

## L-arginine-dependent nitric oxide synthase: a new metabolic pathway in the lung and airways

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**ABSTRACT:** An L-arginine-dependent pathway, metabolising L-arginine to citrulline and nitrogen oxides, has been described in many cell types in different species, including man. Two subtypes of this nitric oxide synthase have been reported: a constitutive enzyme type, releasing nitric oxide after stimulation, is typically found in endothelial and neural cells; another subtype can be induced in macrophages after cytokine treatment.

This review summarizes the literature on the known and proposed roles of this L-arginine-dependent nitric oxide production in different pulmonary processes.

Nitric oxide has been reported to act as a neurotransmitter in the inhibitory nonadrenergic, noncholinergic nerves in the airways of guinea-pig and man. It is released in cytostatic processes by immune-stimulated alveolar macrophages. Recent data on the role of L-arginine-dependent processes in immune-complex-mediated lung injury, histamine-induced activation of guanylate cyclase or cytokine networks in the lung are also discussed. Finally, similarities and differences between tracheal epithelium-derived relaxing factor and nitric oxide are analysed.

The details of the role and distribution of nitric oxide synthase in the (human) lung and airways are not yet completely understood. Nitric oxide is believed to play a role in various pulmonary physiological processes, such as bronchodilation and the cytotoxic action of certain cells. The modulation of nitric oxide release will therefore, most probably lead to application of novel therapies in diseases such as asthma and inflammatory pulmonary diseases.

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In 1987, a new metabolic pathway was described both in the vascular endothelium [1], and in activated murine macrophages [2, 3]. The products of this enzymatic pathway are L-citrulline and the highly reactive and unstable nitric oxide (NO), which decomposes into other nitrogen oxides, such as nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and the potent oxidising agent peroxynitrite (ONOO<sup>-</sup>) (fig. 1). The enzyme responsible for the production of these nitrogen oxides has been named nitric oxide synthase [4]. From the first moments of its discovery, it was seen to be L-arginine-dependent. Indeed, NO is synthesised from the terminal guanidino nitrogen atom of the L-arginine (and not the D-arginine) molecule [4].

Soon it became clear that this metabolic pathway exists in various cells from different embryological origin, and that two forms of NO synthase can be distinguished, based on their mode of NO release [5]. A first subtype is a constitutive, cytosolic calcium-dependent enzyme, releasing nitric oxide after receptor or physical stimulation: this has been found in the vascular endothelium, platelets, neutrophils neural

tissue. The other subtype is an inducible, calcium-independent enzyme, which is found following induction with cytokines, in macrophages, vascular smooth muscle cells, cardiac myocytes, endothelial cells, and fibroblasts, as well as certain epithelial and adenocarcinoma cells [4, 5].

Nitric oxide is a highly reactive and unstable substance with a very short half-life. Except for on-line chemiluminescence, it is mainly measured by indirect techniques (fig. 1). Since NO accounts for the biological activity of endothelium-derived relaxing factor (EDRF), and also inhibits platelet aggregation, both vascular tissue and platelets can be used as indicators for the NO production by other cells. Haemoglobin and other haeme proteins, which absorb nitric oxide to form paramagnetic detectable nitroso-haeme products, and superoxide dismutase, which prolongs NO action by preventing its breakdown to other nitrogen oxides, are then used to further modulate pharmacological effects. Another indirect method is the spectrophotometric determination of nitrite and nitrate, two nitrogen oxides rapidly formed during NO

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oxidation. Considerable evidence has emerged suggesting that the effects of NO in some physiological processes are mediated through the activation of guanylate cyclase, resulting in an increased level of cyclic guanylate monophosphate (cGMP) in the target cell [4, 5]. Therefore, cGMP accumulation in cultured rat lung fibroblasts is frequently used as a model to study the ability of cells to produce NO [6, 7]. Finally, a class of L-arginine analogues is known to inhibit nitric oxide synthase in either a competitive or irreversible way, rendering them useful for exploring the role of L-arginine dependent nitric oxide production.

Recent observations suggest that this metabolic pathway is present in different pulmonary cell-types, and that it may play a role in various regulatory mechanisms in airway and lung tissue. This review will focus on the known and proposed mechanisms of the regulation and role of nitric oxide production in the lung and airways, in humans, as well as in other animal species.

The role of nitric oxide in the pulmonary vascular bed, *i.e.* the impairment of its release in chronic obstructive lung disease [8], and its possible therapeutic use in pulmonary hypertension [9], has recently been reviewed in *The European Respiratory Journal* [10]; it will, not therefore, be considered in this review.

#### A role for nitric oxide in nonadrenergic, noncholinergic neural control in the airways

In addition to the classical cholinergic bronchoconstrictor and adrenergic bronchodilator neural mechanisms, evidence supports the existence, in the airways of different animal species and man, of neural pathways which are neither adrenergic nor cholinergic, the so-called nonadrenergic, noncholinergic (NANC) nerves [11, 12]. NANC stimulation may either induce contraction (excitatory NANC), or relaxation (inhibitory NANC) [12]. While excitatory NANC responses

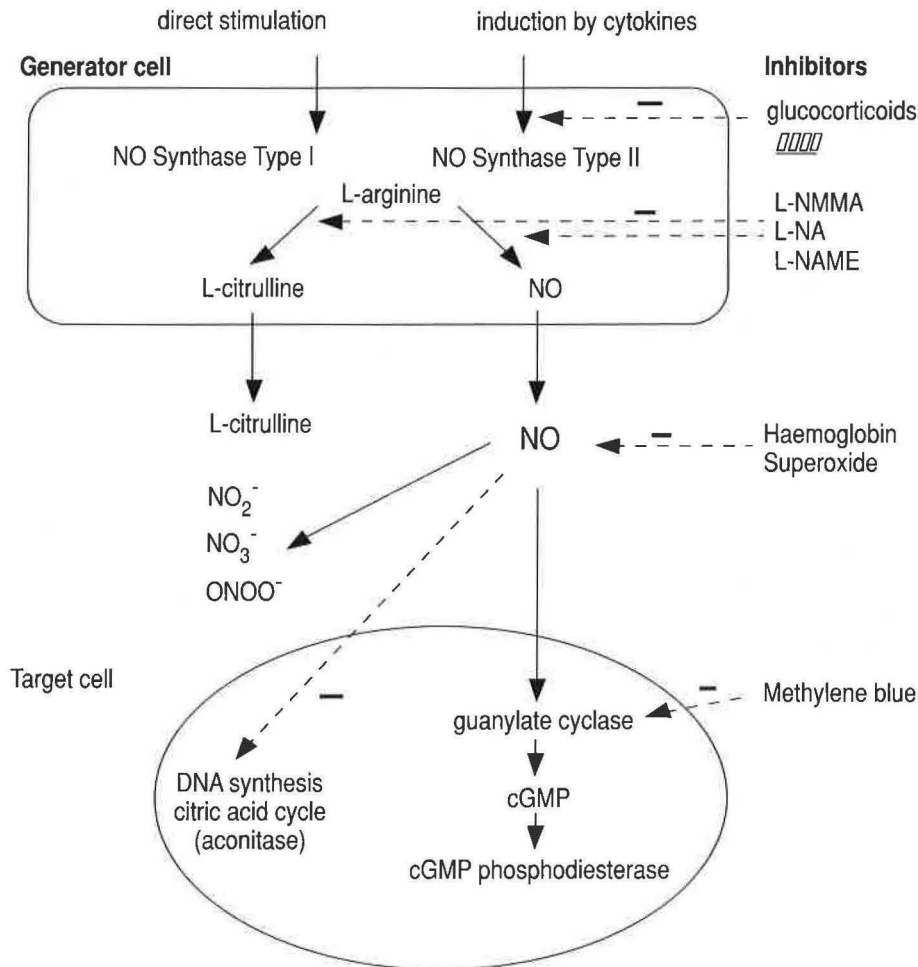


Fig. 1. - Schematic diagram of the proposed L-arginine-dependent nitric oxide synthase pathway. NO is formed in the generator cell from L-arginine, either after direct stimulation (NO synthase type I), or after induction with cytokines (NO synthase type II). After diffusion out of the cell, NO can react with oxygen radicals and is then vulnerable to degradation to nitrogen oxides. NO can stimulate soluble guanylate cyclase activity in certain target cells or inhibit deoxyribonucleic acid (DNA) synthesis and aconitase activity (citric acid cycle) in tumour cells. The induction of NO synthase type II is suppressed by glucocorticoids. Both subtypes of the enzyme NO synthase are suppressed by certain analogues of L-arginine (such as L-NMMA, L-NA, L-NAME). The pathway is also inhibited by haemoglobin and superoxide anion, which inactivate NO, and by methylene blue, a guanylate cyclase inhibitor. L-NMMA: L-N<sup>G</sup> monomethyl arginine; L-NA: L-N<sup>G</sup> nitro-arginine; L-NAME: L-N<sup>G</sup> nitro-arginine methylester; ONOO<sup>-</sup>: peroxyntirite.



are believed to be mediated by the release of different neuropeptides, including substance P, neurokinin A and calcitonin gene-related peptide, the inhibitory NANC responses are believed to be mediated, at least in part, by the release of vasoactive intestinal peptide (VIP) and related peptides, such as peptide histidine isoleucine/methionine. However, ELLIS and FARMER [13] have shown that  $\alpha$ -chymotrypsin, an enzyme capable of degrading peptides, only reduces inhibitory responses to NANC stimulation in the guinea-pig airways by about 35%; raising the possibility that at least part of the response is mediated by a molecule other than a protein. Therefore, it seemed reasonable to assume that NO might play a role in inhibitory NANC neurotransmission in the airways, comparable to its proposed role as a neurotransmitter of the NANC nerves in the anococcygeus muscle [14], and the gut [15].

In the airways of guinea-pigs, the peptidase-resistant component of the NANC inhibitory response is attenuated in a concentration-dependent manner by the L-arginine analogue, L-N<sup>G</sup> nitro-arginine (L-NA), but not by D-N<sup>G</sup> nitro-arginine, and can be reversed by L-but not D-arginine. It is suggested that this effect is presynaptic, because at a concentration producing a 74% reduction in the electrically-induced NANC relaxation, L-N<sup>G</sup> nitro-arginine is without effect on relaxations produced by submaximal concentrations of VIP [16].

LI and RAND [17] demonstrated that L-N<sup>G</sup> monomethyl arginine (L-NMMA) and L-N<sup>G</sup> nitro-arginine methylester (L-NAME), two more competitive inhibitors of NO synthase, also reduce relaxations of guinea-pig tracheal smooth muscle elicited by electrical stimulation of NANC nerves; with L-NAME being 10–30 times more potent than L-NMMA. Recent data also suggest that NO could mediate NANC relaxation of tracheal smooth muscle in other species, such as the pig [18]. Moreover, in guinea-pig airways, L-NAME significantly enhances cholinergic neuro-transmission in the trachea. Therefore, NO could also be an endogenous modulator of the cholinergic neurotransmission in guinea-pig airways which co-exists with NANC innervation in this species [19]. Thus, NO may be co-released with acetylcholine, and act as a braking mechanism for cholinergic reflex bronchoconstriction [19].

Recently, these studies have been extended to human airways, where the prominent inhibitory NANC is the only neural bronchodilator mechanism known. BELVISI *et al.* [20] initially reported that L-NMMA only partially blocked these inhibitory NANC responses in human trachea, but later reported that L-NAME inhibits the tetrodotoxin-sensitive (*i.e.* neural) dilator response to neural stimulation at all frequencies tested in tracheal rings from normal donors for heart-lung transplantation [21]. L-arginine, but not D-arginine, reversed this inhibitory effect of L-NAME. Moreover,  $\alpha$ -chymotrypsin, which degrades VIP and related peptides, had no inhibitory effect in humans [21], contrasting with guinea-pig tracheal neural

responses [21]. BAI *et al.* [22] found that nitric oxide mediates a component of the inhibitory response after stimulation of inhibitory NANC neurons in bronchial smooth muscle, although this inhibition (by L-NAME) was less than 50% of the NANC relaxation in precontracted human bronchial tissues, implying that other mediators are involved in the relaxant response. These data indirectly suggest that NO is a (or the?) neurotransmitter of bronchodilator nerves in human airways.

Nitric oxide-containing vasodilators such as glyceryl-trinitrate and isosorbide dinitrate act as nonspecific smooth muscle relaxants, activating guanylate cyclase and raising cGMP levels [5]. Nitrates have been reported to be effective as relaxants of bovine airway smooth muscle *in vitro* [23], and intravenously administered glyceryl trinitrate was reported to relax the tracheal smooth muscle in nonasthmatic, anaesthetized humans undergoing cardiac surgery [24]. However, reports about the clinical efficacy of sublingual glyceryl trinitrate and isosorbide dinitrate in the treatment of asthma are conflicting [25–30].

S-nitrosothiols are bioactive NO-adducts, which form readily, under physiological conditions, from the reaction of endogenously-derived nitrogen oxides with reduced thiols [31]. These compounds have half-lives that are significantly greater than the unstable NO-radical itself. They have been shown to relax both precontracted guinea-pig tracheal rings [32], and human peripheral bronchi [33], an effect which is mediated in part by activation of guanylate cyclase. A study published by DUPUY *et al.* [34] demonstrated that inhalation of low concentrations of NO rapidly reversed methacholine-induced bronchoconstriction in anaesthetized and mechanically-ventilated guinea-pigs. Inhaling S-nitroso-N-acetyl-penicillamine, an NO donor molecule, also induced prompt and long-lasting bronchodilation. Nitric oxide and NO-donors have, therefore, potential for pharmacological application as bronchodilators, and their utility in the treatment of airflow obstruction certainly deserves further consideration.

All of these investigations indicate that NO, or a NO-like product, might play a role in NANC-induced relaxations of tracheal smooth muscle in different species. They give no answer yet to the questions whether NO: 1) may mediate the smooth muscle relaxant activity of other putative NANC neurotransmitters, such as VIP [35]; 2) may be co-released with transmitters such as VIP from the same neurons [36]; or 3) may even be released from other than neuronal pulmonary cells.

#### **Immune-stimulated alveolar macrophages release L-arginine derived nitric oxide**

KNOWLES *et al.* [37] found that a calcium-independent NO synthase is induced in the lungs of rats treated with lipopolysaccharide (LPS), but it was not clear in which cell type this NO synthase was induced.



Recent work in our laboratory shows that pulmonary rat macrophages, alveolar as well as pleural, can produce L-arginine derived nitrite in a dose- and time-dependent manner, after activation with endotoxin, rat recombinant interferon- $\gamma$  (IFN- $\gamma$ ) and opsonized zymosan *in vitro* [38]. The L-arginine analogue L-NMMA is effective in inhibiting this production.

Consistent with the fact that glucocorticoids are able to inhibit the induction of nitric oxide synthase in the lung *in vivo* after LPS treatment [39], we were able to demonstrate that these anti-inflammatory agents block the induction of nitrite in alveolar macrophages *in vitro* by all of the stimuli mentioned [38]. The mechanism underlying this inhibition is suggested to be receptor-mediated, since the addition of equimolar concentrations of corticosterone (the partial agonist of the glucocorticoid receptor) [38] or RU 38 486 (a complete antagonist of the receptor) [40] to the incubations with dexamethasone partially reverse this inhibitory effect. These data confirm reports on the ability of glucocorticoids to inhibit nitric oxide synthase in murine macrophages [41], and suggest that part of the anti-inflammatory effect and immunosuppressive effects of glucocorticoids are due to their inhibition of the induction of NO-synthase. In the meantime, we have been able to extend these observations by showing that two constituents of food components, soybean trypsin inhibitor and  $\beta$ -amylase, are capable of inducing this L-arginine dependent nitrite production in rat alveolar macrophages *in vitro* in a dose- and time-dependent manner [42]. Whether this *in vitro* observation may explain, in part, the previously observed anti-carcinogenic effects of certain food products, by inducing the production of the cytotoxic molecule NO in phagocytic cells, remains to be established.

Many authors [2, 43] have shown that mouse peritoneal macrophages are cytotoxic to tumour cells and microorganisms *in vitro*, via this L-arginine-dependent pathway, by inactivating iron/sulphur centres in key enzymes associated with cellular respiration and deoxyribonucleic acid (DNA) synthesis. Few studies have been conducted to investigate whether this pathway plays a role in alveolar macrophages. This is of practical importance, since alveolar macrophages are known to play an important role in pulmonary host defence mechanism through the release of numerous secretory products, such as oxygen radicals, enzymes, anti-proteases, cytokines and bioactive lipids [44].

A recent study by BARRATT *et al.* [45] reported that simultaneous application of the immune-activating agent, muramyl dipeptide, and endotoxin, to rat alveolar macrophages *in vitro*, induced cytostatic activity against a rat fibrohistiocytoma line, mediated by the L-arginine NO-generating pathway.

Ozone inhalation increases nitric oxide release from rat alveolar macrophages harvested 48 h following inhalation. HUOT and HACKER [47] were able to prove that alveolar macrophages, harvested by bronchoalveolar lavage from rats exposed to intratracheal

instillation of a single fibrogenic dose of bleomycin, are able to express cytostatic activity for co-cultured neoplastic cells. This is linked to the release of significant amounts of nitrites. Erythrocytes inhibit this alveolar macrophage cytostatic activity, by preventing reactive nitrogen intermediates from reaching target cells, because haemoglobin serves as a sink for reactive nitrogen oxides [48]. Therefore, it seems that reactive nitrogen oxides are important in the cytostatic mechanism of macrophages in certain *in vivo* models of lung injury.

Although fungistasis in mice peritoneal macrophages and tumour cell killing in mice and rat macrophages *in vitro* requires L-arginine, human alveolar macrophage-mediated fungistasis *in vitro* does not require L-arginine, and human alveolar macrophages do not produce amounts of nitrite and nitrate detectable by colorimetry in co-cultures with *Cryptococcus neoformans* [49]. This absence of L-arginine-dependent nitrogen oxides in human alveolar macrophages, (compared to mice peritoneal macrophages), during conditions under which fungistasis occurs suggests that this phenomenon is species-specific, rather than specific to the tissue origin of the macrophage. Various explanations are possible for this observed difference. The lack of demonstrable NO in human macrophages, suggests that NO synthase may be absent in these cells, may respond to alternative stimuli, or may not produce extracellular nitric oxide [50]. Reduced biopterin (5,6,7,8 tetrahydrobiopterin) is required for full-activity of cytokine-induced NO synthase in the murine peritoneal [51] and the rat alveolar [52] macrophage, but human macrophages lack the enzymatic system necessary to produce reduced biopterin [53]. This does not exclude the existence of this system in alveolar macrophages *in vivo*, since reduced biopterin or other co-factors could be obtained *in vivo* from an exogenous biological source. This may not completely account for the difference between human and murine macrophages, since human macrophages treated with interferon- $\gamma$  plus endotoxin, and reconstituted with intracellularly-delivered biopterin, produce no nitrite or nitrate [54].

Recently, SHERMAN *et al.* [55] reported that human recombinant interferon- $\gamma$  increases the citrulline content, but not the nitrite content, in the *in vitro* culture media of human alveolar macrophages; they propose that citrulline production is a main indicator of L-arginine oxidation. In contrast to this cytokine, *Pneumocystis carinii* when placed in co-culture with human alveolar macrophages does induce a small but detectable nitrite production. [55]. This raises the possibility that NO may indeed play a role as a microbicide, produced by human alveolar macrophages, against certain opportunistic pathogens; a mechanism comparable to the one proposed for human blood monocytes *in vitro* inactivation of *Mycobacterium avium* [56].

Chloromethyl ketone derivatives, a class of molecules co-valently binding to the active site of serine proteases, are able to block nitrite production of



immune-stimulated rat alveolar macrophages *in vitro* [57]. These inhibitors may, therefore, be useful in further defining the role of nitric oxide synthase production in (alveolar) macrophages.

### Cytokine networks and nitric oxide

The development of pulmonary inflammation involves a series of cellular events controlled by a variety of mediators, including cytokines. During pulmonary inflammation, a number of these cytokines are expressed and released into the lung and airways. Therefore, the interaction between these cytokines in cytokine networks may play an important role in the modulation of inflammation in the local environment.

Granulocyte macrophage-colony-stimulating factor (GM-CSF) and muramyl dipeptide, a constituent of the bacterial cell wall, are able to enhance IFN- $\gamma$ -induced nitrite production in rat alveolar macrophages *in vitro*, with GM-CSF serving as a priming factor [58]. The interleukins 1, 3 and 4 and tumour necrosis factor are devoid of such activities in rat alveolar macrophages [58].

Results from our laboratory indicate that not only rat alveolar macrophages, but also rat lung fibroblasts, are capable of producing nitrite upon stimulation with IFN- $\gamma$ . This effect is markedly enhanced in fibroblasts by the addition of endotoxin and interleukin-1 $\beta$  [59]. Interleukin-1 $\beta$  can also serve as an efficient priming signal for this IFN- $\gamma$  induced nitrite production. The nitric oxide synthase pathway, therefore, provides an excellent tool for exploring the interaction between cytokines in the lung. Several recent observations suggest that nitric oxide may play a role in pulmonary cell-to-cell interactions and critical defence mechanisms of the host: pyocyanine, a pigment produced by *Pseudomonas aeruginosa*, can inhibit the release of reactive nitrogen intermediates from murine alveolar macrophages [60]; peroxy-nitrite inhibits rat alveolar type II cell respiration [61]; the immunosuppressive effect of cultured rat alveolar macrophages on mitogen-induced proliferative responses of splenic lymphocytes involves the L-arginine dependent nitric oxide pathway [62].

### Other L-arginine dependent processes in the lung

Acute or chronic lung injury has been linked to the presence of proteases and oxygen radicals released from activated phagocytic cells. Recent data suggest that the free radical NO may be added to the list of cytotoxic molecules involved in lung injury. Indeed, L-NAME protects rats against immune-complex-induced lung and skin vascular injury [63], a process which is believed to be polymorphonuclear- and oxygen-radical-dependent. This protective effect is reversed by the presence of L- but not of D-arginine. Additionally, in the absence of L-NAME, this observed injury is enhanced by the presence of L- but not D-arginine. That nitrite and nitrate are found in

high concentrations in the bronchoalveolar lavage (BAL) fluid of animals undergoing immune complex deposition, supports this hypothesis [63].

Histamine seems to be responsible for the production of cGMP both in human [64], and guinea-pig lung tissue [65]. LEURS *et al.* [65] showed that this H<sub>1</sub>-receptor-mediated cGMP response is potently inhibited in the guinea-pig lung by haemoglobin. Their experiments suggest that after an initial H<sub>1</sub> receptor-mediated phospholipase C-dependent, calcium mobilization, the enzymatic conversion of L-arginine to NO is stimulated. This NO production may then cause the increased cGMP production, extending data reported by MAYER and BOHME [66] that the (bovine) lung is capable of converting L-arginine to an activator of guanylate cyclase. Since cGMP has been reported to modulate the immunologically induced release of mediators from mast cells, through an effect on the microtubular assembly [67], this might suggest a role for NO-mediated guanylate cyclase activation in allergic diseases.

Endogenous nitric oxide has been found in expired air samples from rabbit, guinea pig and human, utilising different techniques including chemiluminescence [68]. Although the exact origin remains to be determined, it is likely that NO is produced in the lung during different processes, such as vascular regulation and in host defence. Indeed, many cells apart from endothelium and macrophages, known to play a role in lung defence and injury, have been described as using L-arginine for their cytotoxic action (lymphocytes [69]), or to release NO *in vitro* (neutrophils [70]; platelets [71]; monocytes [70], as well as mast cells [72, 73]).

### Epithelium-derived relaxing factor is probably not nitric oxide

In the past, the airway epithelium was regarded as mainly passive, thereby functioning as a barrier. It is now known that the epithelium actively participates in many airway functions, and that various agents can induce the release of an inhibitory substance from airway epithelium, the so-called epithelium-derived relaxing factor (EpDRF). This substance is capable of relaxing certain tissues, and regulating the tone of the underlying airway smooth muscle, therefore attenuating airway smooth muscle responsiveness [74]. Such a system may, therefore, function in a manner analogous to that described for the influence of the endothelium on vascular smooth muscle by releasing EDRF or nitric oxide. Recent studies on the effect of removal of the airway epithelium on underlying smooth muscle contraction strongly suggest the existence of such an EpDRF, but its identity remains unclear. In some studies, the production of guinea-pig and human tracheal bronchial epithelium-derived relaxing factor has been demonstrated in the presence of inhibition of cyclo- and lipooxygenase, ruling out a prostaglandin as the relaxant responsible [74, 75]. A saturated solution of nitric oxide is able to cause



concentration-dependent and complete relaxation of nonvascular smooth muscle relaxation including bovine epithelium denuded airway strips [76]. Since it is speculated that the airway epithelium has a similar role as endothelium in vascular tissue, nitric oxide is a good candidate for being the epithelium-derived relaxing factor.

However, epithelium-dependent relaxation of vascular or gastrointestinal smooth muscle, used as a bioassay for EpDRF, is not affected by methylene blue [77, 78], which inhibits the effects of EDRF on soluble guanylate cyclase. EpDRF must, therefore, be an agent that produces endothelium-independent relaxation in these systems. Moreover, L-NMMA does not inhibit osmotic-induced epithelium-dependent relaxation in guinea-pig airways [79]. These results suggest that NO is probably not responsible for causing epithelium-derived relaxation of airway smooth muscle, although EpDRF and vascular EDRF have some pharmacological similarities. More work is required to identify the precise characteristics of EpDRF.

### Discussion

It is clear that full exploration of the role of nitric oxide suffers various limitations. Firstly, the measurement and detection of nitric oxide, due to its instability, is based mainly on indirect detection techniques. However, the recent cloning of the nitric oxide synthase enzyme will make it possible to look for its distribution and expression [80].

Secondly, although NO looks promising for measuring diffusion capacity in humans [81, 82], the main limitation is the fact that the toxicity of both the nitric oxide radical and the L-arginine analogues have not been investigated in humans or animals. When considering the investigation of the role of NO in the human lung, one should, indeed, be aware that NO is one of the main constituents of the gas phase of cigarette smoke at a concentration even >50 ppm [83–85], and is recognized as a toxic air pollutant [86]. Nitric oxide is extremely cytotoxic, which explains its toxicity to malignant cells. In addition, nitric oxide is known to cause nitrosative deamination of DNA, resulting in genomic alterations in bacteria which are comparable to those alterations that have been linked, in humans, to a variety of disorders resulting from base deamination [87]. Nitric oxide and the superoxide anion can react to form peroxynitrite, a powerful oxidant [88] and inactivator of  $\alpha_1$ -proteinase inhibitor [89], the most abundant and effective extracellular antiprotease in the lower respiratory tract.

It is likely that multiple functions of the L-arginine-dependent nitric oxide production exist in the lung, since multiple cell types of the lung, involved in different pathological processes, are capable of producing L-arginine-dependent nitric oxide (table 1). Although details about the role and distribution of nitric oxide synthase in the (human) lung are not yet known, the wide array of stimuli that are involved in both the immediate release or the induction

Table 1. – Evidence for stimulus-dependent generation of NO by pulmonary cells

Cell type	Animal species	Stimulus	First Author	Year	[Ref.]
<b>NO synthase type I (direct stimulation)</b>					
NANC neurons	Guinea-pigs	Electrical stimulation	LI	1991	[17]
NANC neurons	Guinea-pigs	Electrical stimulation	TUCKER	1990	[16]
NANC neurons	Pig	Electrical stimulation	KANNAN	1992	[18]
NANC neurons	Human	Electrical stimulation	BELVISI	1992	[21]
NANC neurons	Human	Electrical stimulation	BAI	1992	[22]
<b>NO synthase type II (induction by cytokines)</b>					
Pulmonary cells, cultured <i>in vitro</i>					
Alveolar macrophages	Rat	Endotoxin, interferon- $\gamma$ , opsonized zymosan	JOSENS	1991	[38]
Alveolar macrophages	Rat	Endotoxin	BARRATT	1991	[45]
Alveolar macrophages	Rat	Soybean trypsin inhibitor $\beta$ -amylase	JOSENS	1992	[42]
Alveolar macrophages	Human	Co-culture with <i>Pneumocystis carinii</i>	SHERMAN	1991	[55]
Alveolar macrophages	Rat	Co-culture with splenic lymphocytes	KAWABE	1992	[62]
Alveolar macrophages	Mice	Interferon- $\gamma$	SHELLITO	1992	[60]
Pleural macrophages	Rat	Endotoxin, interferon- $\gamma$	JOSENS	1991	[38]
Lung fibroblasts	Rat	Interferon- $\gamma$	JOSENS	1992	[59]
<i>In vivo</i> models of lung injury					
Whole lung	Rat	After intravenous endotoxin	KNOWLES	1990	[37]
Alveolar macrophages	Rat	After intratracheal bleomycin	HUOT	1990	[47]
Alveolar space, cell type?	Rat	Immune complex lung injury	MULLIGAN	1991	[63]
Alveolar macrophages	Rat	Following ozone inhalation	PENDINO	1992	[46]

NANC: nonadrenergic, noncholinergic.



of nitric oxide production indicate that this pathway may play an important role in the pathogenesis of different pulmonary disorders.

### References

- Palmer RMJ, Ferrige AG, Moncada S. – Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524–526.
- Hibbs JB, Taintor RR, Vavrin Z. – Macrophage cytotoxicity: role for L-arginine deaminase and imino nitrogen oxidation to nitrite. *Science* 1987; 235: 473–476.
- Stuehr DJ, Marletta MA. – Induction of nitrite/nitrate synthesis in murine macrophages by BCG infection, lymphokines or interferon- $\gamma$ . *J Immunol* 1987; 139: 518–525.
- Moncada S, Palmer RMJ, Higgs EA. – Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem Pharmacol* 1989; 11: 1709–1715.
- Moncada S, Palmer RMJ, Higgs EA. – Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; 443: 109–142.
- Leitman DC, Agnost VL, Tuan JJ, Andresen JW, Murad F. – Atrial natriuretic factor and sodium nitroprusside increase cyclic GMP in cultured rat lung fibroblasts by activating different forms of guanylate cyclase. *Biochem J* 1987; 244: 69–74.
- Schroder H, Leitman DC, Bennett BM, Waldman SA, Murad F. – Glyceryl trinitrate-induced desensitization of guanylate cyclase in cultured rat lung fibroblasts. *J Pharmacol Exp Ther* 1988; 245: 413–418.
- Dinh-Xuan AT, Higenbottam TW, Clelland CA, et al. – Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; 324: 1539–1547.
- Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J. – Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet* 1991; 338: 1173–1174.
- Dinh-Xuan AT. – Endothelial modulation of pulmonary vascular tone. *Eur Respir J* 1992; 5: 757–762.
- Barnes PJ. – New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989; 83: 1013–1026.
- Stretton D. – Nonadrenergic, noncholinergic neural control of the airways. *Clin Exp Pharmacol Pathol* 1991; 18: 675–684.
- Ellis JL, Farmer SG. – Effects of peptidases on nonadrenergic, noncholinergic inhibitory responses of tracheal smooth muscle, a comparison with effects on VIP- and PHI-induced relaxation. *Br J Pharmacol* 1989; 96: 521–526.
- Gillespie JS, Liu X, Martin W. – The effects of L-arginine and N<sup>G</sup> monomethyl L-arginine on the response of the rat anococcygeus muscle to NANC nerve stimulation. *Br J Pharmacol* 1989; 98: 1080–1082.
- Bult H, Boeckxstaens GE, Pelckmans PA, et al. – Nitric oxide as an inhibitory nonadrenergic noncholinergic neurotransmitter. *Nature* 1990; 345: 346–347.
- Tucker JF, Brave SR, Charamlambous L, Hobbs AJ, Gibson A. – L-N<sup>G</sup>-nitro-arginine inhibits nonadrenergic, noncholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *Br J Pharmacol* 1990; 100: 663–664.
- Li CG, Rand MJ. – Evidence that part of the NANC relaxant response of guinea-pig trachea to electrical field stimulation is mediated by nitric oxide. *Br J Pharmacol* 1991; 102: 91–94.
- Kannan MS, Johnson DE. – Nitric oxide mediates the neural nonadrenergic, noncholinergic relaxation of pig tracheal smooth muscle. *Am J Physiol* 1992; 262: L511–L514.
- Belvisi MG, Stretton D, Barnes PJ. – Nitric oxide as an endogenous modulator of cholinergic neurotransmission in guinea-pig airways. *Eur J Pharmacol* 1991; 198: 219–221.
- Belvisi MG, Stretton D, Verleden GM, Yacoub MH, Barnes PJ. – Inhibitory NANC nerves in human tracheal smooth muscle: involvement of VIP and NO. *Am Rev Respir Dis* 1991; 143: A355.
- Belvisi MG, Stretton D, Yacoub M, Barnes PJ. – Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. *Eur J Pharmacol* 1992; 210: 221–222.
- Bai TR, Bramley AM, Schellenberg RR, Pare PD. – Nitric oxide mediates nonadrenergic, noncholinergic inhibitory neural responses in human bronchial smooth muscle. *Am Rev Respir Dis* 1992; 145: A383.
- Gruetter CA, Childers CE, Bosserman MK, et al. – Comparison of relaxation induced by glyceryl trinitrate, isosorbide dinitrate and sodium nitroprusside in bovine airways. *Am Rev Respir Dis* 1989; 139: 1192–1197.
- Byrick RJ, Hobbs EG, Martineau R, Noble WH. – Nitroglycerin relaxes large airways. *Anesth Analg* 1983; 62: 421–425.
- Hirschleifer L, Arora Y. – Nitrates in the treatment of bronchial asthma. *Dis Chest* 1961; 39: 275–283.
- Miller WC, Schultz TF. – Failure of nitroglycerin as a bronchodilator. *Am Rev Respir Dis* 1979; 120: 471.
- Kennedy T, Summer WR, Sylvester J, Robertson D. – Airway response to sublingual nitroglycerin in acute asthma. *J Am Med Assoc* 1981; 246: 145–147.
- Tullett WM, Patel KR. – Isosorbide dinitrate and isoxsuprine in exercise-induced asthma. *Br Med J* 1983; 286: 1934–1935.
- Goldstein JA. – Nitroglycerin therapy of asthma. *Chest* 1984; 85: 449.
- Okayama M, Sasaki H, Takishima T. – Bronchodilator effect of sublingual isosorbide dinitrate in asthma. *Eur J Clin Pharmacol* 1989; 26: 151–155.
- Oae S, Shinhama K. – Organic thionitrites and related substances. A review. *Org Prep Proc Int* 1983; 15: 165–198.
- Jansen A, Drazen J, Osborne JA, et al. – The relaxant properties in guinea-pig airways of S-nitrosothiols. *J Pharmacol Exp Ther* 1992; 261: 154–160.
- Gaston B, Drazen JM, Jansen A, et al. – Relaxation of human airways *in vitro* by S-nitrosothiols. *Am Rev Respir Dis* 1992; 145: A383.
- Dupuy PM, Shore SA, Drazen JM, et al. – Bronchodilator action of inhaled nitric oxide in guinea-pigs. *J Clin Invest* 1992; 90: 421–428.
- Lilly CM, Stamler JS, Gaston BM, Loscalzo J, Drazen JM. – NO-synthase modulates vasoactive intestinal peptide (VIP) pulmonary relaxation in superfused guinea-pig lungs. *Am Rev Respir Dis* 1992; 145: A382.
- Sharaf HH, Said SI. – VIP relaxation of airway, pulmonary artery and aortic vascular smooth muscle is unaffected by blockade of nitric oxide synthase. *Am Rev Respir Dis* 1992; 145: A382.
- Knowles RG, Merrett M, Salter M, Moncada S. – Differential induction of brain, lung and liver nitric



- oxide synthase by endotoxin in the rat. *Biochem J* 1990; 270: 833-836.
38. Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. - L-arginine dependent production of nitrogen oxides by rat pulmonary macrophages. *Eur J Pharmacol* 1991; 200: 205-209.
39. Knowles RG, Salter M, Brooks SL, Moncada S. - Anti-inflammatory glucocorticoids inhibit the induction of nitric oxide synthase in the lung, liver and aorta of the rat. *Biochem Biophys Res Commun* 1990; 172: 1042-1048.
40. Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. - Corticosteroids prevent the induction of nitric oxide synthase activity in different pulmonary cells. In: De Deyn PP, Marescau B, Stalon V, Qureshi I, eds. Guanidino Compounds in Biology and Medicine London, Great Britain, John Libbey Publishers, 1992; 97-101.
41. Di Rosa M, Radomski M, Carnuccio R, Moncada S. - Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages. *Biochem Biophys Res Commun* 1990; 172: 1246-1252.
42. Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. - Soybean trypsin inhibitor and beta-amylase induce alveolar macrophages to release nitrogen oxides. *Biochem Pharmacol* 1992; 44: 387-390.
43. Stuehr DJ, Nathan CF. - Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J Exp Med* 1989; 169: 1543-1555.
44. Sibille Y, Reynolds HJ. - Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 1990; 141: 471-501.
45. Barratt GM, Raddassi K, Petit JF, Tenu JP. - MDP and LPS act synergistically to induce arginine-dependent cytostatic activity in rat alveolar macrophages. *Int J Immunopharmacol* 1991; 13: 159-165.
46. Pendino K, Punjabi C, Lavnikova N, et al. - Inhalation of ozone stimulates nitric oxide production by pulmonary alveolar and interstitial macrophages. *Am Rev Respir Dis* 1992; 145: A650.
47. Huot EA, Hacker MP. - Role of reactive nitrogen intermediate production in alveolar macrophage-mediated cytostatic activity induced by bleomycin lung damage in rats. *Cancer Res* 1990; 50: 7863-7866.
48. Huot EA, Kruszyna H, Kruszyna R, Smith RP, Hacker MP. - Formation of nitric oxide hemoglobin in erythrocytes co-cultured with alveolar macrophages taken from bleomycin-treated rats. *Biochem Biophys Res Commun* 1992; 182: 151-157.
49. Cameron ML, Granger DL, Weinberg JB, Kozumbo WJ, Koren HS. - Human alveolar and peritoneal macrophages mediate fungistasis independently of L-arginine oxidation to nitrite and nitrate. *Am Rev Respir Dis* 1990; 142: 1313-1319.
50. Hite RD, Smith RM. - Inducibility of human and murine mononuclear phagocyte nitric oxide synthase by lipopolysaccharide and  $\gamma$ -interferon. *Am Rev Respir Dis* 1992; 145: A284.
51. Tayeh MA, Marletta MA. - Macrophage oxidation of L-arginine to nitric oxide, nitrite and nitrate. Tetrahydrobiopterin is required as a co-factor. *J Biol Chem* 1989; 264: 19654-19658.
52. Jorens PG, Van Overveld FJ, Vermeire PA, Bult H, Herman AG. - Pterins inhibit nitric oxide synthase activity in rat alveolar macrophages. *Br J Pharmacol* 1992; 107: 1088-1091.
53. Nichol CA, Smith GK, Duch DS. - Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. *Ann Rev Biochem* 1985; 54: 729-764.
54. Werner-Felmayer G, Werner ER, Fuchs D, et al. - On multiple forms of NO synthase and their occurrence in human cells. *Res Immunol* 1991; 142: 555-561.
55. Sherman MP, Loro ML, Wong VZ, Tashkin DP. - Cytokine- and *Pneumocystis carinii* induced L-arginine oxidation by murine and human pulmonary alveolar macrophages. *J Protozool* 1991; 38: 2349-2369.
56. Denis M. - Tumor necrosis factor and granulocyte macrophage-colony stimulating factor stimulate human macrophages to restrict growth of virulent *M. avium*. Killing effector mechanism depends on the generation of reactive nitrogen intermediates. *J Leukoc Biol* 1991; 49: 380-387.
57. Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. - Serine protease inhibitors modulate nitric oxide synthase activity in rat alveolar macrophages. *Agents Actions* 1992; 36: 243-247.
58. Jorens PG, Van Overveld FJ, Bult H, Vermeire PA, Herman AG. - Muramyl dipeptide and GM-CSF enhance interferon- $\gamma$ -induced nitric oxide production by rat alveolar macrophages. *Agents Actions* (in press).
59. Jorens PG, Van Overveld FJ, Vermeire PA, Bult H, Herman AG. - Synergism between interleukin-1 $\beta$  and the nitric oxide synthase inducer interferon- $\gamma$  in rat lung fibroblasts. *Eur J Pharmacol* 1992; 224: 7-12.
60. Shellito J, Nelson, Sorensen RU. - Pyocyanine, a product of *Pseudomonas aeruginosa*, inhibits the release of reactive nitrogen intermediates from murine alveolar macrophages. *Am Rev Respir Dis* 1992; 145: A551.
61. Hu P, Zhu L, Ischiropoulos H, Matalon S. - Peroxynitrite inhibition of oxygen consumption and ion transport in alveolar type II (AT II) pneumocytes. *Am Rev Respir Dis* 1992; 145: A568.
62. Kawabe T, Isobe KI, Hasegawa Y, Nakashima I, Shikomota K. - Immunosuppressive activity-induced by nitric oxide in culture supernatants of activated rat alveolar macrophages. *Immunology* 1992; 76: 72-78.
63. Mulligan MS, Hevel JM, Marletta MA, Ward PA. - Tissue injury caused by deposition of immune complexes is L-arginine-dependent. *Proc Natl Acad Sci USA* 1991; 88: 6338-6342.
64. Platshon LF, Kaliner M. - The effect of immunologic release of histamine upon human lung cyclic nucleotide levels and prostaglandin generation. *J Clin Invest* 1978; 62: 1113-1121.
65. Leurs R, Brozius MM, Jansen W, Bast A, Timmerman H. - Histamine H1-receptor-mediated cyclic GMP production in guinea-pig lung tissue is an L-arginine-dependent process. *Biochem Pharmacol* 1991; 42: 271-277.
66. Mayer B, Bohme E. - Calcium-dependent formation of an L-arginine-derived activator of soluble guanylate cyclase in bovine lung. *FEBS Lett* 1989; 256: 211-214.
67. Kaliner M. - Human lung tissue and anaphylaxis. Evidence that cyclic nucleotides modulate the immunologic release of mediators through effects on microtubular assembly. *J Allergy Clin Immunol* 1977; 60: 951-959.
68. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. - Endogenous nitric oxide is present in the exhaled air of rabbits, guinea-pigs and humans. *Biochem Biophys Res Commun* 1991; 181: 852-857.
69. Park KGM, Hayes PD, Garlick PJ, Sewell H, Eremin O. - Stimulation of lymphocyte natural cytotoxicity by L-arginine. *Lancet* 1991; 337: 645-646.
70. Salvemini D, De Nucci G, Gryglewski RJ, Vane JR. - Human neutrophils and mononuclear cells inhibit



- platelet aggregation by releasing a nitric oxide-like factor. *Proc Natl Acad Sci USA* 1989; 86: 6328-6332.
71. Radomski MW, Palmer RMJ, Moncada S. - An L-arginine, nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* 1990; 87: 5193-5197.
72. Bissonnette EY, Hogaboam CM, Wallace JL, Befus AD. - Potentiation of tumor necrosis factor- $\alpha$ -mediated cytotoxicity of mast cells by their production of nitric oxide. *J Immunol* 1991; 147: 3060-3065.
73. Salvemini D, Masini E, Anggard E, Mannaioni PF, Vane J. - Synthesis of a nitric oxide-like factor from L-arginine by rat serosal mast cells: stimulation of guanylate cyclase and inhibition of platelet aggregation. *Biochem Biophys Res Commun* 1990; 169: 596-601.
74. Goldie RG, Fernandes LB, Farmer SG, Hay DWP. - Airway epithelium-derived inhibitory factor. *TIPS* 1990; 11: 67-70.
75. Barnes PJ, Cuss FM, Palmer JB. - The effect of airway epithelium on smooth muscle contractility in bovine trachea. *Br J Pharmacol* 1985; 86: 685-691.
76. Buga GM, Gold ME, Wood KS, Chaudhuri G, Ignarro LJ. - Endothelium-derived nitric oxide relaxes nonvascular smooth muscle. *Eur J Pharmacol* 1989; 161: 61-72.
77. Guc MO, Ilham M, Kayaalp SO. - The rat anococcygeus muscle is a convenient bioassay organ for the airway epithelium-derived relaxant factor. *Eur J Pharmacol* 1988; 148: 405-409.
78. Fernandes LB, Paterson JW, Goldie RG. - Co-axial bioassay of a smooth muscle relaxant factor released from guinea-pig tracheal epithelium. *Br J Pharmacol* 1989; 96: 117-124.
79. Munakata M, Masaki Y, Sakuma I, *et al.* - Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. *J Appl Physiol* 1990; 69: 665-670.
80. Bredt DS, Hwang PM, Glatt CE, *et al.* - Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991; 351: 714-718.
81. Borland CDR, Higenbottam TW. - A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur Respir J* 1989; 2: 56-63.
82. Meyer M, Piper J. - Nitric oxide (NO), a new test gas for study of alveolar-capillary diffusion. *Eur Respir J* 1989; 2: 494-496.
83. Norman V, Keith CM. - Nitrogen oxides in tobacco smoke. *Nature* 1965; 205: 915-916.
84. Borland C, Higenbottam T. - Nitric oxide yields of contemporary UK, US and French cigarettes. *Int J Epidemiol* 1987; 16: 31-34.
85. Alving K, Lundberg JM. - Nitric oxide, a possible mediator of cigarette smoke-induced bronchial and pulmonary vasodilatation in the pig. *Am Rev Respir Dis* 1992; 145: A264.
86. Lippmann M. - Airborne acidity: estimates of exposure and human health effects. *Environ Health Perspect* 1985; 63: 63-70.
87. Wink DA, Kasprzak KS, Maragos CM, *et al.* - DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; 254: 1001-1003.
88. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman PA. - Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide anion. *Proc Natl Acad Sci USA* 1990; 87: 1620-1624.
89. Moreno JJ, Pryor WA. - Inactivation of  $\alpha$ 1-proteinase inhibitor by peroxynitrite. *Chem Res Toxicol* 1992; 5: 425-431.