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Nitric oxide, the biological mediator of the decade: fact or fiction?

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Nitric oxide, the biological mediator of the decade: fact or fiction? S. Singh, T.W. Evans. ©ERS Journals Ltd 1997.

ABSTRACT: Nitric oxide (NO), an atmospheric gas and free radical, is also an important biological mediator in animals and humans. Its enzymatic synthesis by constitutive (c) and inducible (i) isoforms of NO synthase (NOS) and its reactions with other biological molecules such as reactive oxygen species are well characterized. NO modulates pulmonary and systemic vascular tone through its vasodilator property. It has antithrombotic functions and mediates some consequences of the innate and acute inflammatory responses; cytokines and bacterial toxins induce widespread expression of iNOS associated with microvascular and haemodynamic changes in sepsis.

Within the lungs, a diminution of NO production is implicated in pathological states associated with pulmonary hypertension, such as acute respiratory distress syndrome: inhaled NO is a selective pulmonary vasodilator and can improve ventilation-perfusion mismatch. However, it may have deleterious effects through modulating hypoxic pulmonary vasoconstriction. Inhibitors of NOS may be of benefit in inotrope-refractory septic shock, but toxicity of newly developed selective iNOS inhibitors have prevented clinical trials of efficacy.

An expanding literature on the origins and measurement of NO in exhaled breath implicates NO as a potentially useful marker of disease activity in respiratory tract inflammation in the future.

This report reviews some aspects of research into the clinical importance of nitric oxide.

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The ability of mammalian cells to synthesize an endothelially-derived relaxant factor (EDRF) was first demonstrated in 1980. In 1987, it was shown that the actions of EDRF and nitric oxide (NO) were substantially similar [1–3], and during the subsequent decade progress in understanding the biological role of NO has been remarkable. Thus, NO was regarded initially only as an atmospheric pollutant present in vehicle exhaust emissions and cigarette smoke. More recently, NO seems set to be identified as an almost ubiquitous biological mediator, implicated in the pathogenesis of diseases as diverse as hypertension, asthma, septic shock and dementia; and as a potential marker of clinical diseases, that may prove amenable to therapeutic manipulation.

NO production and metabolism

Nitric oxide is synthesized from the terminal guanidine nitrogen of the semiessential amino acid L-arginine, which

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is converted to L-citrulline in a stereospecific reaction catalysed by a family of nitric oxide synthases (NOS) (fig. 1). These are complex haemoproteins, with properties similar to cytochrome P450, and contain both oxidative and reductive domains. NOS require a number of co-factors, such as tightly-bound flavoproteins and tetrahydrobiopterin, while the production of NO itself requires the co-substrates oxygen (O₂) and nicotinamide adenine dinucleotide phosphate (NADPH). Mechanistic studies are consistent with the hypothesis that the reductase domain of NOS provides reducing equivalents from NADPH to the haem domain, where oxidation of L-arginine occurs [4]. Three major isoforms of NOS have so far been identified: endothelial NOS (eNOS or type 3) and neuronal NOS (nNOS or type 1), which are expressed constitutively and are collectively termed constitutive nitric oxide synthase (cNOS); and a macrophage-derived form that is not normally expressed but is induced (iNOS or type 2) by endotoxin and inflammatory mediators, such as

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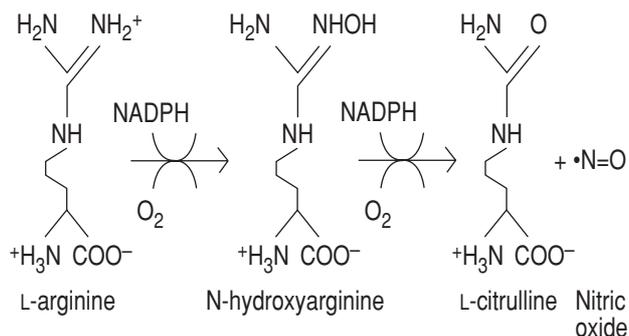


Fig. 1. – Chemical reactions involved in the synthesis of nitric oxide. L-arginine is converted to L-citrulline and nitric oxide. The reaction is catalysed by nitric oxide synthase (NOS) in the presence of oxygen and the co-substrate nicotinamide adenine dinucleotide phosphate (NADPH). NOS requires several co-factors, including flavones and tetrahydrobiopterin.

cytokines [5, 6] (table 1). The genes for these isoforms have been localized to chromosomes 7 (eNOS), 12 (nNOS) and 17 (iNOS) [7].

The constitutive enzymes produce limited amounts of NO and are activated by calcium and calmodulin. Agonists activate cNOS *via* a rapid increase in intracellular calcium concentration, resulting in NO release within seconds or minutes. Endothelially-derived NOS is membrane bound [8], and has been identified in vascular endothelial cells, platelets, myocardium and endocardium. Neuronal type NOS is cytosolic [9], and is found in skeletal muscle as well as in nervous tissue, while cNOS has also been identified in neutrophils and mast cells [10]. Inducible NOS has a 50% homology to cNOS; it is cytosolic and calcium-independent, perhaps due to its tight (noncovalent) binding to calmodulin, which prevents calcium-binding at physiological concentrations.

The inducible isoform was originally isolated from lipopolysaccharide/interferon-gamma (IFN- γ)-treated murine macrophages [11], but has since been identified in many cell types, including vascular smooth muscle, vascular endothelium, endocardium, myocardium, hepatocytes, fibroblasts, astrocytes and immune cells. All three isoforms of NOS have been detected in the human respiratory tract [12, 13]. Following an appropriate stimulus, such as IFN- γ , interleukin (IL)-1 β , tumour necrosis factor-alpha (TNF- α), endotoxin or exotoxin, iNOS is induced in the relevant cell type by gene transcription, probably *via* the transcription factor nuclear factor-kappa B (NF- κ B). This process can be inhibited by glucocorticoids [14]. Inducible NOS generates up to 1,000 times more NO than cNOS, and cellular production continues for hours. The effects of NO produced by iNOS are, therefore, more pronounced and widespread [15], an important concept which determines whether its effects are beneficial or detrimental to health in a particular clinical circumstance.

Nitric oxide is a free radical, by virtue of its unpaired electron; this makes it highly reactive with other radicals, such as superoxide [16]. Its half-life at physiological concentrations is measured in seconds [4], and it decomposes in aqueous solutions to nitrite (NO_2^-) and nitrous oxide (NO_3^-); a reaction catalysed by transition metals, such as iron. There is, consequently, a rapid inactivation of NO by haemoglobin to methaemoglobin, NO_2^- and NO_3^- , *via* an intermediary compound nitrosohaemoglobin.

In the presence of superoxide anion, NO combines three times faster than superoxide dismutase (a scavenger of superoxide), leading to the formation of peroxy-nitrite (OONO^-); an unstable, strongly pro-oxidant species with toxic effects on many molecules (*i.e.* nucleic acids, lipids and proteins) [17] (fig. 2). The cellular effects of

Table 1. – Properties of constitutive and inducible isoforms of nitric oxide synthase

	NOS isoform		
	Type 1 (nNOS) constitutive	Type 2 (eNOS) constitutive	Type 3 (iNOS) inducible
Location			
Chromosome number	12	7	17
Cellular compartment	Cytosolic	Membrane-bound	Cytosolic
Cell type	Neurons Skeletal muscle	Endothelium Platelets Endocardium Myocardium	Immune cells Vascular smooth muscle Endothelium Endo/myocardium Hepatocytes Astrocytes Fibroblasts
Activation	Calcium-dependent	Calcium-dependent	Calcium-independent
Regulation			Induction prevented by corticosteroids and protein synthesis inhibitors
Response to stimulation	Constitutive <i>e.g.</i> glutamate/receptor interaction leads to immediate NO release by neuronal dendrites	Constitutive <i>e.g.</i> ACh/receptor interaction leads to immediate NO release by vascular endothelium	Induction by LPS/cytokine leads to massive NO production after 2–6 h
Nonselective inhibitors	L-arginine analogues	L-arginine analogues	L-arginine analogues
Selective inhibitors			Aminoguanidine Under development

NOS: nitric oxide synthase; nNOS: neuronal NOS; eNOS: endothelial NOS; iNOS: inducible NOS; ACh: acetylcholine; LPS: lipopolysaccharide.

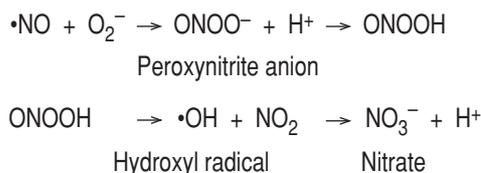


Fig. 2. – Interactions of nitric oxide with reactive oxygen species. $\bullet\text{NO}$: nitric oxide; O_2^- : superoxide anion; NO_2 : nitrogen dioxide.

NO are, in the main, attributable to its activation of guanylyl cyclase after binding to its haem moiety. This enzyme catalyses the conversion of guanylate triphosphate (GTP) to cyclic guanylate 3'5'-monophosphate (c-GMP). The rise in cGMP concentration, an intracellular messenger, results in smooth muscle relaxation and many of the alterations in cellular function produced by NO. Other intracellular second messenger systems, such as protein kinase activation, inositol triphosphate inhibition and intracellular calcium release, have been proposed as effectors of NO function at the cellular level. Indeed, NO is thought to form intermediary complexes with thiols, such as cysteine; the formation of sulphhydryl complexes may prolong the half-life of extracellular nitric oxide, facilitating its role in cell-cell communication prior to inactivation [18].

Thus, through processes at the cell surface, NOS isoforms are activated either *via* a second messenger, such as calcium, (cNOS) or in the case of iNOS through gene transcription. In the presence of appropriate substrates, these enzymes catalyse the formation of NO, which is either inactivated by its reaction with haemoglobin or albumin, acts as a biological mediator *via* its effect on guanylate cyclase, or forms toxic radical derivatives through its reaction with reactive oxygen species (ROS).

NOS inhibition

Nitric oxide synthase inhibitors (NOSIs) have been vital tools in determining the physiological and pathophysiological roles of NO. Furthermore, they are on the verge of clinical use as pressor agents in inotrope-refractory septic shock [19, 20]. NOSIs are analogues of L-arginine and act as false substrates for the enzyme, thus blocking endogenous NO formation. A number of non-selective NOSIs, such as N-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine methylester (L-NAME) have been developed. Their effects are overcome by L-arginine. Agents more selective for iNOS inhibition, such as aminoguanidine, have been investigated in the non-clinical setting, but are currently unavailable for clinical use.

Proposed functions of NO in homeostasis

Physiological roles for NO have been proposed following a number of experimental studies, including the localization of NOS isoforms to various tissues, in varying distributions, by immunohistochemical and biochemical techniques or messenger ribonucleic acid (mRNA) detection. The functional effects of stimulating or inhibiting the L-arginine-NO pathway have also proved invaluable in this respect.

NO and the cardiovascular system

Nitric oxide produced by microvascular endothelial cells is a major determinant of resting vascular tone [21]. The

endothelium produces NO at a basal rate, maintaining background vasodilatation in small arteries and arterioles in animals and humans. By contrast, veins synthesize very little NO basally. Systemic injection of L-NMMA increases blood pressure in experimental animals and healthy volunteers, but produces little change in venous pressure [22]. It seems that pulmonary vascular endothelial cells produce NO continuously, and that hypoxia reduces this release [23]. Furthermore, the normal human pulmonary vasculature expresses cNOS abundantly and its diminution is inversely correlated with total pulmonary vascular resistance (PVR) [24]. Such studies implicate NO as an important mediator of pulmonary blood flow and, consequently, of ventilation-perfusion (V/Q) matching within the lungs. This recent work extends the findings of earlier studies *in vitro*, that demonstrated a reduced vasodilator response to acetylcholine in pulmonary arterial vessels following pretreatment with NOS inhibitors [25–27]. Thus, it is now clear that increased vascular resistance or vasospasm need not be due to an increase in vasoconstrictor effects, but may reflect a loss of NO-mediated basal vasodilator tone [28]. This may underlie the intricacy of alterations in local and regional microvascular perfusion in pathological states, such as pulmonary hypertension.

In addition to its direct haemodynamic effects, NO inhibits the adhesion [29], activation and aggregation of platelets [30, 31], thereby conferring antithrombotic properties on the endothelial surface. Furthermore, the antiaggregatory effect of NO is synergistic with that of prostacyclin, another endothelium-derived vasoactive mediator. Nitric oxide may also be involved in modulating negative inotropic effects [32], as it affects cardiac relaxation during diastole [33].

NO in lung function

Nitric oxide mediates the nonadrenergic, noncholinergic (NANC) neural inhibitory responses of human airways [34], either directly or *via* stable NO carrying intermediates, such as nitrosocysteine. NANC pathways represent the only neural bronchodilator mechanism in human airways [35], and NO is thought to act as a "braking" mechanism to cholinergic bronchoconstriction, rather than having any direct bronchodilator effect *per se*. Nitric oxide derived from resident immune cells may also contribute to host defence or inflammation in the lungs [36].

NO as a neurotransmitter

In addition to the neural bronchodilatation mediated by NO, it seems to be involved in neurally-mediated vasodilatation in the pulmonary circulation. Studies using electrical field stimulation suggest that adenosine triphosphate (ATP) from sympathetic neurones causes the release of endothelially-derived NO, which acts on the pulmonary vasculature causing relaxation [37], whilst NOS inhibition produces an increased adrenergic vasoconstrictor response. Neurones staining for NOS have been identified in the urinary tract and gastrointestinal system, as well as the vascular bed and bronchi.

Through its smooth muscle relaxing propensity, NO is thought to play important roles in adaptive relaxation

of the stomach to accommodate food, and in sphincter relaxation, as suggested in animal studies [38, 39]. In the central nervous system, NO has been proposed as a mediator of memory function [40], electrocortical activation (important as an alerting response in the control of arousal) [41], and as a modulator of pain perception [42, 43].

NO and the immune system

Endogenous NO produced by iNOS in activated murine macrophages is important in host defence [44], and appears to play a crucial role in controlling infection *in vivo*, as demonstrated indirectly by experiments in mice infected with *Leishmania*. Nitric oxide is also involved in mononuclear cell-mediated killing of *Mycobacterium tuberculosis* and other pathogens in rodents [45], is toxic to tumour cell lines *in vitro*, and may selectively inhibit a subset of thymus-derived lymphocytes (type-1 T-helper (Th1)) [46]. Definitive confirmation of these roles in humans is awaited. In the upper respiratory tract (URT), NO appears to be important in maintaining ciliary function [47], and may have a role in sterilizing the mucosa of URT, thus reducing susceptibility to subsequent lower respiratory tract infection.

Nitric oxide also contributes to the acute inflammatory response mediated by endotoxin, cytokines or physicochemical stress, through the activation of iNOS. The resultant NO production causes vasodilatation, plasma exudation [48], and activation of innate, nonadaptive immune mechanisms. This, consequently, has implications for the role of NO in inflammatory disease states, and provides a therapeutic target for suppression of inflammation by manipulation of the NO pathway.

NO in other homeostatic mechanisms

NO is linked to homeostasis in many physiological systems. In the gastrointestinal tract, in addition to its role in modulating gut motility in mice, the possibility of NO being a defence mechanism against ingested organisms has been proposed [49]. In the endocrine system, endogenous NO appears to be involved in regulating renin production and sodium homeostasis in the kidney [50], in so far as it has been shown to determine sensitivity to the pressor effects of a sodium challenge [51]. In pregnancy, NO may be responsible for the associated vasodilatation, reduced systemic vascular resistance, and increased cardiac output [52]. Oestrogens increase the expression of eNOS. Nitric oxide may also provide a basal tocolytic tone, and a decrease in the activity of the L-arginine-NO pathway appears to coincide with the onset of labour.

Nitric oxide and disease

Excess or deficient NO has recently been linked to numerous pathological conditions, representing an extension of the roles discussed in homeostasis. Within the cardiovascular system, diminished NO production is implicated in conditions associated with increased vascular tone, vasospasm and thrombosis due to enhanced adhesion of platelets and white cells to the vessel wall. Abnormalities of arterial blood flow in the human forearm following infusions of L-NMMA have been de-

tected in patients with essential hypertension, diabetes mellitus, heart failure and overt atheroma [53, 54].

In the gastrointestinal tract, infantile hypertrophic pyloric stenosis and achalasia are associated with loss of nitrenergic nerves [55], in keeping with findings in mice deficient in the gene for nNOS, which have grossly distended stomachs and hypertrophy of the pyloric circular muscle [38]. Abnormalities of peripheral nitrenergic nerves are also found in erectile dysfunction [39]. In the central nervous system, relatively little has been learned from human studies, but excess NO appears to protect against epilepsy in rats and could contribute to programmed cell death in the central nervous system. In Huntington's chorea, NOS-staining neurones are spared for an as yet unknown reason [56].

The role of NO in the respiratory system and in sepsis has been studied in some depth. Endotoxaemia and a number of cytokines stimulate the induction of iNOS [57]. In severe sepsis and other inflammatory states, during which widespread induction of iNOS occurs, very large amounts of NO (up to 1,000 times the physiological concentrations) are produced. Thus, increased plasma concentrations of nitrate and cGMP have been demonstrated in septic patients, and increased conversion of L-arginine to nitrate following treatment with IL-2 [58]. The uncontrolled production of NO is thought to contribute to the vascular hyporeactivity and myocardial depression that cause systemic hypotension seen in these circumstances [59–61].

The adult respiratory distress syndrome (ARDS) represents the pulmonary manifestation of a global inflammatory process, in which widespread induction of iNOS probably occurs in the injured lung [62]. It is defined by refractory hypoxaemia in the presence of radiographic evidence of pulmonary infiltrates [63], and is characterized by high permeability alveolar oedema and a loss of hypoxic pulmonary vasoconstriction (HPV), which produces a marked increase in V'/Q' mismatch [64]. The uncontrolled release of iNOS-derived NO may contribute to its pathogenesis. Additionally, a loss of local vascular regulation, vasoconstriction and mechanical blockage of vessels all cause a reduction in the cross sectional area of the pulmonary vascular bed, an increase in PVR, pulmonary hypertension and consequent right ventricular dysfunction, which can lead to impaired left ventricular function [65, 66].

Pulmonary hypertension, whether primary or secondary, is characterized by increased thickening of the walls of pulmonary arteries, narrowing of the pulmonary arterial luminal diameter and increased PVR [67, 68]. Endothelially-derived vasoactive factors mediate pulmonary vascular control [69]. Exogenous NO, administered by inhalation, has successfully reduced PVR and improved refractory hypoxaemia in children with persistent pulmonary hypertension of the newborn (PPHN) [70]. Reduced expression of NOS has been demonstrated in the pulmonary arteries of patients with primary and secondary pulmonary hypertension [24]. An inverse correlation was shown between diminished eNOS mRNA expression and the morphological severity of pulmonary hypertension. These findings represent an elegant addition to the existing literature, although the question of whether endothelial dysfunction (*i.e.* diminished basal NO production) leads to the development of pulmonary hypertension (as a result of the subsequent vasoconstriction,

in situ thrombosis and vascular smooth muscle proliferation caused by the lack of NO [71]) or is the consequence of secondarily elevated pulmonary arterial pressures remains unresolved [72]. Nevertheless, progression of the disease is accompanied by further endothelial disruption that itself promotes disease progression.

In normal airways, NO limits bronchoconstriction through the nitrenergic neural bronchodilator mechanism *via* prevailing cNOS expression. By contrast, in asthma, an inflammatory disorder of airways, increased levels of exhaled NO [73] are thought to be produced following induction of iNOS, and bronchial biopsy material displays increased expression of iNOS compared with that from nonasthmatics [74]. Furthermore, exhaled NO concentrations increase during the inflammatory late response to allergen [75] and in acute exacerbations of asthma [76]; and are reduced by inhaled and oral glucocorticoids known to inhibit iNOS induction, but not cNOS mRNA expression [77]. Interestingly, NO production does not appear to reflect asthma severity directly nor relate directly to airway hyperresponsiveness to methacholine challenge. Taken together, this suggests that the role of NO in asthma is more likely to be as a marker of cytokine-mediated airway inflammation than as a direct detrimental influence on airway function. Whether NO contributes to the airway narrowing (secondary to vasodilatation and plasma exudation) in the late response to allergen is unknown.

Further work demonstrating increased exhaled NO levels in a rodent model of endotoxic sepsis syndrome, and in patients with bronchiectasis (NO levels correlate with disease extent) [78] suggests that exhaled NO is likely to be an important marker of airway inflammation in pulmonary disease.

Therapeutic potential of NO

NO donors

Amyl nitrate was used in the treatment of asthma as long ago as 1866 [79], and is widely quoted as the earliest therapeutic use of a NO donor in respiratory disease. NO is also the active moiety of glyceryl trinitrate and nitroprusside, which act as nitrovasodilators. Glyceryl trinitrate preferentially dilates veins, and its relative venoselectivity can be explained by the low basal output of NO in venous smooth muscle, with an upregulation of guanylate cyclase enabling an enhanced response to exogenously administered NO [80]. Other drugs increase the production of endogenous NO, such as inhibitors of angiotensin-converting enzyme (ACE) that block the breakdown of bradykinin, which in turn stimulates endothelial release of NO. This may contribute to their anti-hypertensive properties.

L-arginine

The therapeutic potential of L-arginine, the substrate for NOS, in fuelling NO production is now being realized; reports suggest that arginine prevents the onset of atheroma in experimental models, and restores certain aspects of endothelial function in humans [81]. It has also been shown to improve endothelially-derived vasodilator function in animal models of pulmonary hypertension [82], and a deficiency of L-arginine has been

shown in PPHN [83]. Clearly, a prerequisite would be sufficient NOS present for the substrate; a deficiency of NOS, as demonstrated in patients with pulmonary hypertension, would suggest the use of inhaled NO as an alternative therapy. The potential therapeutic effect of providing L-arginine supplements in parenteral nutrition, to fuel NOS in septic patients, has been raised [84], although, in this situation, an abundance of NO prevails through the induction of iNOS.

Inhaled NO

Inhaled NO has been used in the intensive care setting to treat a number of disorders, including ARDS, primary pulmonary hypertension, PPHN, and pulmonary hypertension in congenital heart disease, together with post/perioperative pulmonary hypertension in paediatric and adult cardiac surgery and pulmonary hypertension associated with exacerbations of chronic obstructive pulmonary disease (COPD) [85–88].

The concept that inhaled NO produces selective pulmonary vasodilatation is an attractive one. A lack of pharmacological specificity for the pulmonary circulation becomes less relevant, so long as the agent is inactivated prior to its reaching the systemic circulation. Conventional intravenous vasodilator therapy to reduce PVR and improve hypoxaemia and right ventricular dysfunction in patients with pulmonary hypertension is limited by two problems. Firstly, the global pulmonary vasodilatation induced by intravenous agents may abolish the protective effect of HPV in underventilated alveoli, causing further deterioration in V/Q matching and worsening hypoxaemia. Secondly, the doses required to produce a beneficial effect in the pulmonary vascular bed frequently cause systemic vasodilatation and cardiovascular instability. The action of inhaled NO, which is rapidly inactivated by haemoglobin, is thus confined to the area of deposition, recruiting blood to functional lung units to which the inspired gas has access, without deleterious systemic effects. This should lead to a simultaneous improvement in V/Q matching, a reduction in shunt fraction and a fall in PVR [89]. Indeed, early animal studies using inhaled NO (at concentrations of 5–80 parts per million (ppm)) in acute hypoxic and pharmacologically-induced pulmonary hypertension demonstrated rapidly reversible pulmonary vasodilatation, with no systemic effects [90, 91].

The effect of inhaled NO in human volunteers during hypoxia was similar, except that much lower concentrations (10 ppm) of NO were required for maximum effect [92]. In a retrospective trial of inhaled NO in ARDS, concentrations of 18 ppm for 40 min produced significant reductions in mean pulmonary artery pressure, and an increase in arterial oxygen tension (P_{a,O_2}) to fraction of inspired oxygen (F_{I,O_2}) ratio, enabling F_{I,O_2} to be reduced by 15% [93]. Similar results have been reported at lower concentrations (100 parts per billion (ppb) to 18 ppm) [94, 95], although no study has yet demonstrated a clear reduction in mortality or morbidity. A trend towards reduced mortality has been shown in a subgroup of inotrope/vasopressor resuscitated septic patients with ARDS, who responded to inhaled NO (>20% rise in arterial oxygen tension (P_{a,O_2}) and/or >15% fall in mean pulmonary artery pressure) [96]. A

response to NO was associated with increased right ventricular function, other cardiac indices and improved oxygen delivery; while lack of response to NO and higher mortality were characteristic of patients with depressed cardiac reserves.

However, there is also some evidence of impaired gas exchange after the use of inhaled NO from small clinical trials of patients with ARDS [97, 98]. Trials are currently ongoing to address outcome, although death may be an insensitive end-point for ARDS, due to the influence of its diverse aetiologies on mortality [99].

There is also a growing body of evidence of the beneficial role of inhaled NO in PPHN. Outside the intensive care setting, use of inhaled NO in patients with COPD and secondary pulmonary hypertension produced pulmonary vasodilatation, although P_{a,O_2} remained unchanged [88]. The potential for preventing, or reducing, right ventricular dysfunction in this context, akin to that seen in long-term oxygen therapy, is an enticing prospect if the safety of long-term inhaled NO can be addressed and appropriate delivery systems designed.

However, inhaled NO therapy may well be detrimental in chronic respiratory failure [100]. Thus, worsening pulmonary gas exchange with inhaled NO (40 ppm) has been demonstrated in 13 advanced COPD patients, due to a reversion of HPV and subsequent deterioration of V'/Q' matching. The authors speculated that differences in NO responsiveness between COPD and ARDS may be due to the underlying mechanism of hypoxaemia. It seems that patients with prominent intrapulmonary shunt contributing to hypoxaemia, as in ARDS, are more likely to benefit from the selective pulmonary vasodilatory effect of inhaled NO. By contrast, COPD is characterized by broad heterogeneity in V'/Q' but negligible shunt, and inhaled NO might inappropriately dilate pulmonary vessels in chronically underventilated alveolar lung units, thus worsening V'/Q' matching.

Inhaled NO also has a beneficial role in reducing the morbidity associated with high altitude pulmonary oedema [101]. An improvement in hypoxia-induced pulmonary hypertension and arterial oxygenation is thought to occur through a favourable action of inhaled NO on redistribution of pulmonary blood flow from oedematous to nonoedematous alveolar units.

It is clear that the potential benefit to be gained by the clinical use of inhaled NO may be tempered by its detrimental effects in certain conditions. This necessitates careful risk/benefit analysis prior to use and identification of the lowest effective clinical doses for each indication. Indeed, concerns over toxicity have led to an interest in alternative short-acting inhaled vasodilator, such as nebulized prostacyclin. A recent study in ARDS has suggested that inhaled NO and nebulized prostacyclin have similar efficacy [102].

NOS inhibition

NOSs have been invaluable in unravelling the pathophysiological effects of NO, and have been proposed as therapeutic interventions in inflammatory conditions where excessive production of NO by iNOS is deleterious, such as septic shock, ARDS and asthma. Substrate analogues, such as L-NMMA and L-NAME, reverse the local inflammation-associated vasodilatation and pro-

found hypotension seen in experimental models of septic shock. In patients with septic shock supported by large doses of inotropes, L-NMMA and L-NAME were first shown to restore blood pressure by increasing PVR [19]. Similar results were demonstrated in a series of 15 patients with septic shock, but there was an associated fall in cardiac output, and no conclusions could be drawn regarding morbidity and mortality. Methylene blue, a guanylyl cyclase inhibitor, has been used with similar haemodynamic effects in small studies. A major drawback of such nonselective NOSs is the detrimental effect of inhibiting constitutive (endothelial) NOS, potentially leading to systemic hypertension and vasospasm [103, 104]. Aminoguanidine is 10–100 times more selective for iNOS inhibition than L-NMMA and L-NAME, but toxicity remains a major drawback to its use in clinical trials in septic shock. The development of selective iNOS inhibitors, with the beneficial effects of inhibiting excessive NO production in inflammatory disorders without the systemic effects of inhibiting cNOS, is in progress.

Exhaled NO

Exhaled NO has been proposed as a simple, noninvasive means of measuring airway inflammation [77, 105]. Because of its nonspecific increase in inflammation due to asthma, bronchiectasis and respiratory tract infections [78]; it has been suggested that serial measurements, rather than absolute values may be useful in monitoring whether treatment is adequate, or indeed whether newer anti-inflammatory inhaled medications are effective. This would require the development of smaller, inexpensive NO analysers.

Toxicity and safe use of NO

High concentrations of inhaled NO are likely to have toxic effects resulting from the formation of nitrogen dioxide (NO_2 , which dissolves to form toxic nitrous oxide), peroxy nitrite radicals (in the presence of superoxide), and methaemaglobinaemia (interaction of nitrate and haemoglobin, causing a reduced oxygen carrying capacity). The deleterious pro-oxidant outcome *versus* the cytoprotective antioxidant outcome seems critically dependent on the prevailing oxygen concentrations and relative concentrations of individual ROS. NO combines with oxygen in high FI_{O_2} s to produce potentially toxic NO_2 , and levels in inhaled gas should, therefore, be measured using fuel cell analysers and soda lime to remove excess. Inhalation circuits also accurately measure inspired NO concentrations by chemiluminescence or electrochemical analysers. Although there is evidence to suggest that NO is an effective scavenger of ROS [106] and may inhibit xanthine oxidase [107] (and so reduce ROS); under hypoxic conditions, NO reacts with superoxide to form peroxy nitrite [108], which damages lipids, surfactants, nucleic acids and proteins through its pro-oxidant capacity. However, frequently quoted toxicity studies have been carried out principally in nondiseased animals exposed to inhaled NO [109]. Thus, no histological, ultrastructural or gravimetric evidence of pulmonary toxicity was evident after exposure of rats to 1,000 ppm NO for 30 min [116], rabbits to 43 ppm for 6 days [111], or long-term studies in mice for 6 months [112]. This lack

of demonstrable pulmonary toxicity may have little relevance to the clinical setting, in which the propensity of inflamed lungs to produce ROS in the presence of high $F_{I,O_2,S}$ would appear to be considerable. Until further information regarding the toxic potential of inhaled NO in the inflammatory lung setting emerges, cautious monitoring of the products derived must continue in the presence of the lowest effective concentrations of inspired NO. Another important safety issue pertains to slow weaning of inhaled NO therapy in acute hypoxic pulmonary hypertension in view of the rebound effect of its rapid withdrawal.

Summary

Nitric oxide is an important inter- and intracellular mediator, governing a range of physiological functions in animals and humans, from controlling smooth muscle tone in the cardiovascular, gastrointestinal, respiratory and genitourinary systems, to neurotransmission and a role in immune function and inflammation. Overproduction can lead to deleterious effects in severe inflammatory disorders. The role of nitric oxide as a therapeutic agent in conditions in which local endogenous supply is diminished (e.g. pulmonary hypertension) is also emerging. Exhaled nitric oxide may in future, be regarded as a clinically useful marker of lung inflammation. Selective inducible nitric oxide synthase inhibitors are under development.

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