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Inhaled and exhaled nitric oxide

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Abstract. Inhaled nitric oxide (NO) is used to treat various cardiopulmonary disorders associated with pulmonary hypertension. The rationale is based on the fact that NO, given by inhalation, only dilates those pulmonary vessels that perfuse well-ventilated lung units. As a result, pulmonary gas exchange is improved while pulmonary vascular resistance is reduced and pulmonary blood flow is increased. Inhaled NO has been succesfully applied to treat persistent pulmonary hypertension of the newborn, reducing the need for extracorporeal life support. Although pulmonary hypertension and altered vasoreactivity contribute to profound hypoxaemia in adult and paediatric acute respiratory distress syndrome (ARDS), the benefit of inhaled NO still remains to be established in patients with ARDS. ARDS is a complex response of the lung to direct or indirect insults, leading to pulmonary vasoconstriction and various inflammatory responses. Recent randomized trials suggest that inhaled NO only causes a transient improvement in oxygenation. Whether this effect is important in the long-term management of ARDS remains to be established. NO, measured in the exhaled breath, is an elegant and non-invasive means to monitor inflammation of the upper and lower respiratory tract. In the normal upper airways, the bulk of exhaled NO originates from the paranasal sinuses. Exhaled NO is increased in nasal allergy and decreased in cystic fibrosis, nasal polyposis and chronic sinusitis. That NO production is increased in asthmatic airways is also well established. However, several questions still need to be addressed, in particular evaluation of the sensitivity and specificity of the measurement techniques, and assessment of the bronchodilator action of endogenous NO.

Key words. Nitric oxide; persistent pulmonary hypertension of the newborn; acute respiratory distress syndrome; sinusitis; asthma.

Introduction

The biological functions of nitric oxide (NO) are so diverse and complex that it is now increasingly difficult to briefly delineate its physiological roles and pathophysiological implications. In respiratory medicine, NO can either be viewed as a paracrine factor (derived from e.g. endothelium, epithelium, nerves and inflammatory cells), a therapeutic gas or a marker of inflammation. As a paracrine factor, endothelium-derived NO, among others, plays a central role in the modulation of pulmonary vascular tone. Epithelium-derived NO represents an original means of non-specific host defence of airways which are constantly exposed to aeropathogenic agents, while it can also modulate bronchial smooth muscle tone. As a gaseous molecule, NO has been extensively investigated in clinical settings and used as inhalational therapy to relieve pulmonary hypertension and/or refractory hypoxaemia in children and adults; measurement of exhaled NO has more recently gained interest as a new lung function test. As a marker of inflammation, exhaled NO has become an elegant way to monitor airway inflammation in asthma and other conditions associated with inflammation of the respira-

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tory tract. The aim of this review is twofold. First, we focus on inhaled NO (iNO) and the rationale for its use in clinical settings and highlight the results of recently published multicentre trials on iNO in the newborn and the adult. Second, exhaled NO and its implications for human physiology and pathophysiology will also be discussed.

Inhaled NO

The main therapeutic interest of exogenous NO is based on its potent vasodilator properties and the unique possibility for its delivery as a gas to the lung vessels. In the past 10 years, iNO has become a popular treatment for various cardiopulmonary disorders that are characterized by pulmonary hypertension or altered pulmonary vasoregulation, especially persistent pulmonary hypertension of the newborn (PPHN) and acute respiratory distress syndrome (ARDS). Treatment of these syndromes has been hampered by the lack of effective agents which can lower pulmonary vascular resistance (PVR) without causing systemic hypotension or impairing gas exchange. When these patients do not respond to 'optimal' medical management, veno-arterial extracorporeal membrane oxygenation (ECMO) is applied as the treatment of last resort. This technique is time-consuming, expensive and associated with significant risks. Many pharmacologic agents cause potent vasodilation, but their clinical use is limited by systemic hypotension or worsened gas exchange by deflecting blood from well-ventilated areas to poor-ventilated areas (fig. 1). Therefore, the 'ideal' pulmonary vasodilator has to be selective, with action not only restricted to the pulmonary circulation but also causing vasodilation only in well-ventilated lung units in order not to impair gas exchange [1]. iNO is, to date, the best agent available for inducing selective pulmonary vasodilatation (i) without affecting the systemic circulation, because of its rapid inactivation by haemoglobin confining its action to the pulmonary vascular bed (fig. 2) [2], and (ii) without worsening gas exchange because, administred as a gas, NO will reach only the well-ventilated lung units. In the neonate, iNO has been a breakthrough in the treatment of PPHN. In adult ARDS, the results of the recently published multicentre trial are rather conflicting.

iNO in paediatric patients

In the neonate

Rational for the use of iNO. In the foetus, the pulmonary circulation is characterized by high PVR. At birth, PVR drops dramatically, and pulmonary blood

flow increases accordingly. Failure of these processes in the normal transitional circulation results in PPHN. This syndrome is characterized by pulmonary hypertension, right-to-left shunting across the ductus arteriosus and/or foramen ovale and severe hypoxaemia, and it contributes significantly to neonatal morbidity and mortality. Numerous respiratory diseases, such as hyaline membrane disease, meconium aspiration syndrome, sepsis and congenital diaphragmatic hernia, are associated with PPHN. To date, the mechanisms that maintain high PVR in the foetus and contribute to the transition of the pulmonary circulation remain poorly understood. There is however consistent evidence suggesting that the NO/cGMP pathway plays a major role in the regulation of vascular tone in the perinatal pulmonary circulation. For example, at birth, inhibition of the constitutive endothelial NO synthase (eNOS or NOS III) significantly impedes the fall in PVR and the resulting rise in pulmonary blood flow [3]. In experimental PPHN induced by occlusion of the ductus arteriosus in foetal lambs, NOS III is impaired [4] and mice with targeted disruption of the NOS III gene show pulmonary hypertension [5].

Therapeutic use of iNO in PPHN. On the basis of these findings, experimental and clinical studies have demonstrated that iNO can lower PVR, increase pulmonary blood flow, enhance cardiac output and improve oxygenation [6–8]. Furthermore, iNO enhances ventilation-perfusion mismatch in the treatment of severe lung
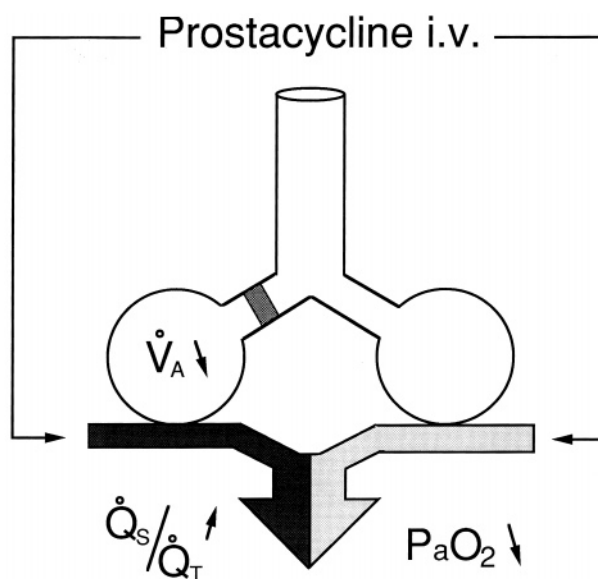

Figure 1. Effects of infused systemic vasodilators (e.g. prostacyclin) on intrapulmonary shunting ($\dot{Q}_s/\dot{Q}_t$) and partial pressure of arterial oxygen (PaO$_2$) [from ref. 1, with permission].

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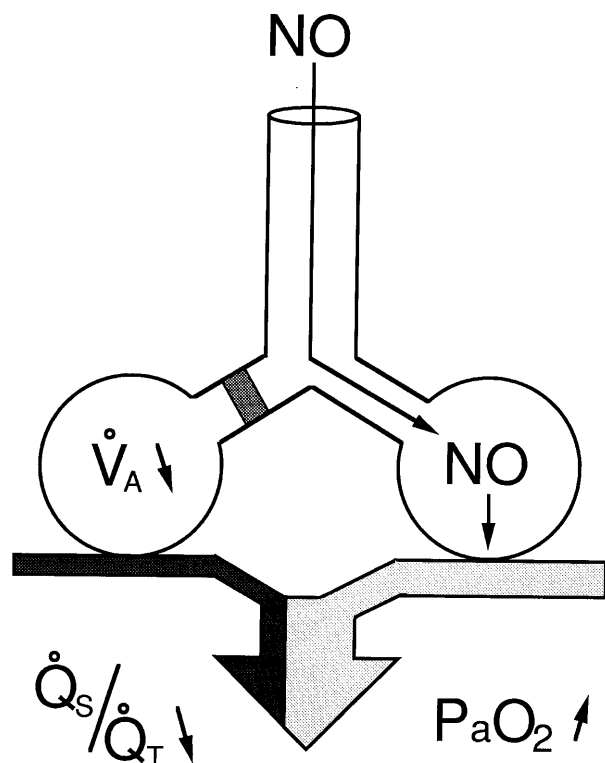Multi-author Review Article   1105

Figure 2. Effects of iNO on intrapulmonary shunting ($\dot{Q}_s/\dot{Q}_t$) and partial pressure of arterial oxygen (PaO$_2$).

disease, an effect known as the 'microselective effect' [9]. The results of these early observations have now been confirmed by randomized multicentre clinical trials [10–15]. Although the diversity of these trials renders them difficult to compare, various questions can be answered. All these studies conclude that iNO significantly lowers PVR and significantly improves oxygenation in term and preterm neonates with refractory hypoxaemia. A recent meta-analysis showed that 58% of all hypoxaemic newborns responded to iNO within 30–60 min [16]. Doses of 5 ppm were as efficient as 80 ppm [12]. Furthermore, the efficacy of iNO is disease related and depends upon the applied ventilator strategy, highlighting the importance of analysing the pathophysiology underlying PPHN [13]. In addition, the as yet unpublished French-Belgian study showed that early introduction of iNO prevents the evolution towards refractory hypoxaemia and may lower the incidence of brochopulmonary dysplasia [15]. The most striking impact of iNO is reduction in the use of ECMO, thereby reducing the cost and the risk for these patients. In France, the introduction of iNO in 1992 caused a 80% decrease in the use of ECMO [17]. The same holds true for the United States [18].

Despite its spectacular impact on oxygenation, however, iNO did not lower the mortality rate in these hypoxaemic newborns. These infants are more likely to benefit from a more global approach, ante- and postnatal, including in utero transfer to a level III unit for optimal management (antenatal glucocorticoids, maternal antibiotics, early exogenous surfactant, early iNO).

Concerns remain as to the potential toxic effects of iNO, including methaemoglobinaemia and lung injury caused by NO$_2$, peroxynitrite and hydroxyl radical formation [19]. NO also inhibits platelet aggregation and prolongs bleeding time. This is of concern especially in premature neonates which may be at high risk for intracranial haemorrhage [20]. However, iNO proved to be safe in all the multicentre trials and none of the above-cited side-effects occurred. Particular attention has to be payed to the potential mutagenic effects of NO [21]. Further studies are needed to confirm the absence of long-term effects.

Other indications for iNO in paediatrics

Congenital diaphragmatic hernia. Unlike other causes of PPHN, infants with congenital diaphragmatic hernia (CDH) have only a transient or no response to iNO [22]. This malformation, which has long been thought to be merely a hole in the diaphragm, appears now as a complex disease including lung hypoplasia, surfactant deficiency, and functional and morphological abnormalities of the pulmonary vascular bed sometimes associated with left heart hypoplasia [23]. These various features may account for the lack of response to iNO.

Congenital heart defects. iNO has been succesfully used for diagnostic (to determine the reversibility of pulmonary hypertension) and therapeutic purposes (to prevent post-operative pulmonary hypertension and lower-right ventricular afterload) [24].

Sickle cell disease. Recent data have focussed on the important role of the vascular endothelium in the pathophysiology of vaso-occlusive accidents. Hence, encouraging preliminary results with iNO in the acute chest syndrome herald interesting therapeutic perspectives in a disease that has so far been regarded and treated as only a haemoglobin abnormality.

ARDS in children. Although pulmonary hypertension and altered vasoreactivity contribute to profound hypoxaemia in ARDS, the utility of iNO in this disorder remains questioned. There are only a few data evaluating the efficacy of iNO pediatric patients with ARDS [25, 26]. Improvements in oxygenation were only modest and larger trials are necessary to ascertain whether these acute physiologic improvements can alter clinical outcome.

iNO in adult ARDS

Definition and rationale for the use of iNO in ARDS

ARDS is a complex response of the lung to direct or indirect insults. Because of its complex pathophysiology and the heterogeneity of the underlying aetiologies, the American-European concensus conference on ARDS attempted to clearly define this entity as an acute-onset respiratory failure [partial arterial oxygen concentration/fraction of inspired oxygen ($PaO_2/FiO_2 \leq 200$ mm Hg] with bilateral infiltrates and non-cardiogenic pulmonary oedema [27].

The mechanisms underlying ARDS remain largely unknown, although pulmonary lesions may be mainly due to inflammatory interaction between platelets, leucocytes, mononuclear cells, macrophages and endothelial cells, where oxidative stress is prominent.

The rationale for using an inhaled vasodilator in ARDS is based on the observation that pulmonary vasoconstriction represents an important component of the rise in pulmonary artery pressure in ARDS. These alterations may adversely affect patients with ARDS because the increase in PVR may (i) produce excessive afterload on the right ventricle and decrease cardiac output or right ventricular failure, (ii) interfere with ventilation/perfusion mismatching, and (iii) transmit high pulmonary artery pressure to the microvasculature contributing to protein leak and pulmonary oedema. iNO produces microvascular dilation in those areas accessible to the gas, thereby reducing pulmonary artery pressure and shunt, reducing right ventricular afterload and avoiding cardiac failure, without affecting the systemic circulation. In addition, the improvement in oxygenation would allow physicians to decrease the intensity of mechanical ventilation, and could, in turn, decrease the amount of secondary iatrogenic pulmonary injury and facilitate recovery. However, animal studies revealed conflicting results: some reported improvement in the severity of lung injury with iNO, while a similar number of studies reported no benefit, or even a worsening of lung injury in some instances [28–32].

Therapeutic use of iNO in adult ARDS

Rossaint et al. [33] reported the first use of iNO in ten patients with severe ARDS [33]. At a concentration of 18 ppm, iNO produced a statistically significant reduction in pulmonary artery pressure and intrapulmonary shunting while the ratio of PaO_2 to FiO_2 increased and mean systemic arterial pressure and cardiac output remained unchanged. Continuous administration of NO produced consistent, but limited, decreases in pulmonary artery pressure and limited increases in the ratio of PaO_2 to FiO_2 for 3–53 days. Gerlach et al. [34] also reported an improvement in the survival rate up to

80% with iNO in ARDS. The effect of iNO has been shown to be dose dependent [35]. The increase in PaO_2 was less when higher concentrations of iNO were used, presumably due to a diffusion of NO into poorly ventilated portions of the lung with a loss of its 'micro-selective' effect. Since these first enthusiastic reports, only a few appropriately designed studies addressing the clinical outcome of patients with ARDS treated with iNO have been published. Unlike those in the newborn, the results of these randomized, multicentre clinical trials are conflicting.

Dellinger et al. [36] enrolled 177 patients from 30 centres in a phase II trial to examine the effect of several iNO doses (1.25, 5, 20, 40 and 80 ppm). There was no difference between the groups treated with iNO and placebo (nitrogen alone) with respect to mortality, the number of days alive and off mechanical ventilation, or the number of days alive after meeting oxygenation criteria for extubation. Interestingly, post hoc analysis revealed that the 5 ppm sub-group of iNO-treated patients had a higher percentage of patients who were alive and off mechanical ventilation at day 28. Another recent randomized, controlled trial, showed that iNO improves oxygenation, but does not affect mortality [37]. Michael et al. [38] could not find any substantial differences between the iNO group and the conventionally treated groups in terms of improvement in the PaO_2/FiO_2 ratio and reduction in FiO_2. Finally, two as yet unpublished prospective, double-blind, randomized phase III studies also failed to demonstrate any effect of iNO on mortality or duration of mechanical ventilation [39, 40].

In summary, the recent trials suggest that iNO only improves oxygenation in adult ARDS temporarily and raise many questions regarding the involvement of NO in the pathophysiology of ARDS and the rationale for its use in this disease. However, if one considers that the proportion of ARDS patients dying from severe hypoxia is low ($< 5\%$), it becomes apparent that even were iNO to completely cure ARDS, it would be difficult to discern an effect on survival. In addition, none of the previous treatments tested in ARDS has been shown to succesfully reduce morbidity and mortality. In fact, NO is 'only' a selective pulmonary vasodilator capable of 'no more' than reducing PVR and pulmonary arterial pressure, and reversing hypoxaemia in a disease multifactorial in origin and where patients die from multiple organ failure. Nevertheless, iNO has reduced the need for ECMO in adult ARDS, which is a primary outcome effect in the newborn. Therefore, like positive end-expiratory pressure, permissive hypercapnia, and patient positioning, iNO should be regarded as one treatment among several in the therapeutical arsenal for ARDS. Whether modest improvements in oxygenation will prove to be important in the long-term management of ARDS patients awaits further study.

Exhaled NO

Since 1991, measurement of in vivo NO production in humans has proven technically feasible by means of ex vivo manoeuvres, i.e. by sampling the exhaled breath and analysing it for NO content using a chemiluminescent NO analyser [41]. As the technique is non-invasive it was immediately applied to patients and evidence soon accumulated to suggest that measurement of exhaled NO could be viewed as a new lung function test [42] to monitor airway inflammation in asthma and other conditions associated with inflammation of the respiratory tract.

It is still difficult to know the actual source of endogenous NO which is detected in the exhaled air [43]. As NO is synthesized by many lung cells, it could originate from virtually anywhere in the respiratory tract, from alveolar space to the nose. Several recent and carefully conducted studies have clearly shown how the techniques of measurement are likely to affect the amount and origin of exhaled NO. This has prompted the European Respiratory Society to issue in 1997 specific recommendations for measurement of exhaled and nasal NO [44], an initiative which has been followed in 1998 by the American Thoracic Society.

NO in the upper airways

The evaluation of NO in the airways initially focussed on orally exhaled air and was believed to reflect lower airways production. However, Lundberg et al. [45] have clearly shown that most of the NO in the exhaled air of healthy subjects originates from the upper respiratory tract and more precisely in the paranasal sinuses, with only a minor contribution from the lower airways. Thus, 50–90% of the exhaled NO comes from the nose during mouth breathing with an open posterior nasopharynx [46]. When evaluating NO excretion in the lower airways during an exhalation, appropriate measures such as exhaling against a resistance to close the soft palate must be taken to separate the nasal passages from the rest of the respiratory tract.

NO production in normal upper airways. In healthy subjects, the sinuses are generally considered to be sterile, despite their proximity to the nasal cavity which is normally colonized by bacteria. The process that maintain sinus sterility is not fully understood, although ciliary activity and secretory immunoglobulins, working in concert with intact ostium, are known to participate to the cleaning of these cavities. Lundberg et al. [45] reported that NO is produced by epithelial cells in the paranasal sinuses and is present in sinus air at very high concentrations (about 10 ppm or 10,000 ppb, closed to the highest permissible atmospheric pollution levels. In immunohistochemical and mRNA in situ hybridization

studies, they showed that an NO synthase similar to the inducible isoform is constitutively expressed apically in the sinus epithelium. In contrast, only weak NO synthase activity was found in the epithelium of the nasal cavity. However, paranasal sinus NO diffuses into the nasal cavities, where it can be easily measured. According to the flow rate of aspiration (100–700 ml/min), nasal NO concentrations of 1000 to 200 ppb, respectively, are measured [47]. The sinus NO concentrations reported in Lundberg's study were markedly reduced (− 78%) by local instillation of an NOS inhibitor (L-nitro-arginine methyl ester, L-NAME) into the maxillary sinus, whereas nasal nebulization of L-NAME only reduced the NO concentration by 22%. Interestingly, and in contrast to the usual regulation of classical inducible NO synthase, high doses of oral (in order to access the paranasal epithelium) corticosteroids do not alter nasal NO concentration and thus probably do not down-regulate the sinus epithelial NO synthase [48].

Although the direct proof remains to be established, NO could play a critical role in the physiology and pathology of the upper respiratory tract because NO also stimulates ciliary motility [49] and plays a major role in immunity and host defence [50].

NO and nasal allergy. It is easy and simple to evaluate nasal NO concentration using a chemiluminescent analyser sampling air from each nasal cavity. Recently, patients with allergic rhinitis have been shown to have a two-fold increase in nasal NO concentration [51]. This increase results from increased expression of inducible NO synthase in the nasal mucosa [52] as it is suppressed by nasal or systemic corticosteroids, a regulation which is lost in the sinus epithelial NOS. Increased NO levels during nasal allergy may have several pathophysiological consequences. It is worth emphasizing that non-symptomatic patients also had increased nasal production, suggesting that NO synthase overexpression and/or overactivity persisted at the level of the mucosa and did not return to normal between two allergic rises [51]. On the one hand, increased release of nasal NO in allergic patients may increase airway blood flow causing hyperaemia, airway oedema by plasma exudation and increased release of tachykinins [53]. Consequently, increased NO at the level of the nasal cavity not only contributes to nasal congestion, rhinorrhoea and sneezing, but also worsens mucus retention and infection by decreasing the permeability of the sinuses. Furthermore, it was recently reported that NO prevents Fas-receptor-mediated apoptosis in freshly isolated human eosinophils, and increased NO concentrations contribute to the accumulation of eosinophils in the mucosa [54]. On the other hand, increased NO can prevent infection of the upper airways due to its cytotoxic effect on bacteria. Altogether, one can speculate that NO could favour a vicious circle inducing inflam-

mation/retention/infection, if its direct bacteriostatic properties are counterbalanced by deleterious consequences to the permeability of sinus cavities. Further work is required to clarify the precise role of NO in paranasal sinus disorders.

The beneficial or deleterious role of increased NO levels in nasal allergy can be directly assessed by NO synthase inhibition. Nasal nebulization of L-NAME is a simple and easy method to block increased nasal NO production. This route of administration is safe as it neither alters the NO concentration in paranasal sinuses (at least in the maxillary sinuses) nor induces the adverse effects of systemic NO blockade (e.g. hypertension). This approach could represent an alternative to the use of corticosteroids which have been shown to lower the increased nasal NO in allergic rhinitis, and should be tested in the near future.

NO and mucociliary function. Lundberg et al. [48] also showed that patients with Kartagener's syndrome (referred to as an 'immobile ciliary syndrome' and characterized by situs inversus, sinusitis and bronchiectasis) had very low nasal NO concentration (10–20 ppb). The sharp decrease in NO production in patients with Kartagener's syndrome or in certain patients with nasal polyps could have important pathophysiological consequences. As mentionned above, NO plays an important role in immunity and host defense and stimulates ciliary motility. Lindberg et al. [55] have demonstrated the physiological importance of NO on mucociliary function in the upper airways. A decrease in nasal NO concentration has been reported in cystic fibrosis, chronic sinusitis and nasal polyposis [56–58]. In this latter case, the drop in the nasal NO concentration was mainly due to mechanical occlusion of the sinuses by the polyps in the absence of associated allergy. In all cases, decreased NO levels in the upper airways could favour mucociliary dysfunction and participate in chronic infections.

Paranasal sinus defence by NO represents a paradigm for non-specific host defence. Resident macrophages are the first line of defence in most of the cavities (e.g. pulmonary alveoli, peritoneal cavity) in mammals. Macrophages express NADPH oxidase and generate high levels of superoxide anion and other reactive oxygen species which kill the phagocytosed pathogen agents [59]. Upon inflammatory stimuli, macrophages and, to a lesser extent, most of the cells respond by expressing the inducible NO synthase. NO represents a second line of defence which helps the first to cleanse pathogenic agents that are difficult to kill. An inverse situation characterizes the paranasal sinus cavities. The first line of defence is represented by epithelium-derived NO, whereas resident macrophages are absent under normal conditions. However, acute and chronic sinusitis are characterized by the recruitment of phagocytic cells,

in particular eosinophils [60]. Among phagocytic cells, eosinophils express the highest NADPH oxidase activities, and thus generate the highest amounts of superoxide anion [61]. As superoxide anion is recognized as one of the major inactivators of NO, it can be speculated that inflammation could contribute to decrease NO levels. Reciprocal breakdown of both NO and superoxide anion could contribute to altering these two major non-specific host defences [62]. Alternatively, peroxynitrite, the possible by-product of NO and superoxide anion interaction, could be generated and has been shown to be far more toxic to bacteria than NO or reactive oxygen species separately, although other synergisms have been described [63].

Exhaled NO in the newborn. Exhaled NO has also been investigated in the upper airways of healthy newborns within the first minutes after birth and at postnatal ages of 1 and 24 h [64]. A 30% increase was noted between 1 and 24 h of age. As in the adult, it is suggested that most airway NO is formed in the upper airways, especially in the nose. Hypothetical roles of NO in the newborn upper airway include enhanced non-specific host defence mechanisms, reduced bronchial hyperreactivity, as well as pulmonary vasodilation and stimulation of mucociliary transport. However, because neonates breathe at a frequency of 30–40 breaths/min, much faster methods must be developed to measure peak pharyngeal NO concentrations accurately. In subsequent work, Schedin et al. [65] showed that premature infants (post-conceptional age range 25–36 weeks) also excrete significant amounts of NO from the upper airways which increased with post-conceptional age.

NO in lower airways

In contrast to the ongoing debate on how to measure exhaled NO, recent studies strongly support the diagnostic value of this measurement in asthma. Compelling evidence clearly demonstrates that asthmatic subjects who are not treated with inhaled glucocorticosteroids exhale, on average, more NO than healthy controls [66–69]. This evidence is based on both empirical and theoretical grounds. High levels of exhaled NO have been consistently found by several investigators using different techniques. The inducible NOS II is markedly expressed in asthmatic airways [70] and unlike the constitutive eNOS III and the neuronal NOS I which synthesize NO only in minute amounts to meet physiological demands, expression of inducible NOS II by asthmatic epithelial cells leads to massive synthesis of NO, thus explaining high levels of exhaled NO in asthma. This observation has practical implications. Because NO production is increased in inflammatory diseases, measuring exhaled NO can be viewed as a non-invasive, though indirect, means to detect inflam-

mation of the respiratory tract. Because NOS II is induced by several inflammatory cytokines, monitoring exhaled NO is also an elegant way to assess the efficacy of anti-inflammatory agents with the assumption that the more potent the medication, the less NO will be exhaled. Consistent with this contention is the observation of a dose-dependent inhibitory effect of inhaled glucocorticosteroids on exhaled NO in asthmatic patients [71]. However, the measurement of exhaled NO may in some instances be difficult to interpret and there are a few questions that still need to be addressed.

Measurement of exhaled NO, however, lacks both sensitivity and specificity. There are at least two categories of patients in whom airway inflammation does not seem to be associated with high levels of exhaled NO. These are smokers [72] and patients with cystic fibrosis [73] who consistently have reduced exhaled NO compared with healthy subjects. NO and carbon monoxide are two major components of tobacco smoke. Both are putative inhibitors of NO synthase activity. Inhibition of NO synthase by cigarette smoke occurs at a post-transcriptional level and probably accounts for reduced NO production in smokers. The mechanims of reduced exhaled NO in cystic fibrosis patients are not known. Circumstancial evidence, however, suggests that accelerated oxidation—rather then reduced production—of NO is a likely mechanism for the relatively low level of exhaled NO in cystic fibrosis [74]. Also, not all medications with putative anti-inflammatory properties would result in reduced exhaled NO in asthmatic patients. For example, it has been shown that the sulphidopeptide leukotriene receptor antagonists do not alter the production of NO in asthmatic patients [75].

The second question relates to the physiology of endogenous NO with respect to its bronchodilatory effect. There is evidence to suggest that maintaining a certain level of NO production within the tracheobronchial tree is critical to modulate bronchial tone, especially when the airways are stimulated by brochoconstrictor stimuli. First, inhibition of NO generation by various L-arginine analogues often aggravates brochial hyperreactivity to various agents, including histamine [76], metacholine [77] and bradykinin [77]. Basal bronchomotor tone, however, does not seem to be affected by NO synthase inhibitors [78]. Second, exhaled NO is increased during upper respiratory tract viral infections [79], but this increased production seems to be rather beneficial for asthmatic patients. This was first suggested by the demonstration that experimental virus-induced airway hyperresponsiveness is related to a relative deficiency in NO [80]. The protective role in this condition is further supported by the recent finding that the greater the increase in exhaled NO, the lower the severity of airway hyperresponsiveness to histamine following experimental rhinovirus infection in asthmatic subjects [81]. Stim-

ulus such as thermal water also causes an increase in exhaled NO which parallels an improvement in lung function in asthmatic children [82]. Evidence is therefore accumulating that endogenous NO is not only beneficial, but may also be particularly necessary when the bronchi are harmed by exogenous aggression.

Such aggression can simply be strenuous exercise with its associated hyperventilation that may cause exercise-induced asthma in susceptible subjects [83]. Therminarias et al. [84] showed recently that airway obstruction is associated with a relative decrease in NO output during exercise in eight healthy subjects breathing cold ($-10\,^{\circ}$C) as compared to ambient ($22\,^{\circ}$C) air. Due to its bronchodilatory effect, it is tempting to speculate that such a relative deficiency in NO production may favour airway obstruction in these subjects. This protective effect is further supported by the recent demonstration that the release of endogenous NO by kinins inhibits bronchoconstriction induced by cold-air inhalation in guinea pigs [85]. However, several studies [86, 87] have shown an increase from baseline of the actual NO output during exercise, even with cold-air breathing. The explanation is therefore likely to be far more subtle than the mere hypothesis of a relative deficiency of a bronchodilator agent being responsible for bronchial constriction.

Conclusion

When clinicians face an insoluble problem at the patient's bedside, the solution can be sought at the bench. In its Nobel prize year, NO became a wonderful example of how discoveries in basic sciences can bring new tools for the understanding and the treatment of human diseases. As an inhaled gas, NO has become one of the major therapeutic strategies in diseases associated with pulmonary hypertension and hypoxaemia. If its beneficial effect on long-term outcome of patients with ARDS remains to be proven, there is, since the recently published multicentre trials, no doubt as to its efficacy in PPHN. As an exhaled gas, NO has become an elegant and non-invasive lung function test and has brought some light to the understanding of inflammatory diseases of the upper and lower airways.

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