Nitric Oxide in the Nasal Airway: A New Dimension in Otorhinolaryngology

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The discovery that the gas nitric oxide (NO) is an important signaling molecule in the cardiovascular system earned its Nobel prize in 1998. NO has since been found to play important roles in a variety of physiologic and pathophysiologic processes in the body including vasoregulation, hemostasis, neurotransmission, immune defense, and respiration.

The surprisingly high concentrations of NO in the nasal airway and paranasal sinuses has important implications for the field of otorhinolaryngology. NO provides a first-line defense against micro-organisms through its antiviral and antimicrobial activity and by its upregulation of ciliary motility. Nasal treatments such as polypectomy, sinus surgery, removal of hypertrophic adenoids and tonsils, and treatment of allergic rhinitis may alter NO output and, therefore, the microbial colonization of the upper airways. Nasal surgery aimed at relieving nasal obstruction may do the same but would also be expected to improve pulmonary function in patients with asthma and upper airway obstruction.

NO output rises in a number of conditions associated with chronic airway inflammation, but not all of them. Concentrations are increased in asthma, allergic rhinitis, and viral respiratory infections, but reduced in sinusitis, cystic fibrosis, primary ciliary dysfunction, chronic cough, and after exposure to tobacco and alcohol. Therefore, NO, similar to several other inflammatory mediators, probably subserves different functions as local conditions dictate. At present, it seems that the measurement of NO in the upper airway may prove valuable as a simple, noninvasive diagnostic marker of airway pathologies.

The objective of this review is to highlight some aspects of the origin, physiology, and functions of upper airway NO, and to discuss the particular methodological problems that result from the complex anatomy.

In 1978, Murad et al[1] hypothesized that nitric oxide (NO) is involved in vasoregulation. In 1980, Furchgott et al[2] showed that vasodilatation is regulated by a substance derived from the endothelium. A few years later, Ignarro et al[3] and Palmer et al,[4] almost simultaneously, showed that this was in large measure attributable to nitric oxide (NO). The investigators received the Nobel Prize when it was determined that this, most important of the autacoids, has a vital role in a multiplicity of other physiologic and pathophysiologic processes in the body.[5,6]

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NO plays an important role in vasoregulation, regulating the arterial blood flow and pressure by mediating the resting vasodilator tone.[7] NO controls hemostasis by inhibiting platelet-endothelial cell contact, preventing leukocyte adhesion and activation on endothelial cell surfaces[8] and by its action on the cascade of coagulation.[9,10]

NO has been proposed as the transmitter of certain types of central neurotransmission, such as pain perception, memory, learning, and depression. In the peripheral autonomic neurones referred to as the nonadrenergic, noncholinergic system (NANC). In the respiratory system, it is probably involved in neural bronchodilatation.[8,11]

NO has been shown to provide a first-line defense against micro-organisms by antiviral and antimicrobial activity[12,13] and by upregulation of the ciliary motility. The evidence for the latter comes from in vitro[14] and saccharin transport studies.[15,16]

Chronic inflammation may lead to the pro-

duction of several metabolites, among them NO, that may have cytotoxic and even genetoxic effects, thereby damaging DNA.[17] NO is also implicated in the tumoricidal activity of the immune system and in other aspects of the tumor biology such as angiogenesis and the development of metastases. NO may have either pro- or antiinflammatory properties of which prodominates will depend on such factors as the type and phase of inflammation, the individual response and its local concentration. Thus, similar to other mediators, NO has a dualistic function.[6]

NO is involved in many physiologic events such as ventilation/perfusion matching within the lungs, host defense, pregnancy, penile erection, peristalsis, tolerance to morphine, and many others.[8]

NO in the nose and sinuses is found not only in the tissues, interstitial fluid, and cells of intravascular compartment, but is also a constituent gas of the airway itself. The objective of this review is to highlight some aspects of the origin, physiology, and functions of upper airway NO, and discuss the particular methodological problems resulting from the complex anatomy.

Exhaled NO (ENO)

NO was first shown in exhaled air in 1991.[18] Two years later, Alving et al[19] showed that exhaled NO (ENO) levels were elevated in asthmatics, and that they decreased after treatment with steroids. In the same study, Alving reported a high concentration of NO in upper airways.[19]

A large number of studies[19-38] have confirmed that ENO is increased in such airway inflammations as asthma,[30,39] bronchiectasis,[40] viral respiratory infections,[23,41] and in many other clinical situations as systemic lupus erythematosis,[42,43] active tuberculosis,[44] acute lung allograft rejection,[45] rhinitis,[21] sleep apnea,[46] and after exercise.[47-49] A recent study found significantly higher levels of ENO in otherwise healthy children with positive skin prick test to common allergens.[50]

In chronic obstructive lung disease (COLD), the results are conflicting. Some studies show increased levels of NO[51,52]; others do not.[53,54] Exhaled NO (ENO) is decreased in chronic cough,[55] in primary pulmonary hypertension,[56,57] after exposure to tobacco[58-60] or alcohol,[61] in acute[62] and chronic sinusitis,[63] in human immunodeficiency virus (HIV) infection,[64,65] cystic fibrosis,[66,67] and primary ciliary immobility.[16,68] However, infants with cystic fibrosis and bronchopulmonary dysplasia had significantly higher levels of exhaled NO than healthy control patients.[69] Recent studies reported raised nitrite levels in breath condensate in stable patients with cystic fibrosis, whereas the ENO was in the normal range.[70,71] This suggests that poor diffusion of nitric oxide across viscous secretions and removal by reactive oxygen species may contribute to the low gaseous NO levels observed in some suppurative conditions, such as cystic fibroses[62,63] and sinusitis.[58,59] Similarly, differences in airway wall edema has been suggested as a possible explanation for the large variations in the effect of inhaled NO on lower airway tone.[72]

Thus, potential functions and mechanisms of action of NO appear to be legion and by and large its role and significance to each remain to be determined.

NASAL NO

The finding of high levels of NO in the nasal airways[19] and paranasal sinuses[73] in normal humans and animals[74,75] as compared with the lower airways evoked renewed interest in the physiology and respiratory role of the upper airways and its potential interaction with the lower airways. Some of the mechanisms of this link are controversial,[76,77] as is the function and physiologic role of the large, air-filled paranasal sinuses.[78]

A growing number of connections are being found between the intra- and extrathoracic airways. A relationship has been shown between increased bronchialhyperresponsiveness, decreased FEV1, and nasal polyposis and allergic rhinitis.[79] Nasal treatments such as polypectomy and sinus surgery may improve pulmonary function in patients with asthma.[78] One of the dominant links may prove to be NO.

NO produced in the upper airways has been shown to be a modulator of pulmonary function improving ventilation perfusion V/Q matching.[80-82] A reduction of the autoinhalation of nasally produced NO may contribute

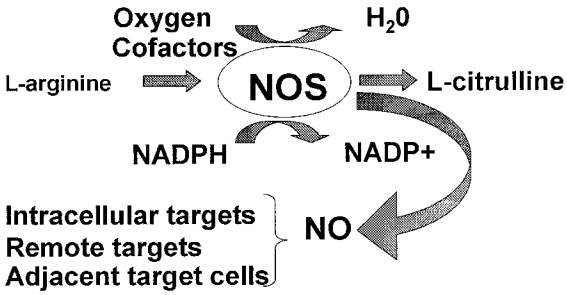

Fig 1. Nitric oxide synthesis.

to the negative effects of mouth breathing in sleep disorders and after tracheotomy.[81,83]

NO is also a recognized air pollutant derived from car exhaust and domestic gas use, and is a major constituent of cigarette smoke. Concentrations as high as 50 ppm may be found in the mainstream smoke, and it has been speculated that these high levels may suppress normal endogenous NO production,[56] explaining reduced ENO levels in smokers.[54] In non-smokers, concentrations approaching these levels have been identified in the normal maxillary antrum and frontal sinus, where they may serve to maintain sterility.[84]

The abnormal levels of NO found in various airway diseases and the response of both to steroids suggest a possible role for NO as a diagnostic tool as well as a marker of airway inflammation.[30] More studies are necessary in order to show the relationship between NO and the classic markers of inflammation.[39,85]

THE ORIGIN OF NASAL NO

Biochemical Pathway

NO is synthesized from the semiessential amino acid L-arginine by the action of one of several forms of nitric oxide synthase (NOS) with the production of L-citrulline. For this chemical reaction there are several cofactors, among which are oxygen and nicotinamide adenine dinucieotide phosphate (NADPH).[86] The NO produced is oxidized to nitrite ($NO2-$), which can be used to monitor NO formation, nitrate ($NO3-$), and peroxynitrite ($ONOO-$) ions (Fig 1).[87]

The NOS exists in at least 2 isotypes—the constitutive NOS (cNOS) and inducible NOS (iNOS). cNOS may be named according to its location as endothelial NOS (eNOS) and neuronal NOS (nNOS). These enzymes are the expression of different chromosomes whose genes are localized at chromosomes 7 (eNOS), 12 (nNOS), and 17 (iNOS).[86,88-91] They have different requirements for their activation. Whereas nNOS and eNOS are calcium-calmodulin–dependent and produce small amounts of NO, iNOS is calcium-calmodulin–independent and is activated by proinflammatory cytokines and endotoxins. Calmodulin is a chemical identified in close juxtaposition to the cilia of the nasal and upper airway epithelium, and is thought to be involved in ciliary motility. When activated, iNOS produces up to 1,000 times more NO than cNOS.[92] The 2 isoforms presumably serve different physiologic roles.[93] This may result in different physiologic effects.[94] L-arginine analogues like NG-nitrio-L-arginine methyl ester (L-NAME) and N-monomethyl-L-arginine (L-NMMA) are competitive inhibitors of all NOS isotypes, whereas amino-guanidine and corticosteroids only inhibit iNOS.[86] Although the iNOS found in the paranasal sinuses can produce large amounts of NO, it has some characteristics in common with cNOS. For example, it is calcium-dependent, not inhibited by steroids,[95] and continuously expressed. The induction of iNOS requires gene transcription. Hence, the increase in NO production takes hours, but it may continue for days.

Cellular Origin

Many cells in the upper and lower respiratory system such as the parasympathetic vasodilator nerves,[96] endothelial cells, and ciliated mucosa cells produce cNOS.[92,97] The inducible form of the NO synthase, iNOS, has been described not only in epithelium but also in macrophages, fibroblasts, neutrophils, endothelium, and vascular smooth muscle.[5] Nevertheless, the precise site of its main generation has not yet been determined. It is likely that the NO measured in the exhaled air is produced superficially in the mucosa, because the portion generated in deeper structures is probably trapped by hemoglobin.[5]

Anatomic Origin

The origin of the NO measured from the nasal airway has been controversial. Lundberg et al[73] found NO concentrations inside the sinus that was several hundred times higher than in exhaled air from lower airways (3,000-25,000 ppb), and suggested that the majority of nasal NO originates in the sinuses. There was also immunohistochemical evidence of higher concentrations of iNOS in sinus mucosa[73,98] compared with nasal.

However, recent evidence suggests that the sinuses are not the site of nasal NO production. High levels of NO are found in the nose of neonates shortly after birth,[99] before the sinuses have developed.[100] Moreover, in the first direct measurement of sinus NO output and of ostial function in relation to NO,[84] it was shown that, when all the sinus ostia are blocked, nasal NO output is decreased by a mere 12%.[84] In these experiments, further protection against sinus NO entering the nose was achieved by pharmacologic inhibition (70%-90%) with lidocaine. This local anesthetic greatly reduces sinus NO output but has no effect on nasal output.[84]

In addition, the frontal and maxillary sinuses were studied individually.[84] When their ostia had been plugged and each sinus punctured in 2 places so that the composition of the air entering and leaving was known, it was found that NO output in the frontal sinus was a little less than in the nose but, in the maxillary sinus, it was 3 times greater.[84] Calculations suggested that the NO output per cm^2 of mucosa was lower in the nasal cavity than in the sinuses, and that the higher output was attributable to the much larger nasal surface area. In the nose, this is around 300 cm^2, whereas in the maxillary it is about 30 cm^2.[101] This interpretation is in agreement with the relative activity of NOS identified in tissue extracted from these sites.[98]

Diffusion of NO through the sinus ostia has also been studied.[84] In the punctured frontal and maxillary sinuses, artificially raising their NO concentration did not increase nasal NO output, and flushing the sinuses constantly with NO free air did not lower it. Therefore, it seems that NO in the nasal airway is derived from the nasal mucosa and not from the sinuses.[84] This is not surprising because the majority of sinus ostia are small and are located deep in the middle meatus, where ventilation is probably restricted, especially at low flows. Thus, although some sinus NO does escape into the nose, this can not remove it as fast as it is generated. Therefore, it seemed probable that its output must be limited, possibly by a negative feedback mechanism, which appears to be the case. When the ostia were occluded, serial measurements of sinus NO showed that its concentration had reached a plateau of approximately 30,000 ppb after 5 to 10 minutes of accumulation, above which it did not rise.[83]

These findings strongly indicate that, although the NO production per square unit of mucosa is smaller, the majority of NO in the nasal passage originates from the nasal mucosa itself. The contribution from the nasopharynx appears to be minor,[102-104] which coincides with the termination of the Schneiderian ciliated mucosa.[105]

NO Production in the Nasal Mucosa

The data on NOS activity in healthy nasal mucosa are sparse. In specimens taken form patients undergoing septum surgery, the NOS activity detected was all cNOS,[106] whereas calcium-independent and steroid-insensitive iNOS was found in high concentrations in healthy sinus mucosa.[95,105] In samples from diseased inferior nasal turbinates, strong immunostaining for eNOS was localized to vascular endothelium, surface epithelium, and submucosal glands in all patients.[91] In the specimens from patients with chronic rhinitis, moderate immunostaining for iNOS was seen in surface epithelium, glandular, inflammatory, and vascular endothelial cells.

In polyposis, Ramis et al[106] found that nasal polyps contained higher levels of total NOS activity than nasal mucosa. Nasal polyps mainly contained iNOS activity, whereas in nasal mucosa, all NOS activity detected was in the constitutive form. In both, NOS activity was localized in the epithelial cells.

In healthy volunteers, Runer et al[107] increased nasal NO by spraying the NO donor sodium nitroprusside into the nose. Both mucosal blood flow and mucocilary beat frequency increased concurrently, suggesting that NO in high doses can alter these param-

eters. In vitro experiments on canine nasal sepal mucosa suggest that the release of NO from the nitroxidergic nerve endings is augmented by cold exposure.[108] Thus, NO-induced vasodilatation may contribute to the swelling of the nasal mucosa in cold conditions.[76,109] These findings suggest a possible role for eNOS and iNOS in the regulation of blood flow, nasal secretion, and ciliary movement in health[15,107] and disease.[91]

PRODUCTION, ABSORPTION, AND OUTPUT OF NO FROM THE AIRWAYS

The nasal airway protects the lower airway by humidifying, warming, and filtering the inspired air. The particular aerodynamic characteristics of the complex slit-like nasal airway results in maximum contact between the inspired air at the nasal mucosa. With this method, not only particles but also inhaled noxious gases are removed before they reach the delicate structures of the alveolar bed of the lungs. The efficiency of the removal of particles depends mainly on their size, whereas solubility and the concentration of gases will influence the removal of gaseous pollutants.[76]

As NO was until recently considered only a noxious air pollutant, it was a surprise to find concentrations as high as 20 to 30 ppm within healthy sinuses[84,110] or nasal cavities[111] after a few minutes of stagnation. However, the concentration of NO measured in the nose and sinuses reflects output and not production, and at high concentrations, there is some NO reabsorption by the nasal mucosa.[112] The velocity of the air stream will have a marked impact on the concentration of NO measured in the exhaled or aspirated gas. Presumably, in each segment of nasal mucosa, there are elements that can produce and absorb NO, and the nasal passages act as a linear integrator.[113] A portion may undergo chemical reaction, part may be absorbed by the blood flowing through the mucosa, and part probably goes into solution, and none of this activity can be measured with the usual techniques of nasal NO determination[112] and remains to be confirmed.

Exhaled NO output from the lungs appears to be influenced by the aspiration flow.[114] In the nose, flow itself probably does not alter NO production; it merely represents the rate at which the NO released from the nasal mucosa is removed.

AERODYNAMIC CONSIDERATIONS RELEVANT TO NASAL NO OUTPUT

Regardless of the measuring technique used, it is essential to record the NO concentration only when it has been ascertained that a steady-state plateau has been achieved. In several studies, nasal aspiration has been performed by using the built-in sampling flow of the analyzer, which is usually in the range from 0.2 to 0.7 L/min.[5,32,58,64,103,112,115-117] At such low rates, laminar flow regimen will prevent air penetration to the deeper parts of the nose. Local turbulence is essential for achieving maximum nasal NO output.[76,118] Aspiration flows below 1 L/min, fail to mimic the aerodynamics of nasal respiration, and consequently, the plateau achieved may not be representative of the maximum nasal NO output.[118] This situation is enhanced in the presence of nasal congestion when the narrowed peripheral parts of the nasal airway may remain unventilated and the ventilated parts may undergo dynamic collapse because of the Bernoulli effect. As a consequence, the obtained NO measurements are unreliable.

Consequently, aspiration flow must be individualized. For practical purposes, flows between 3.0 and 6.0 L/min,[118] a range more similar to the maximum physiologic flow rates (6 L/min at least), will ensure that a stable plateau is reached within 20 seconds in most subjects.[119]

Changes in Nasal Volume

Changes in nasal patency occur constantly and spontaneously as part of the nasal cycle as well as in response to changes in posture, body temperature, exercise, rhinitis, decongestion, and histamine challenge.[101] It has been reported that these changes in nasal volume and resistance influence the nasal NO output at low sampling flows.[120] This is probably because, at these flows, the air aspirated from the nose gains access to only some of its mucosa.[118] When physiologic aspiration flows are used in the range of 2 to 6 L/min, it is found that neither increases (saline) nor

decreases in volume (posture) alter the NO output.[121]

Likewise, with xylometazoline, the reduction of 10% to 15% in NO concentration found with this topical decongestant,[20,121-123] is not related to volume changes at physiologic aspiration flow rates.[121]

Exercise

Nasal respiration is the normal and preferred respiratory route during rest and sleep. Healthy subjects switch from nasal to oronasal breathing when the rate of ventilation quadruples, just as it may do in exercise.[76]

Exercise reduces resistance by vasoconstriction of the capacitance vessels, but the nasal blood flow remains unchanged.[124] NO output is diminished. In cases of allergic inflammation of the upper airways, the decongestant effect of exercise seems to be maintained.[125] This can not be explained by dilution of the nasal air by ventilation nor by changes in nasal volume.

Based on an observed concurrent reduction in nasal NO output and resistance, Imada et al[116] suggested that NO output might be involved in the control of nasal resistance, but the lack of correlation between unilateral NO output and unilateral nasal volumes makes this explanation seem unlikely. If the contact time between air and mucosa was the limiting factor, reduced resistance should result in lower tidal nasal flows and, therefore, in an increase in NO output rather than a decrease. It may be that the increase in volume accompanied by the reduced resistance, reduction in airflow velocity, and the local turbulence required to penetrate the more remote parts of the nasal passage are important factors in the explanation of this phenomenon.[118]

In the lower airways, constriction is a muscular phenomenon not—a vascular one. Asthmatic-exercise-induced—bronchoconstriction[126] might be related to NO physiology. Several studies have shown that NO concentration in exhaled air from the lower airways increases during exercise.[49,127-131] This increase in NO output is probably more closely related to increasing ventilation[49,129,132] than to the increased blood flow.[135] The lack of response to administration of NO in patients with pulmonary fibrosis has been attributed to loss of normal functional pulmonary capillary bed.[133] The finding that changes in pulmonary blood flow failed to alter the output of NO exhaled from the lungs at rest or during exercise reinforces this hypothesis.[134] Reduced absorption attributable to diminished contact time between air and mucosa during high flows is another suggested mechanism for NO changes during exercise.[129] A detailed discussion of exercise and the lower airways is outside the purview of this article.

NO IN RELATION TO NASAL PHYSIOLOGY AND UPPER AIRWAY PATHOLOGY

The nasal valves, acting as an anterior resistor, account for approximately half of the total airways resistance.[135] They have important aerodynamic functions in inspiration and expiration. In the former, the abrupt increase in nasal dimensions within the cavum and its complicated geometry dissipate the laminar airflow and promote turbulence. This enhances mucosa-to-air contact. This change in airflow characteristics protects the lower airways from particles, noxious gases, and extreme temperatures.[76,136,137] During death, the process is reversed to limit the heat and fluid loss, and nasal resistance supplements vocal cord adduction in providing a brake to the elastic recoil of the rib cage and lung tissue, thereby contributing to the improved oxygenation by allowing more time for gas exchange in the alveoli.[137]

During the brief air passage through the 6 to 9 cm long nasal airway, the concentration of NO increases by approximately 100 ppb, provided that the mean air flow rate is in the physiologic range of 6 L/min. After its return from the lung, the NO concentration is more than halved. The nose is a net producer of NO, whereas the lung is a consumer.

The Role of NO in the Nasal Cavities

NO accumulates physiologically during periods of nonventilation of the nasal cavity, just as it does during the nasal cycle, speech, swallowing, or mouth breathing. The subsequent resumption of nasal breathing will then result in the inhalation of nasal NO to the lower airways and lungs. This may have physiologic

effects in the lower airways, such as bronchodilation and regulation of the ventilation/perfusion relationship, or play a role in host defense. It is possible to speculate that one of the purposes of the nasal cycle may be to create an alternating high NO-concentration in the nasal passages. During nasal respiration, the majority of the airflow is through the most patent side, and it is on this side that bacteria, viruses, pollutants, and particulate matter will adhere to the thick inner secretions of the 2-layer fluid blanket.[76] When the cycle shifts, this side obstructs. This permits a local increase in NO concentration, potentially reaching levels sufficient to reduce bacterial growth, viral replication, and enhance mucociliary clearance.[12-15]

NO and the Paranasal Sinuses

Both the discovery of the very high concentration of NO inside the sinus and the high output of NO from the nasal airway have added a novel dimension to the physiology and function of the upper airways. The secluded sinus cavities communicate with the nasal airways only through narrow passages, thereby rendering drainage difficult. Therefore, it is surprising that nasal infections do not involve them more frequently. Perhaps the very high NO concentration found in the sinuses might be protective against microbial invasion through its enhancement of sinufugal ciliary action and its antibacterial and antiviral properties. The lowered NO concentrations associated with cystic fibrosis and nasal polyposis[138] may explain their greater susceptibility to infection. The significance of the low NO values found intranasally during an acute sinusitis as well as its increase after antibiotic therapy[58] remains to be evaluated.

Similarly, it may be speculated that adenoidal hypertrophy deprives the middle ear cleft of nasal NO, thus cutting it off from a possible important defensive mechanism. This may explain perhaps the reduction in the frequency of serous and acute otitis media after adenoidectomy.

The sinuses are major producers of NO, and it could be speculated that large antrostomies deprive them of the high NO concentrations needed to protect them against infection. Therefore, despite successful evaluation of fluid and pus and improved oxygenation, infection may persist.

Advantages of Nasal Breathing in Relation to NO

Autoinhalation of NO may represent an important physiologic advantage of nasal breathing compared with strict oral breathing. Lundberg et al[82] have shown that nasal breathing improves oxygen saturation compared with mouth breathing and infusion of nasal air into the tube of intubated patients improved their oxygen uptake.

Inhalation of nitric oxide in high concentrations (5-20 ppm) have been found to increase the blood oxygen tensions of premature infants with respiratory distress syndrome.[139,140] In mechanically ventilated children, removal of even low concentrations of occult NO in compressed hospital air used for ventilation (13-79 ppb) induced a significant and reversible decrease in $PaSO_2$.[141] Likewise, the reduced aerocrine function associated with nasal obstruction and diminished nasal ventilation will deprive the infant lung of the high levels of nasal NO that are present during early childhood and may be a contributory cause of sudden infant death syndrome. Pulmonary hypertension associated with low levels of NO[52] has been observed in children and adults with severe, long-standing upper airway obstruction during sleep, and relief of severe nasal obstruction by external nasal dilation in heavy adult snorers improves the $PaSO_2$ significantly.[83] Clearly, an increasing body of evidence suggests that nasal NO may have beneficial effects on pulmonary function.

MEASUREMENT OF NASAL NO

Current knowledge suggests that the measurement of exhaled NO and/or some other gases such as CO,[142] ethane, and penthane[143] may have value as diagnostic tools for monitoring the cause of disease and in evaluating the impact of treatment. It is already used for the latter in asthma and rhinitis. Furthermore, the measurement of these gases from the airways may become important in assessing the effect of indoor and outdoor exposure to pollutants.

Because NO concentration is flow-dependent, it is essential to measure it at a known and fixed flow rate.[114] By reporting the product of the NO concentration and the flow, ie, the NO rate of output,[116] measurements at different flows become comparable, provided the aspiration flow is within a flow range that gives maximal and stable NO outputs.[119]

Methods for Direct Measurement of Nasal NO (Online)

The dual nasal passage with openings both anterior and posterior to the nasopharynx provides opportunities for a variety of different methods of NO collection. These have been described elsewhere with both their advantages and disadvantages.[144] Their salient features will be discussed below.

Bilateral Nasal NO Measurements

Contamination or dilution from the oral cavity and lower airways during nasal NO collection can be avoided by closure of the nasopharyngeal velum. A positive pressure of 5 to 10 cmH_2O is created within the oral cavity by blowing against a closed mouth or a resistor (Fig 2),[114] although some patients can learn to close their velum voluntarily while mouth breathing. During NO measurements, one problem is to know if and when air from the lower airways has been permitted to enter the nasal airstream. The presence of CO_2 can be used, or the precipitation of water vapor on the walls of a small piece of transparent tubing placed in the contralateral nostril during aspiration also gives a good indication.[119]

Aspiration Through Nasal Airways in Series

NO can be easily collected by aspiration at a fixed flow through the nasal passages in series (Fig 2). In nasally congested patients, suction may induce alar collapse, and this impedes measurements at high flows. This complication can usually be avoided by use of an external dilator of by introducing a nozzle in the contralateral nostril to expand and stabilize the valve area.[119] The aspiration technique can be used successfully in most adults and has been performed in children as young as 6 years of age.[119]

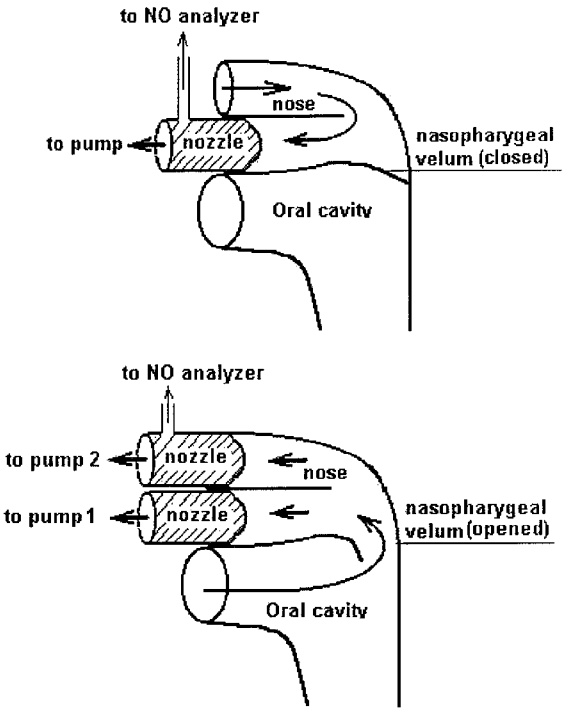

Fig 2. Illustration showing the principles of bilateral nasal NO measurement with nasal aspiration from nasal passages in series with closed velum (upper panel) and unilateral nasal NO measurement with dual pump system (lower panel).

Alternatively, room air or NO-free air at a constant flow can be insufflated through the nasal passages in series while velum closure is secured by exhalation against a resistor. NO is sampled from a tube that is connected to an olive introduced into the other nostril, on the exit side of the system.[114,121,144] This technique increases the risk of velum opening and is only recommended if suction can not be achieved.

Other Methods for Bilateral NO Measurements

When the suction or insufflation techniques can not be performed successfully, alternative methods of bilateral nasal NO output measurement should be used.

NO measurement with a nasal exhalation maneuver against a resistor using a fixed flow (nasal flow in parallel) incurs several problems. First, the nasal NO output will be contaminated, or rather diluted, by the NO content in the lower airways.[144] Although used in

many studies, it is still a matter of debate if simple subtraction of the orally exhaled NO output from the nasally exhaled NO output is valid, and it requires an additional measurement. Of course, the same is true when subtracting the ambient NO concentration from nasal NO readings.

Several studies have addressed the possible interference by high environmental concentrations of inhaled NO on the exhaled levels. Most data refer to the lung.[145-147] The situation in the nose is different as long as ambient NO is relatively low (<150 ppb) because the nasal NO output is much higher. The problem can be easily solved by using a NO scrubber or feeding NO-free air into the contralateral nostril.

Secondly, the partition of the exhaled flow through each of the nasal passages is not known. Hence, in cases of asymmetrical nasal resistance attributable to cycling changes or unilateral pathology, the uneven flow distribution may avoid penetration of airflow to all segments on the less-ventilated side. This may result in an underestimated actual nasal NO output.[118]

Another possible method of obtaining NO for measurements is to aspirate nasal air at a constant flow via the oral cavity with the glottis closed. The built-in pump of the analyzer generally delivers suction flow too low for reliable readings and it is best connected as a side-arm to a stronger pump.[144]

Unilateral Nasal NO Measurements

Unilateral measurements can be of interest when addressing unilateral physiology and pathology.[59,148,144] Some investigators have attempted to sample NO unilaterally by suction through one nostril via the mouth with the contralateral nostril left either open or closed.[59] Although simple and attractive from a practical point of view, this method risks overestimate the unilateral NO output because of the potential contamination of NO from the contralateral nasal airway. NO accumulates very rapidly in the nasal passages,[111] and the high concentration in the contralateral passage will inevitably diffuse to the nasopharynx and mix with the air that is aspirated through the other nostril.

Dual Aspiration System

To avoid this source of error, we have introduced a dual aspiration method.[148] Dual suction is provided by 2 pumps or alternatively by one strong suction source (wall suction), with 2 independent rotameters (Fig 2). Hence, the flow through each nasal cavity is known and can be adjusted individually so that the maximum NO output is obtained from both sides at the same time. The air is aspirated through the nasal airways in parallel and the unilateral nasal NO determined. NO is detected from one side at each measurement (provided 2 analyzers are not available). By using this method, it has been shown that unilateral nasal NO outputs were similar in both sides in normal adults and is half the NO output of the 2 sides measured in series.[148] Thus, the technique has clinical and research potential.

METHODS FOR REMOTE COLLECTION AND DELAYED ANALYSIS OF NO (OFFLINE)

NO can be passed through the analyzer as soon as it has been extracted from the nose or stored before analysis. The latter presents 2 problems. A suitable container must be used to store the gas. This must neither allow NO to escape nor permit other gases to diffuse in. Secondly, the NO sampling rate remains as critical as ever. The equipment used for direct online measurements of NO in air exhaled is technically advanced, expensive, and requires maintenance from skilled personnel. Therefore, transporting it to the patient is neither practical nor advisable.

These points led to the search of a simple and reproducible way of sampling NO and other gases in remote locations for later analysis.[149]

Several technical issues have to be addressed, such as the properties of the storage material for the transportation of the gas to the analyzer, the size of sample needed given the response time of the analyzer and the lag period necessary to reach a reliable steadstate, the need to control the rate of the flow at the time of collection, and the necessary of eliminating the dead space.[33,149] The solubility of NO and its reaction with other gases and

materials limits the collection technique and choice of storage reservoir materials.[33]

Recently, a new method of solving these problems has shown excellent agreement between direct online NO measurements and offline measurements. This method allows remote collection of NO and other exhaled or aspirated gases from both upper and lower airways, provided the collection reservoir is adapted to the properties of the gas in question.

CONCLUSIONS

The discovery of higher NO concentrations in the sinuses and the nasal airway than anywhere else in the body suggests an important role for this gas in the local immune defense. Within the nose and sinuses, its bactericidal and virocidal activity supplement its augmentation of ciliary beat frequency and protect against some infections. Its tumoricidal action may account for the low incidence of malignancy observed in the Schneiderian muciosa. Nasal NO is transported by the inspired air to the lung, where it plays an important part in matching blood flow to ventilation. Because of this, NO is increasingly added to the inspired air in patients who can not breathe nasally because of intubation or tracheotomy, as in the intensive care unit or operating room. These benefits provide the best evidence to date for considering the nose as the preferred and natural airway in humans.

Clinically, changes in airway NO can serve as an indication of inflammation, an index of disease progression, or an indication of therapeutic efficacy. The degree and direction of change may also have potential as a diagnostic marker. These functions are rendered more valuable by the simplicity and noninvasive nature of its measurement.

NO, similar to several other inflammatory mediators, probably subserves different functions as local conditions dictate. This fascinating aspect of its pathophysiology is still tantalizingly elusive.

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