Nitric Oxide in Health and Disease from the Point of View of the Otorhinolaryngologist

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Abstract: Nitric oxide (NO) plays role in a great range of important functions in the organism, such as vasodilatation, relaxation of muscles, neurotransmission, neuromediation, and host defense reactions. In the upper airways, nasal cavities and paranasal sinuses are the main sources of this biological mediator. Although the exact role of NO in nasal physiology remains poorly understood, the functions are thought to be host defense, ciliary motility and improved ventilation–perfusion ratio in the lungs by auto-inhalation. Low NO concentrations were reported in certain diseases such as primary ciliary dyskinesia, cystic fibrosis, and acute and chronic maxillary sinusitis whereas high concentrations were detected in upper airway infection, allergic rhinitis and nasal polyposis. Additionally this ubiquitous radical is being implicated in the regulation of cochlear blood flow, sensorineural hearing loss, middle ear effusions, and outer hair cell and vestibular functions. Solid tumors is another area where NO appears to have both tumor-promoting and tumor-inhibiting effects.

The presence of NO with high levels within the nose and paranasal sinuses makes it reasonable to believe that this pluripotent gas is involved in a variety of physiological as well as pathophysiological events in the airways. Although NO has an ever-increasing role in various areas related to the practice of otolaryngology, further research is required to understand fully the role of NO in the upper airways.

Key Words: Nitric oxide, nose, ear, paranasal sinuses, head and neck cancer, and otorhinolaryngology.

INTRODUCTION

Nitric oxide (NO), a biological mediator, exhibits a great range of important functions in the organism. It is synthesized from the substrate L-arginine by the catalyzed reaction by NO-synthases (NOS) leading to the generation of NO and L-citrulline [1, 2]. It plays role in vasodilatation, relaxation of muscles, neurotransmission, neuromediation, and host defense reactions against bacteria and fungi [1, 2]. Three isoforms of NOS have been identified: neuronal NOS (nNOS or NOS-I) is expressed constitutively by neurons in the brain and enteric nervous system, whereas endothelial NOS (eNOS or NOS-III) exhibits constitutive expression that is confined to the endothelial cells lining the vasculature; the third isoform is inducible NOS (iNOS or NOS-II) is expressed only in response to certain inflammatory stimuli such as bacteria products, cytokines and lipid mediators [3-5]. Once induced, iNOS can produce large amounts of NO for prolonged periods. Although this profuse NO production is an integral part of the host defense mechanism, sustained release can also be harmful to healthy tissue in certain situations [3, 6, 7]. That means excess or diminished NO production can have detrimental effects in physiological or pathological processes. It has been suggested that NO, produced in large amounts by iNOS, is a toxic agent whereas eNOS is seen as a protective enzyme. This paradoxical role of NO led many efforts to understand the role of NO and its synthases in the pathogenesis of a variety of diseases [8].

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Although it had been generally accepted that NO is solely generated in biological tissues by specific NOS, NO can also be generated in tissues by either direct disproportionation or reduction of nitrite to NO under the acidic and highly reduced conditions which occur in disease states, such as ischemia [9]. It was observed that the generation and accumulation of NO from typical nitrite concentrations found in biological tissues increases 100-fold when the pH falls from 7.4 to 5.5 [9]. Non-enzymatic NO production was also demonstrated in the stomach following reduction of salivary-derived nitrite [10]. Intragastric NO production is initiated in the oral cavity by the reduction of nitrate to nitrite with the contribution of some bacteria [10].

DONORS AND ANTAGONISTS

Because of the limited utility of authentic NO gas in many experimental systems and the short half-life of NO *in vivo*, compounds that have the capacity to release NO have been widely used as therapeutic agents and as pharmacological tools to investigate the role of NO in physiological and pathophysiological conditions [11]. Direct NO donors (NO gas, sodium nitroprusside, and sodium trioxodinitrate) are pharmacological agents with either a nitroso or nitrosyl functional group. In contrast to organic nitrates, which require metabolism for activity, these agents spontaneously release NO. Donors requiring metabolism (organic nitrate and nitrite esters) are prodrugs requiring enzymatic metabolism to generate bioactive NO [11].

Compounds able to prevent the biological activity of NO can be grouped into six main categories according to their mechanism of action [12]. These include drugs preventing

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uptake of L-arginine into cells, thus denying NOS its substrate; agents that reduce the supply of cofactors required for NOS-catalyzed oxidation of L-arginine; inhibitors of electron flow via NADPH/flavins and agents interfering with the heme moiety; inhibitors of the expression of NOS; drugs preventing the binding of substrate to NOS; and scavengers of NO [12]. The first described NOS inhibitor, NG-monomethyl-L-arginine (L-NMMA), has been used to identify many of the physiological actions of NO and to investigate its role in some pathophysiological processes [12].

NO MEASUREMENT METHODS

NO can be measured directly or indirectly. Indirect methods have been used to measure NO levels *in vivo* in the fluid phase where it is very short-lived. These include the measurement of nitrate and nitrite, which are the stable end-products of NO metabolism, or the use of immunohistochemical techniques to localize NOS. Alternatively, it is possible to examine the effects of inhibiting NOS through any of these mechanisms [13]. In contrast to the NO produced within the tissues, gaseous NO (a radical) is more stable, the reason for which is, it is easier to measure in the airways by a technique called chemiluminescence [14]. Other techniques that have been used to establish that NO is present in the exhaled breath of humans include mass spectroscopy and gas chromatography-mass spectroscopy [15, 16].

There is no standardized technique for measuring nasal NO and several different methods have been used. There are two main approaches in nasal NO assessment: direct sampling from the nose with an air stream generated by the analyzer whilst mouth breathing or breath holding or use of NO-free air stream infused into one nostril at a certain flow rate and exited from the other nostril where it is analyzed during mouth breathing or breath holding [15, 17, 18]. The commonest method in use is that of direct nasal aspiration using the NO analyzer pump [13, 19]. Appropriate *in vivo* measurement of nasal and sinus NO is still controversial. The measurement is complicated by many factors such as the production of NO by lower airways and NO partly passing into the oropharynx during mouth breathing [20]. The European Respiratory Society Task Force's 'Measurement of NO in Exhaled Air' [14] has proposed recommended measurement guidelines for both nasal and exhaled NO. It proposes sampling directly from the nose whilst the patient holds their breath in full inspiration (which results in soft palate closure). Silkoff *et al.* [19] have compared NO measurement methods and recommended sampling fixed nasal flow (nasal exhalation against resistance resulting in a flow rate of 100 ml/s) in preference to direct nasal sampling. Recently two protocols for reliable NO measurement in nasal research have been reported [20]. In the mentioned study, the authors emphasized that the nasal NO measurement could not be reliably achieved during mouth breathing and nasal decongestants reduced data error. They also concluded that with adequate data analysis equipment, peak levels within the nasal cavity and maxillary sinus could be optimally assessed after decongestion and during breath holding [20].

Recently, it was also demonstrated that oscillating airflow produced by humming enhanced sinus ventilation and increased nasal NO levels 15-fold when compared with quiet exhalation [21]. Thus, nasal NO measurement during humming was proposed as a useful noninvasive test of sinus NO production and ostial patency [21].

While interpreting the results of NO measurement, some factors should be considered: The nasal NO concentration is different in newborns, children and adults [16]. The nasal NO concentration calculated in relation to body weight revealed that the concentration in children around 10 years of age was twice as high as that in newborn infants and adults [16]. While gender has no effect on nasal NO [16], acute physical exercise causes reduction in NO output [16, 22]. Nasal NO levels have been reported to be lower in cigarette smokers increasing one week after cessation [16, 23].

SOURCE OF NO IN UPPER AIRWAYS

This small molecule is produced in many systems including the upper and lower airways [15, 17]. The presence of NO in exhaled breath of humans was first demonstrated by Gustafsson *et al.* [15] in 1991. In the respiratory tract, NO is formed and released by various sources including endothelial and epithelial cells, nerves, airway smooth muscle, and inflammatory cells [24]. Of the three isoforms of NOS, it is not clear which is responsible for baseline levels of exhaled NO [25]. Steudel and coworkers [26] studied mice with a congenital absence of each isoform. NOS-I-deficient mice exhaled 64% more, NOS-II-deficient mice exhaled 9% less, and NOS-III-deficient mice exhaled more than twice the amount. The authors concluded that NOS-II was important for baseline levels of exhaled nitric oxide [26]. By immunohistochemistry, both constitutive (NOS-III) and inducible (NOS-II) forms of NOS have been identified in the nasal mucosa [27]. Further studies, in which in situ hybridization was used, found that type III NOS mRNA was present in endothelium, surface epithelium, and glands, whereas type II NOS was largely found in inflammatory cells [27].

NO concentration in the upper respiratory tract is higher than NO level in the lower part [28]. Nasal cavities, nasopharynx and paranasal sinuses excrete this ubiquitous multifunctional biological mediator [29]. Although Lundberg *et al.* [30], who punctured maxillary sinuses and found continuous production of NO, suggested that the main origin of NO in the airways was the paranasal sinuses, the debate is still open as to which degree the sinuses contribute to nasal NO [16, 31]. In a volunteer, it was demonstrated that with the occlusion of osteomeatal complex and sphenoethmoidal recess, NO concentration decreased by 12% [31]. This finding meant that only about 12% of nasal NO was derived from the sinuses; the rest was produced within the nose itself, thus suggested the exact origin of NO as the nose [31].

The enzymes for the production of NO are present in both the nose and the sinuses; although the enzyme concentrations are higher in the sinuses, their net NO output is comparable to that of the nose because of the much larger nasal surface area [31]. The concentration of NO, on the other hand, is much higher in the sinuses because of their poor ventilation. The NO, produced in the sinuses, tends to stay there and accumulate; a very little amount escapes into the nose [31].

Human saliva contains nitrate that is converted into nitrite by the activity of facultative, anaerobic bacteria of the oral cavity such as *S. salivarius, S. mitis* and *S. bovis* [32].

Nitrite can be reduced to NO by bacteria at neutral pH values and may contribute to the antibacterial activity of saliva [32]. However some NO may also form in the mouth at acidic pH values [32]. Bacteria, which are able to denitrify nitrate to nitrite and then to gaseous products, including NO, possess a nitric oxide reductase pathway, which converts nitric oxide to nitrous oxide (N_2O) and ultimately on to nitrogen [33].

FUNCTIONS OF NASAL NO

Although the exact role of NO in nasal physiology remains poorly understood, the functions of nasal NO are thought to be host defense [30], ciliary motility [34] and improved ventilation–perfusion ratio in the lungs by auto-inhalation [35-37]. It is also thought to be an aerocrine messenger between the upper and lower airways [28].

NO is involved in numerous physiological functions, including vasodilatation, bacteriostatic and fungistatic functions, cell-mediated immunity against neoplasms [4] and neurotransmission [38]. Each of these functions has relevance to the nasal cavity, but none has been linked directly to the high levels of NO found in the nasal passages. Although NO is a potent vasodilatator, nasal NO does not seem to influence the congestion state of the nasal mucosa [39-41]. Conversely, studies suggest that the congestion state of the nasal mucosa influences the nasal NO concentration [39-41]. Almost all vasoconstrictors induce a reduction in nasal NO concentration, but varying nasal NO concentration does not change nasal congestion [38, 41-43]. However, Holden *et al.* [38] found that inhibition of NO release was associated with cooling of nasal air. This finding suggested a potential role for NO in modulation of the vascular changes necessary for temperature conditioning of nasal air and, possibly by extension, thermoregulation in humans [38].

It was shown that NO substrate L-arginine increased ciliary beat frequency *in vitro*, and the NO donor, sodium nitroprusside (SNP), increased mucociliary activity *in vivo* in the maxillary sinus of the rabbit [15, 44]. Runer *et al.* [44] found that mucociliary activity in the nasal septum increased with a short latency after challenges with SNP, possibly even faster than the increase in blood flow. This meant that there was no unequivocal relationship between the effect of NO on the mucociliary system and its effect on the smooth muscle of mucosal blood vessels [36]. NOS inhibitor, L-NNMA, was shown to reduce the increased ciliary beat frequency, which was then eliminated by SNP and by L-arginine [44, 45]. These studies suggest that NO acts as a regulator of mucociliary function in the nasal airway. .

Some *in vitro* studies suggested NO to mediate the effects of mediators or transmitters such as acetylcholine, bradykinin, and endothelin [45-47]. It was also shown that inflammatory mediators can upregulate mucociliary activity through a pathway mediated by NO [45].

PRIMARY CILIARY DYSKINESIA

After Lundberg *et al.* [48] reported three children suffering from a genetic disturbance of mucociliary function having very low NO concentrations in their exhaled air, it was demonstrated that arginine, a precursor of NO synthesis, increased nasal NO and improved ciliary beat frequency and nasal mucociliary clearance in 10 patients with primary ciliary dyskinesia (PCD) [49]. Although Balfour-Lynn *et al.* [50] reported lower nasal NO in children with cystic fibrosis (CF), in a recent study investigating the role of nasal NO measurement as a screening test for PCD showed that nasal NO was significantly lower in PCD (64.0 ± 36.6 ppb) compared with normal controls (759 ± 145.8 ppb), idiopathic bronchiectasis (734 ± 163.7 ppb), CF (447.5 ± 162.6 ppb), sinusitis (1487 ± 734 ppb) and Young's syndrome (644 ± 129.9 ppb) [51]. As there was no overlap of nasal measurements between PCD and other disease controls, authors concluded that NO might used clinically in various specialities to screen suspected patients for PCD [51].

All these data and the study demonstrating that the inhalation of nebulized SNP in human volunteers increased nasal mucociliary activity *in vivo* by 57% [44] suggest the protective role of NO in the airways and possible therapeutic role of NO precursors or donors in otolaryngology.

UPPER RESPIRATORY TRACT INFECTION

Pathogenic organisms have been shown to induce NOS in the respiratory tract [13, 52]. In a study performed in patients with acute upper respiratory tract infection, the amount of NO in exhaled air was found higher than an age and sex-matched control group [53]. However there are also controversial data in the literature [54].

ALLERGIC RHINITIS

Studies performed to assess the nasal NO levels in allergic rhinitis revealed controversial results. While some of them showed an increase [42, 55, 56], others showed no difference between NO levels of patients and controls [36, 57, 58]. Palm *et al.* [59] revealed no difference in nasal but higher orally exhaled NO with a larger interindividual variation in patients with allergic rhinitis. They interpreted this finding with the probable occlusion of sinus ostia with secretions or mucosal swelling. They supported their interpretation with the finding that nasal NO was decreased in healthy subjects by topical decongestants, however the same drug caused increase in NO in patients with symptomatic allergic rhinitis [39].

Although several studies showed that in patients with allergic rhinitis treated with topical nasal glucocorticoids, the nasal NO was significantly lower compared with the untreated group [42, 60], decrease in nasal NO in response to topical nasal steroids is also controversial [36, 42, 48, 57, 60]. The lower NO concentration was attributed to steroid inhibition of iNOS, and thus diminished NO production.

With the use of immunohistochemical techniques, a significant positive correlation between the degree of inflammation in specimens and the cellular expression of iNOS was shown in patients with chronic rhinitis. Patients with allergic rhinitis had more marked staining for this enzyme compared with controls [27, 61].

The presence of a direct correlation between nasal NO and symptom score is controversial, as well [42, 59, 62].

Vural and Gungor [63] measured daily NO output in an individual with allergic rhinitis over a period of $5^{1/2}$ months to determine the variability from day to day. Nasal NO levels were detected higher compared to healthy adult values.

During the study the subject had a short period of a cold and a short period of purulent rhinitis. The highest value throughout the study was noted in the prodromal period of the cold and a significant decrease (the lowest values) during the cold. Change during the purulent rhinitis was less remarkable than change during the cold. No correlations between environmental conditions (humidity, atmospheric pressure, pollen count) and NO levels were found. NO changes were also independent of specific and cumulative nasal symptom scores [63, 64].

NO modulates leukotrien B4 (LTB4) induced neutrophil recruitment and fluid secretion in the nose [65]. Although hypersecretion of nasal fluids is one of the cardinal symptoms of allergic rhinitis, rhinorrhea should not be viewed solely as a pathological condition. Continuous nasal secretion plays an important role in the protection of the nasal and respiratory mucosa, and assists in the elimination of materials filtered from inhaled air. The role of neutrophils in chronic sinusitis is also well established; further elucidation of the role of NO in relation to neutrophil-dependent secretion may lead to novel pharmacotherapeutic approaches to the treatment of both allergic rhinitis and chronic sinusitis [65]

Although exhaled NO has been proposed as a useful noninvasive marker of lower airway inflammation [42, 59] whether or not it can be clinically useful in monitoring rhinitis is unclear.

NASAL POLYPOSIS

The commonest form of nasal polyposis is inflammatory in nature with many eosinophils, mast cells and lymphocytes in a stroma covered by an epithelium, which stains heavily for iNOS [66-68]. Nasal polyps have been shown to have higher NOS activity compared with nasal mucosa from controls and this is mainly the inducible subtype [68]. In aspirin-sensitive nasal polyposis, Parikh *et al.* [69] also found that iNOS was raised. In a large series of patients, nasal NO levels were found lower in patients with polyps than those found in uncomplicated allergic rhinitis. In the mentioned study low levels of NO despite high iNOS levels were attributed to the blockage of the osteomeatal complex and failure of NO generated constitutively in the sinuses to reach the nasal airway. The authors of the study proposed the following scenario: Nasal NO levels are the result of two processes: inducible NO production by inflamed nasal mucosa plus constitutive sinus production, detectable in normals. In uncomplicated allergic rhinitis with patent sinus ostia NO levels tend to be elevated but when inflammation is sufficient to obstruct sinus ostia (as in nasal polyps) NO levels fall because sinus NO makes the major contribution [66]. Because regionally produced NO can be considered as one of the first-line host defenses of the respiratory tract against inhaled microorganisms [70], it would not be wrong to speculate that low level of NO seen with nasal polyposis predispose frequent sinus infections.

FUNCTIONS OF PARANASAL SINUS NO

The debate as to which degree the sinuses contribute to nasal NO was mentioned above. It was also mentioned that NO substrate L-arginine increased ciliary beat frequency *in vitro*, and the NO donor, SNP, increased mucociliary activity *in vivo* in the maxillary sinus of the rabbit [15, 44]. It is generally accepted that high NO in the sinuses has an important role in keeping these areas clean as will be discussed below.

MAXILLARY SINUSITIS

The source of NO in sinuses is the apical part of the sinus epithelial cell [13]. Both in acute and chronic sinusitis, reduced NO levels were reported [71, 72]. Deja *et al.* [71] also provided evidence that iNOS was normally present within cilia and microvilli of the maxillary sinus epithelium. Baraldi *et al.* [72] demonstrated that in patients with acute sinusitis, nasal NO levels increased when the patients improved following treatment with antibiotics. Despite low NO levels detected in acute and chronic sinusitis, it is still uncertain whether these low levels are a consequence of reduced maxillary NO production or are caused mainly by an obstruction of the sinus ostia by local edema, nasal congestion, and mucus accumulation within the sinus cavities. The swelling of the nasal mucosa during rhinitis might also lead to partial blockage of the sinus ostia, which would result in reduced passage of sinus NO to the nasal cavity where it is measured [16]. The latter was supported by the results of studies performed in patients with nasal polyposis, in whom the nasally measured NO concentration was inversely correlated with the number of occluded paranasal sinuses [72]. However the first was supported by the demonstration of messenger ribonucleic acids for the iNOS-suppressing cytokines in maxillary sinus mucosa of patients with chronic sinusitis [73].

In a recent study, Lundberg *et al.* [74] showed that during quiet exhalation, mean output of nasal NO was similar in healthy adults and patients with sinusitis and bilateral polyps. In the same study nasal NO increased 7-fold during humming in controls but remained completely unchanged in the patients [74]. These results suggested that humming caused the air to oscillate which in turn greatly accelerated the exchange of air between sinuses and the nasal cavity in healthy controls; the lack of an air passage between the sinuses and nasal cavity in patients with sinusitis and bilateral polyps caused unchanged nasal NO [74].

Recently we demonstrated that in experimentally induced acute sinusitis, sinus NO level was significantly lower compared to controls [75]. While the most severe inflammatory changes were found in untreated sinusitis group, SNP administration hastened the bacteriological and histological recovery. Milder histopathological findings observed in SNP-administered rabbits compared to untreated ones and decrement in the severity of findings earlier than that of untreated rabbits suggested the inhibitor effect of SNP on inflammation in sinuses [75].

It is now accepted that the high NO concentrations in the sinuses probably play an important part in keeping these areas free of disease and infection. NO in airways and sinuses may be bacteriostatic or bactericidal and reduce colonization and infection. Some authors think that primary role of nasal NO is probably to improve ventilation-perfusion matching in the lung, so its concentrations are kept low [13]. In the sinuses, poor ventilation causes NO to build

up to toxic levels, and this plays a part in keeping these stagnant areas clean. NO level positively influences mucociliary function [13].

SLEEP APNEA

Because of the link between sleep apnea and cardiovascular disease, Ip and coworkers [76] investigated circulating NO of 30 patients with sleep apnea and found that serum NO was correlated with apnea-hypopnea index, oxygen desaturation time, and systolic blood pressure. One night of nasal CPAP (continuous positive air pressure) produced a 2.7-fold increase in serum NO in 22 patients. These data suggested a role for NO as a mediator of the cardiovascular abnormalities in patients with sleep apnea.

EAR

This ubiquitous radical is being implicated in the regulation of cochlear blood flow [77], sensorineural hearing loss from bacterial meningitis [78], and in middle ear effusions [77]. It also plays role in outer hair cell [79] and vestibular functions [80].

MIDDLE EAR

Otitis Media (OM)

Inflammatory mediators found in middle ear effusions, including tumor necrosis factor alpha (TNF^{α}), interleukin-8 (IL-8), interleukin-6 (IL-6) and NO have been reported to increase mucus production in cell lines and primary tissue cultures [81, 82]. Both *in vitro* and *in vivo* animal studies have shown that mucin production is increased by NO donation [81, 83], and the increase in mucin production caused by addition of cytokines is blocked by addition of NOS inhibitors [81]. Further evidence for the role of NO as an inflammatory mediator in OM is provided by the presence of NO metabolites in middle ear effusions [81]. Modulation of the pathways involved in NO stimulation of mucin production could offer potential therapeutic options for OM.

It has been shown that mRNA encoding NOS is produced in the middle ear during OM [84, 85]. In an experimental study it was demonstrated that inhibition of NOS resulted in significantly increased middle ear effusion [84]. This result suggested that NO is involved in regulating the permeability of the middle ear vascular, the transudation of serum into middle ear mucosa, and the movement of extracellular fluid across the middle ear mucosal epithelium

In a human study the higher levels of nitrates and nitrites were noted in mucoid OM, followed by serous OM and purulent OM [86]. It is known that most children with a persistent effusion after treatment of acute OM develop mucoid OM, and that mucoid OM represents the most advanced disease state in the spectrum of OM. Highest NO level in mucoid OM also suggests that NO may mediate mucin secretion in the pathogenesis of mucoid OM.

A study performed in the human and rat tympanosclerotic middle ear revealed that iNOS is detected more frequently in the cases with tympanosclerosis, that is a later phase of the disease, compared to those with secretory OM, the early phase [87]. Topical administration of N-acetylcysteine (NAC) to perforated rat tympanic membrane reduced the levels of NO products and caused fewer occurrences of sclerotic plaques compared to controls [88, 89].

Sensorineural hearing loss is associated with many causes such as increasing age, OM with effusion, chronic OM and bacterial meningitis [90]. The pathophysiologic mechanism of sensorineural hearing loss in OM is not completely understood. It has been suggested that sensorineural hearing loss in OM is caused by toxic substances, such as NO present in the middle ear effusion [90]. Jung *et al.* [91] demonstrated that exposure to NO caused irreversible morphologic changes in isolated cochlear outer hair cells, suggesting possible involvement of NO radical in the development of sensorineural hearing loss as a sequel of chronic OM. Furthermore, the lipopolysaccharide-induced production of mucin and mucous cell hyperplasia was inhibited in ears treated with lipopolysaccharide and L-N-nitro-arginine-methyl ester (L-NAME) [92]. All these results suggest that NO is a mediator in the pathway of mucin secretion in chronic OM with effusion.

INNER EAR

Cochlear Functions-Pathophysiology

NO/cGMP pathway is involved in both the ascending and descending pathways of the auditory brainstem [93]. The most compelling physiological evidence supports a role for the cochlear NO/cyclic GMP pathway in the regulation of cochlear blood flow [94]. In the organ of Corti, overstimulation of glutamate receptors as well as ischemia both degenerate the afferent connections to the inner hair cells resulting in an elevation in the threshold of the compound action potential (CAP) of the auditory nerve [95]. NO is thought to be responsible for the observed pathology [94]. This idea was supported by the observations that the afferent connections to the inner hair cells are susceptible to toxicity elicited by high concentrations of the NO donor, SNP [96]. Alternatively, it has been suggested that overstimulation of soluble guanylate cyclase may mediate NO-induced cochlear toxicity [97]. Amaee *et al.* [78] demonstrated a protective effect of N^{G}-methyl-L-arginine, an inhibitor of NO release, on cochlear injury. Although NO appears to be important in the formation of middle ear effusions and can cause cochlear injury, the effect on the inner ear of NO passing from the middle ear space has yet to be documented. The findings of Hanson *et al.* [90] supported that NO, when applied to the round window membrane, passes through it into the perilymph and induces significant changes in cochlear function. The unilateral auditory threshold loss found in the mentioned study suggested the involvement of the NO radical as a local cytotoxic agent in the pathogenesis of sensorineural hearing loss associated with OM [90].

Most studies investigating toxic effects of NO on cochlear potentials [79, 96-99] showed a significant decrease in cochlear potentials during application on the round window membrane or with intracochlear perfusion. Demonstration of the rapid reduction of endocochlear potential accompanied by a large increase in cochlear blood flow with SNP implies that SNP has a direct toxic effect on function of the stria vascularis [96]. Ruan *et al.* [98] saw morphological damage in the inner and outer hair cells with augmentation

of the concentration of SNP. In contrast to these data, Gosepath *et al.* [100] found no alteration in cochlear potentials with the use of NOS inhibitor L-NAME and the NO donor SNAP. Since they found an increase in NOS III immunoreactivity in the apical part of outer hair cells and Deiter's cells after intravenous application of the NO donor SNAP, they concluded that the main part of the NO metabolism happened in this area. Furthermore, they speculated that one role of NO in the cochlea was the participation in the transformation of signals and to lesser extent their transduction [100]. Dais *et al.* [97] demonstrated that high NO concentrations raised hearing thresholds.

Impaired of cochlear blood flow has been thought to contribute to certain forms of sudden hearing loss as well as other inner ear disorders, including tinnitus, presbyacusis and Meniere's disease because NO contributes to the regulation of cochlear blood flow [77, 101, 102]. SNP has been shown to increase cochlear blood flow in a dose dependent manner [103], and in normal [101, 104] and presbyacusic mice [105].

Although evidence suggests that as an inflammatory mediator, NO increases middle ear vascular permeability and mucin secretion in endotoxin-induced OM with effusion, the exact role of NO, in the pathogenesis of middle ear inflammation and in cochlear injury is still unknown.

VESTIBULAR FUNCTIONS-PATHOPHYSIOLOGY

In the rat ear NOS has been identified both in the vestibular organs and the cochlea [80, 106, 107]. It has been suggested that NO mediates neurotransmission in the ear sensory cells and ganglia [80, 106, 107]. In the stria vascularis NO may play an important role in maintaining endolymph and ion homeostasis [106]. In the vestibular organ, NO is synthesized by eNOS and nNOS, which are localized in sensory cells, ganglion cells, vestibular fluid transporting cells, and epithelial cells of the endolymphatic sac [7]. Inducible NOS has not been detected in the healthy vestibular organs [7]. In 1998, Takumida *et al.* [7] could induce formation of iNOS by inoculating bacterial lipopolysaccharide into the vestibular organ of the guinea pigs. They stated that NO was a mediator of LPS-induced morphological and functional disturbances in the vestibule and that iNOS is critical to the development of LPS-induced vestibular disorders, as the vestibular sensory cells expressed iNOS immunoreactivity in their cytoplasm following inoculation with LPS. From that point of view, it can be speculated that large amounts of NO from iNOS in the vestibular fluid-transporting cells for prolonged periods have a toxic effect on the function of these cells, with consequent functional and morphological changes. Whether iNOS production by vestibular fluid transporting cells affects vestibular vascular tone, fluid absorption, or resistance to infection remains to be determined [7]. Speculatively NO generation, mainly from iNOS, may contribute to the pathophysiology of other inner ear diseases, such as Meniere's disease [108-110]. These results suggested that inner ear sensory, fluid transporting and epithelial cells and macrophages might be an important source of NO initiating inflammation, infection or immune responses in the inner ear [7]. Thus, the use of pharmacological agents modulating NO synthesis could explain the role of NO in vestibular systems and represent a new approach to the treatment of certain vestibular pathologies.

HEAD AND NECK CANCER

NO has been implicated in the growth and metastasis of solid tumors. However, this area is complicated, because NO appears to have both tumor-promoting and tumor-inhibiting effects [111]. It has been suggested that the levels of NO found in many human cancers facilitate increased angiogenesis and enhance tumor growth [112].

In patients with squamous cell carcinoma high levels of NOS activity, including iNOS have been detected [113, 114]. Expression of NOS has been found correlated with the grade of head and neck cancer [113].

Bentz *et al.* [115] determined that the expression of the endothelial constitutive isoenzyme (ecNOS or NOS-III) was significantly increased during the progression from normal oral mucosa, through reactive squamous lesions, squamous dysplasia, and eventually to squamous cell carcinoma. Gallo *et al.* [114] showed that an increase in NOS activity in head and neck cancer, is associated with elevated cGMP levels, and correlates with tumor vascularization. Demonstration of correlation between NOS activity with lymph node metastases, suggested that an increased metastatic behavior was associated with a high NOS activity and angiogenesis [114]. The involvement of the p53 tumor suppressor gene in the regulation of NOS expression [116] and angiogenesis raises the possibility that the p53 mutation found in approximately 50% of head and neck cancers [117] could result in an increase in NO production and, thus, in NO-related angiogenesis, ultimately leading to tumor progression.

These data suggest that, in head and neck cancer, the increased NOS activity can be regarded as a novel biologic marker for tumor progression related to angiogenesis, and that inhibitors of NOS are potential antiangiogenic drugs [114]. Furthermore some authors suggest that monitoring NOS activity might provide further information regarding the biologic behavior of head and neck cancer in relation to angiogenesis and tumor spread [114].

Tumor cytokine therapy has been shown to induce NOS in man. Several of these studies associate increased NO production with the toxic effects of cytokine therapy, such as circulatory changes and hypotension. However, some studies indicated increased NO level suggested the response to the therapy [114, 118]. On the other hand, it has been demonstrated that the administration of NOS inhibitors in mice could decrease IL-2/NO-induced capillary leakage and improve the IL-2-induced anti tumor effector cell activation, stimulating by this way tumor regression [119, 120]. It has been proposed that selected NO-blocking drugs may be useful in the treatment of certain human cancers – either as single agents or as a part of combined therapies. In accordance with that, treatment of tumor-bearing mice with NO-blocking agents reduced the growth and vascularity of primary tumors and their spontaneous metastases [119, 120].

CONCLUSIONS

The presence of NO with high levels within the nose and paranasal sinuses makes it reasonable to believe that this

Table 1. Some Known and Suggested Physiological and Pathophysiological Functions of NO in Otolaryngology

NO	Known or suggested physiological and pathophysiological functions	Increased NO	Decreased NO
Nose	• Host defense • Ciliary motility • Modulation of the vascular changes necessary for temperature conditioning of nasal air • Improving ventilation-perfusion rate in the lungs • Aerocrine messenger between the upper and lower airways	• Acute upper respiratory tract infection* • Allergic rhinitis*	• Primary ciliary dyskinesia • Cystic fibrosis • Nasal polyposis
Paranasal sinuses	• Host defense • Ciliary motility		• Acute and chronic sinusitis
Middle ear	• Regulation of cochlear blood flow • Mucus production • Regulation of permeability of the middle ear vascular	• Mucoid otitis media • Tympanosclerosis	
Cochlea	• Mediation of neurotransmission in the ear sensory cells and ganglia • Participation in the transformation of signals and to lesser extent their transduction • Toxic effects on cochlear potentials • Contribution to the pathophysiology of certain forms of sudden hearing loss, tinnitus, and presbyacusis	• Aminoglycoside therapy	
Vestibule	• Maintaining endolymph and ion homeostasis in the stria vascularis • Regulation of vestibular vascular tone, fluid absorption or resistance to infection • Contribution to the pathophysiology of Meniere's disease		
Head and neck	• Tumor-promoting and tumor-inhibiting effects • Angiogenesis	• Head and neck cancer • Metastatic behavior	

*Conflicting results

pluripotent gas is involved in a variety of physiological as well as pathophysiological events in the airways. Some known and suggested physiological and pathophysiological functions of NO in upper airways are summarized in Table (**1**). Although it has an ever-increasing role in diverse areas relevant to the practice of otolaryngology, further research is required to understand fully the role of NO in the upper airways. At present there is some evidence that inflammatory activity within the lower and upper airways can be monitored by serial NO recording, the role of NO measurement for nasal diseases in clinical practice is less clear. It is also reasonable to believe that drugs that affect endogenous NO synthesis, will be used clinically in the future. From that point of view further studies investigating NO precursors or donors, as therapeutic options in otolaryngology are needed.

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