et al., 1995a, 1996b). At this time, it was highly surprising to find an iNOS in a healthy tissue since the dogma was that this enzyme is only expressed in inflamed tissues or in activated white blood cells (Moncada and Higgs, 1993) High-nasal NO levels can be measured immediately after birth (Lundberg et al., 1995a) even in babies delivered by caesarian section (Artlich et al., 2001) and studies in monkeys indicate that iNOS is expressed in the airways already during the third trimester (Shaul et al., 2002). Thus, its expression and activity does not seem to require activation by luminal factors such as bacteria. It should be noted that still today there are uncertainties regarding the relative contribution from the various sources of NO in the nasal passages to the NO we measure in nasal air. It is likely that the individual variation is considerable. As an example, infants have rather high-nasal NO levels despite the fact that the sinuses are poorly developed at birth. This very high NO production measured in the nose and sinuses seems to be rather unique to humans and most other primates, whereas considerably lower levels are found in other animals including rats, mice, rabbits, and dogs (Schedin et al., 1997) Lewandowski et al. measured nasal NO in baboons and found only low levels (Lewandowski et al., 1998). Interestingly, baboons are the only mammal known to lack paranasal sinuses. The demonstration of

NO production in the human sinuses immediately stimulated discussions as to its physiological role.

NO IN SINUS HOST DEFENSE

Although the exact physiological role of NO in the sinuses still remains to be elucidated, a number of facts support an important role for this gas in local host defense. The evidence for this may be summarized as follows: First, from immunohistochemical studies and mRNA studies it seems clear that an iNOS is constantly expressed apically in the sinus epithelium (Lundberg et al., 1995b; Deja et al., 2003). This is the same enzyme used by activated white blood cells to produce NO in response to invasion of virus or bacteria (Nathan, 1997). The central role of iNOS in these cells is clearly illustrated in genetically engineered animals lacking this enzyme. Those animals are more susceptible to bacterial and viral infections (MacMicking et al., 1995; Wei et al., 1995). Second, in patients suffering from sinusitis of different etiology, the nasal NO levels are generally very low (Lundberg and Weitzberg, 1999). The most striking example is primary ciliary dyskinesia (PCD) where NO release in the nasal airways is virtually absent (Lundberg et al., 1994b). Similarily, in cystic fibrosis nasal NO is also markedly reduced (Lundberg et al., 1996c). Interestingly, both these disorders are characterized by a great susceptibility to sinus infections. Whether the reduced sinus NO is a cause or consequence of sinusitis in these patients, however, still remains to be clarified. Deja et al. elegantly showed that inflammation of the sinus mucosa, as observed in radiologic maxillary sinusitis, is associated with dramatic inhibition of the expression of the epithelial iNOS (Deja et al., 2003). As a consequence, sinus NO levels were very low. The authors speculated that this lack of NO would decrease the resistance against sinus infections. A recent case study supports that inhibition of sinus NO indeed can have negative consequences for host defense (Lundberg, 2005). A healthy subject applied an NO synthase inhibitor topically in the right nostril and then saline on the left control side. Nasal NO levels immediately dropped markedly on the right side but stayed normal on the left side. Interestingly (but unfortunate for the subject, who happened to be the author), the subject developed a CT proven right-sided maxillary sinusitis 3 days later. Third, numerous bacteria, including many airway pathogens, are sensitive to NO and chemically related nitrogen oxides when applied in an experimental setting (Fang, 1997; Lundberg et al., 2004). In fact, some bacteria are sensitive to authentic NO gas in concentrations as low as 100 parts per billion (Mancinelli and McKay, 1983), which is orders of magnitude lower than the NO levels in a healthy maxillary sinus (Lundberg et al., 1995b). Fourth, mucocilary clearance is a vital part of sinus host defense. Besides acting directly on microorganisms, NO may also stimulate ciliary motility (Jain et al., 1993). A study by Runer et al. showed that application of an NO donor in the nasal mucosa of humans did causes an increase in ciliary beat frequency (Runer et al., 1998; Runer and Lindberg, 1998, 1999). Furthermore, the same group has shown that low levels of nasal NO correlate with impaired mucociliary function in the human upper airways (Lindberg et al., 1997).

All together, there is evidence to suggest that NO is involved in sinus host defense and that this gas may help to keep these cavities sterile under normal conditions. Conversely, low-sinus NO levels may lead to increased susceptibility to sinus infections. Future studies will elucidate if stimulation of endogenous NO production in the nose and sinuses or topical application of NO donating drugs could be used therapeutically to treat or prevent sinusitis.

NO AS AN "AEROCRINE" MESSENGER

Among biologists, NO has received greatest attention for its vasodilator properties (Ignarro, 2002). The release of NO from the vascular endothelium in response to agonists or shear stress helps to control blood flow via diffusion to the underlying smooth muscle cells, activation of guanylyl cyclase, and the generation of cGMP. The powerful vasodilating effects of NO and the fact that it is a gas can be used therapeutically. When exogenous NO gas is delivered to inhaled air, it dilates vessels in the lung, leading to increased oxygen uptake and a reduction in pulmonary vascular resistance (Frostell et al., 1991). Currently, inhaled NO therapy is clinically approved for use in newborn children with persistent pulmonary hypertension (Roberts et al., 1997) and other indications are also being evaluated (Kinsella et al., 2006). Several investigators have found clear effects on arterial oxygenation and pulmonary arterial pressure using concentrations of inhaled NO as low as 10–100 ppb (Gerlach et al., 1993; Puybasset et al., 1994). Intriguingly, during normal breathing endogenous NO produced by the paranasal sinuses is inhaled at similar concentrations (Gerlach et al., 1994; Busch et al., 2000; Tornberg et al., 2002). We have shown that nasal breathing reduces pulmonary vascular resistance and improves arterial oxygenation compared with oral breathing in subjects without lung disease (Lundberg et al., 1995c, 1996d). The addition of 100 ppb NO during oral breathing mimicked the effect of nasal breathing, whereas moistened air during oral breathing had no effect (Lundberg et al., 1996a). Intubated and mechanically ventilated patients are deprived of the natural inhalation of endogenous upper airway NO. Supplementation of NO-containing nasal air to these patients improves arterial oxygenation and reduces pulmonary vascular resistance (Lundberg et al., 1995c). In addition, Pinsky et al. have shown that the hospital pressurized air may contain NO levels similar to those described earlier (6–500 ppb) which may consequently have effects on arterial oxygenation and pulmonary arterial pressure in mechanically ventilated patients (Lee et al., 1997; Pinsky et al., 1997; Lum et al., 1998; Tan et al., 2002). In another study, nostril widening with breathe easy nasal strips improved arterial oxygenation in spontaneously breathing patients, likely by enhancing ventilation through the nasal airways thereby increasing NO delivery from the nasal airways to the lungs (Herulf et al., 1999). Interestingly, Törnberg et al. recently showed that more NO is released from the nasal passages during nasal inhalation compared with exhalation (Tornberg et al., 2002). This is likely because an inhalation manoeuvre creates a negative pressure in the sinuses thereby forcing NO-containing gas out of the cavities (Lundberg and Weitzberg unpublished observation). Thus, the anatomy of the

nasal airways and conchae seems to create aerodynamic effects favoring sinus air donation during inhalation.

All together, these results clearly show that NO derived from the upper airways is capable of improving oxygen uptake and reducing pulmonary vascular resistance. It is tempting to speculate that the production of NO in the paranasal sinuses has the purpose of modulating lung function in humans. As NO is inhaled from a proximal source, per definition it will only affect pulmonary vessels in contact with ventilated alveoli, thereby improving ventilation/perfusion matching. This newly described physiological effect of NO has been termed "aerocrine," to illustrate the airborne transport of a biological messenger in the human respiratory tract (Lundberg et al., 1995c). This whole concept of nasal breathing controlling pulmonary function and oxygen uptake has links to many of the breathing patterns practiced in traditional yoga. It also may have relevance to the massive cardiopulmonary physiological adaptions that occur immediately after birth. The endogenous nasal NO autoinhaled by the newborn baby may aid in pulmonary vasodilation and oxygen uptake. Although this provocative hypothesis remains to be proven, it is an intriguing fact that newborns are obligate nasal breathers (Lacey and Brown, 2000), as opposed to adults who regularly switch between nasal and oral breathing.

THE DIAGNOSTIC USE OF NASAL NO

The levels of NO in the nose can easily be measured noninvasively and online by simply aspirating air from the nostril or by a nasal exhalation (Palm et al., 2000). The gold-standard measuring method for exhaled ad nasal NO has been chemiluminescence, but sensitive electrochemical analyzers are now also available. Using these methods, it has been found that nasal NO is altered in several airway disorders, including allergic rhinitis, PCD, cystic fibrosis, and sinusitis (Lundberg and Weitzberg, 1999; Djupesland et al., 2001). For most indications, nasal NO is still to be regarded as an interesting research tool with potential clinical importance. However, in PCD, the situation is strikingly different because nasal NO is uniformly extremely low (Lundberg et al., 1994b). The sensitivity and specificity of a nasal NO test in the diagnosis of PCD has now proven so good (Wodehouse et al., 2003; Noone et al., 2004; Stehling et al., 2006) that this test is now used routinely in specialized centers. Extensive literature on measurements procedures and the diagnostic use of exhaled and nasal NO is available elsewhere for the interested reader (Lundberg and Weitzberg, 1999; Djupesland et al., 2001; Maniscalco et al., 2007). When measuring NO by aspirating air from a nostril, the value is a sum of all combined sources of NO in the nose. Although much is likely coming from the large sinus source via the ostia, the nasal mucosa can also contribute. So, is there a way to more accurately measure NO that is coming from the sinuses? In 2002, our laboratory made a remarkable observation while measuring nasal NO. (Weitzberg and Lundberg, 2002). We noticed by serendipity that if a person was humming during a nasal NO measurement, the nasal NO increased dramatically. In a series of experiments, we have now characterized the mechanisms for this phenomenon (Weitzberg and Lundberg, 2002; Maniscalco et al., 2003a,b, 2004, 2006). The NO peak is coming from the paranasal sinuses as a consequence of the oscillating sound waves produced during humming. These sound waves dramatically speedup the exchange of gases over the sinus ostium and sinus gas (containing very much NO) is immediately washed out into the nasal cavity where we detect it as a large peak. This may have a diagnostic value in evaluating how well the sinuses are ventilated. Why then would we be interested in measuring sinus ventilation? A central underlying development in the pathogenesis of sinusitis is a poor ventilation of the sinuses due to obstruction of the ostia (Kaliner et al., 1997). When this happens, the oxygen levels drop in the sinuses (Aust and Drettner, 1974a,b,c) and at the same time carbon dioxide increases thereby depriving many of the mucosal host defense mechanisms (including iNOS activity), which are dependent on oxygen delivery also from the lumen side. This situation creates favorable conditions for bacterial invasion and growth (Drettner and Aust, 1977). In a recent study, we could show that the large humming peak in nasal NO was completely absent in patients with chronic sinusitis and CT proven sinus obstruction (Lundberg et al., 2003). This clearly shows that this simple test is useful to evaluate ostial patency. It remains to be studied if this test could be clinically useful in identifying patients at risk of developing sinusitis and in that case if an early intervention improves the long term outcome in these patients.

A more provocative view on humming is that it might by itself help to prevent or resolve sinusitis. The mechanism would simply be that humming speeds up the gas exchange in the sinuses enormously so that fresh air can enter, thereby preventing the pathological processes associated with reduced oxygen levels as described earlier. It should be noted that during silent nasal breathing the time it takes to exchange all sinus gases is between 5 and 30 min and much longer in patients with partly obstructed ostia (Paulsson et al., 2001) With humming, this occurs in one single exhalation (Weitzberg and Lundberg, 2002; Maniscalco et al., 2003b).

CONCLUSION

Research over the past decade has shown that the potent bioactive gas messenger NO is produced continuously in the human paranasal sinuses by an inducible NO synthase expressed in the sinus epithelium. The high-NO levels locally in the sinuses may have important functions in host defense and conversely, a reduced NO production may increase susceptibility to sinus infections. NO gas from the sinuses and nose is inhaled with every breath and reaches the lungs in a more diluted form. Intriguingly, this NO can function as an "aerocrine" hormone to enhance pulmonary oxygen uptake and reduce pulmonary vascular resistance. Thus, a physiological role of the paranasal sinuses in regulation of pulmonary function is suggested.

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