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Occasional review

Nitric oxide and lung disease

Peter J Barnes, Maria G Belvisi

Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY
P J Barnes
M G Belvisi

Reprint requests to:
Dr P J Barnes

Even five years ago few could have realised that the simple gas nitric oxide (NO) would be involved in the regulation of so many physiological functions and pathophysiological processes. There has been an explosion of information about nitric oxide which appears to be involved in an extraordinary range of functions including vascular regulation, neurotransmission, host defence, and cytotoxicity.[1 2] An increasing volume of literature has shown the importance of nitric oxide in the regulation of various pulmonary functions and suggested its involvement in several pulmonary diseases.[3 4] The purpose of this review is to highlight some of the recent advances in this rapidly moving field.

For over 20 years it has been recognised that the vasodilator responses to many agents are mediated by the release of a vasodilator substance from endothelial cells.[5] The identity of endothelium derived relaxant factor (EDRF) remained elusive, largely because of its short half life, until 1987 when two groups independently suggested EDRF had the characteristics of nitric oxide.[6 7] Many were surprised that such a simple molecule could account for all of the actions of EDRF, but extensive investigations in many species have now provided compelling supportive evidence.[1] Parallel investigations established that nitric oxide was also important in macrophage mediated cytotoxicity.[8 9]

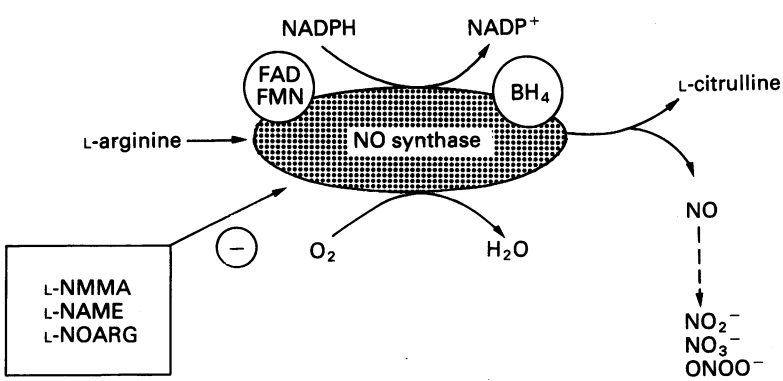

Figure 1 Nitric oxide (NO) formation by NO synthase involves conversion of L-arginine to L-citrulline. Several cofactors are necessary for enzyme activity, including flavones (FAD, FMN) and tetrahydrobiopterin (BH_4). NO synthase is inhibited competitively by arginine derivatives N^G-mono-methyl-L-arginine (L-NMMA), N^G- nitroarginine methyl ester (L-NAME), and N^G-nitroarginine (L-NOARG). Nitric oxide itself may be oxidised to nitrite (NO_2^-), nitrate (NO_3^-), and peroxynitrite ($ONOO^-$) ions.

Formation of nitric oxide

Nitric oxide is a highly reactive radical (more accurately designated NO$^{\cdot}$) formed from the semi-essential amino acid L-arginine by the action of an enzyme nitric oxide synthase (NOS)[10] (fig 1). The enzyme catalysing the reaction is stereospecific as L-arginine is a substrate but D-arginine is not. Nitric oxide is produced in neuronal and non-neuronal tissue as a product of the action of NOS catalysing the conversion of L-arginine to L-citrulline.[11] Conversion of [^{3}H]arginine to [^{3}H]citrulline can be monitored in order to measure NOS activity as [^{3}H]citrulline is formed stoichiometrically with nitric oxide and is measured more easily than the short lived nitric oxide.[12] Nitric oxide is rapidly oxidised to nitrite (NO_2^-) which can also be used to monitor nitric oxide formation. The nitrogen of nitric oxide is derived from the terminal guanidino nitrogen of L-arginine and the oxygen is derived from molecular oxygen.

Nitric oxide is formed in the lungs and can be detected in the expired air of several species, including humans.[13] Nitrite and *S*-nitrosothiols have also been detected in bronchoalveolar lavage fluid of normal human volunteers.[14] The source of nitric oxide is not certain but it may be derived from the alveoli (epithelium or endothelium), from macrophages, or from airway epithelium.

NITRIC OXIDE SYNTHASES (NOS)

Several species of NOS have now been characterised and several distinct NOS genes have been identified.[2 15] NOS exists as constitutive forms (cNOS) which are basally expressed in endothelial, neuronal and other cells and are Ca^{2+}-calmodulin dependent. The purified enzyme migrates as a single 150 kDa band on gel electrophoresis, and the native enzyme appear to be a monomer.[16] The messenger RNA is colocalised with NADPH diaphorase, and neuronal NOS and NADPH diaphorase appear to be one and the same enzyme.[17] The histochemical location of NADPH diaphorase by the formation of blue formazans from tetrazoliums can therefore be used to localise neuronal NOS.[18] A form of cNOS distinct from the neuronal form is localised to vascular endothelial cells.[19 20] cNOS from brain and endothelial cells have been cloned[19–22] and these are distinct enzymes with about 60% homology, which are also homologous to cytochrome P-450

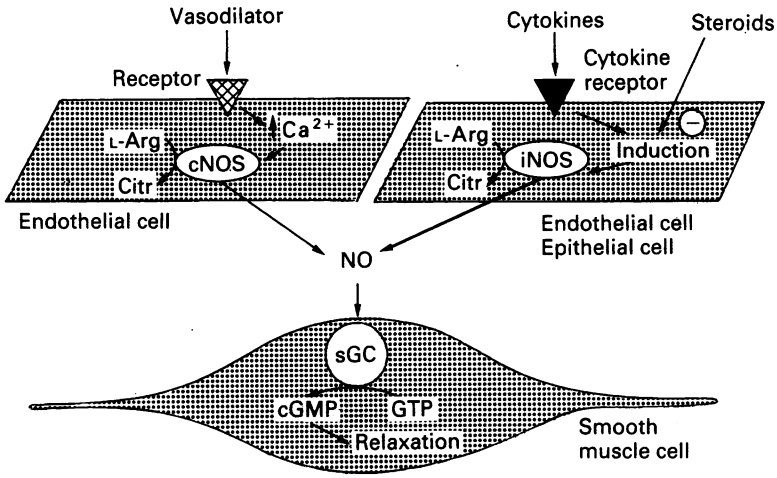

Figure 2 Constitutive nitric oxide synthase (cNOS) is activated by a rise in intracellular calcium ions (Ca^{2+}) in response to activation of a surface receptor (for example, bradykinin receptor on vascular endothelial cells). Inducible NOS (iNOS) is induced in response to activation by cytokines or lipopolysaccharide, resulting in larger amounts of nitric oxide (NO) formation than with cNOS activation. Steroids block the induction of iNOS. Nitric oxide activates soluble guanylyl cyclase (sGC) in target cells, resulting in an increase in cyclic guanosine 3'5'-monophosphate (cGMP) and smooth muscle relaxation.

reductases. Both of these cNOS enzymes are activated by Ca^{2+} and by calmodulin (fig 2). Agonists may activate cNOS via a rapid increase in intracellular Ca^{2+} concentration resulting in release of nitric oxide within seconds or minutes. cNOS has been identified in several cell types, including not only endothelial cells and certain neurones, but also neutrophils, platelets, and mast cells.[1 2]

In addition there are inducible forms (iNOS) which may be expressed after exposure to certain cytokines and endotoxin (fig 2). iNOS does not require calmodulin but does require tetrahydrobiopterin.[23] Both enzymes are dependent on NADPH as a cofactor and contain tightly bound flavin cofactors. iNOS is not dependent on intracellular Ca^{2+} or calmodulin, but the enzyme is induced by several cytokines including interferon γ (IFNγ), interleukin 1β (IL-1β), and tumour necrosis factor α (TNFα) as well as by endotoxin. Induction of iNOS results in the capacity to form much larger amounts of nitric oxide than activation of cNOS and this may result in different pathophysiological effects. iNOS has approximately 50% homology with cNOS[24 25] and has been described in macrophages, fibroblasts, smooth muscle cells, endothelial cells, and neutrophils. Several forms of iNOS have been described and both soluble and particulate forms may occur. Induction of iNOS involves gene transcription, and increased production of nitric oxide therefore occurs several hours after exposure but may continue for several days. Corticosteroids and cytokines such as transforming growth factor β inhibit the induction of iNOS but have no effect on cNOS.[26-28]

NOS INHIBITORS

The development of drugs that inhibit NOS has been the most important strategy in determining the physiological and pathophysiological roles of nitric oxide. Analogues of L-arginine act as false substrates for the enzyme and therefore block the formation of endogenous nitric oxide. This blockade can be overcome by adding back L-arginine, but not by adding D-arginine which is not a substrate for the enzyme. Several arginine analogues have been developed, including N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NOARG), N^G-nitro-L-arginine methyl ester (L-NAME), and N^G-iminoethyl-L-ornithine (L-NIO), all of which are extremely useful in revealing the role of endogenous nitric oxide in various processes.[29] These inhibitors are stereospecific, the corresponding analogues of D-arginine being inactive. In addition, L-NMMA and L-NIO have been shown to inhibit L-arginine transport.[30] Recently, hydroxycobalamin has been used as a tool to distinguish between the source of nitric oxide as it has a differential action in blocking responses to endothelium derived nitric oxide in rat aortic rings but not neurogenic nitric oxide in the rat anococcygeus muscle.[31] Alternatively, the NOS inhibitor L-N^G-nitroarginine *p*-nitroanilide (L-NAPNA) appears to be a selective inhibitor of NOS in the brain, as shown by its antinociceptive activity in mice, with little effect on the endothelium dependent relaxation of blood vessels.[32] Selective inhibitors of iNOS have recently been reported, and aminoguanidine has a 10–100-fold selectivity for iNOS compared with cNOS[33] and may be the basis for potentially important therapeutic agents in the future.

CELLULAR EFFECTS OF NITRIC OXIDE

Nitric oxide activates soluble guanylyl cyclase after binding to its haem moiety to initiate a three dimensional change in the shape of the enzyme which increases its activity and consequently the production of cyclic guanosine 3'5'-monophosphate (cGMP). The rise in cGMP results in relaxation of smooth muscle, but the mechanism by which this happens is unknown. Many possibilities have been suggested including the inhibition of inositol trisphosphate (IP_3), inhibition of a cAMP phosphodiesterase, dephosphorylation of the light chain of myosin, activation of protein kinases, stimulation of membrane Ca^{2+}-ATPase, opening of K^+ channels, increased sequestration of cytosolic Ca^{2+}, and inhibition of Ca^{2+} influx.[34] This increase in cGMP underlies many of the neural and cardiovascular actions of nitric oxide. There have also been recent reports of effects of nitric oxide that are not dependent on cGMP.[35] However, the second messenger systems involved in nitric oxide induced tissue damage and cell death are not as well understood. It had long been recognised that directly acting nitrovasodilators such as glyceryl trinitrate are metabolised to nitric oxide within smooth muscle, endothelium, and plasma, and others like sodium nitroprusside liberate nitric oxide spontaneously.[36] It has been suggested that nitric oxide may form an intermediary complex with thiols such as cysteine, and it has been suggested that *S*-nitrosocysteine more

closely mimics the properties of EDRF than does nitric oxide itself.[37] The formation of these sulphydryl complexes may prolong the half life of nitric oxide released from cells.

The fact that a simple gas such as nitric oxide is a physiological messenger has raised the possibility that other gases could have similar functions. Carbon monoxide is also an activator of guanylyl cyclase and is formed by the action of heme oxygenase. Localisation of the mRNA for the constitutive form of heme oxygenase has been found throughout the brain, and the localisation is similar to that for soluble guanylyl cyclase mRNA, suggesting that carbon monoxide may function as a neurotransmitter.[38]

Role as a vasodilator

The observation that EDRF is nitric oxide immediately suggested that it may play a part in the regulation of the pulmonary circulation and this has been extensively investigated. NOS inhibitors reduce the vasodilator response to acetylcholine in animal and human pulmonary vessels in vitro[39–41] and against endothelin-3 in rat pulmonary vessels.[42] Endogenous nitric oxide appears to act as a braking mechanism against pulmonary vasoconstriction.[40] Release of nitric oxide from endothelial cells in the pulmonary circulation appears to counteract hypoxic vasoconstriction[43 44] and nitric oxide release is apparently decreased in hypoxia.[45] In chronic experimental hypoxia there is a reduced response to nitrovasodilators which appears to result from impaired function of soluble guanylyl cyclase.[46] There is circumstantial evidence that release of nitric oxide from pulmonary vessels may be impaired in patients with chronic obstructive pulmonary disease (COPD) and cystic fibrosis.[47] Impaired endothelium dependent relaxation has also been observed in isolated pulmonary arteries obtained from patients undergoing heart-lung transplantation for end stage chronic lung disease, including cystic fibrosis.[48 49]

Since nitric oxide is a potent pulmonary vasodilator, inhalation of nitric oxide might be effective as a selective pulmonary vasodilator in view of its short half life. Rapid combination of nitric oxide with haemoglobin contained in red blood cells would rapidly inactivate any nitric oxide reaching the systemic circulation, thereby limiting vasodilatation to pulmonary vessels. Inhaled nitric oxide (5–80 ppm) has been shown dose dependently to inhibit pulmonary vasoconstriction induced by either hypoxia or by infusion of a thromboxane analogue in conscious spontaneously breathing lambs[50] and in normal volunteers with induced hypoxia[51] without causing systemic hypotension. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction in sheep without impairing gas exchange.[52] Inhalation of nitric oxide (40 ppm) has also been shown to cause selective pulmonary vasodilatation in patients with pulmonary hypertension[53] and in neonates with persistent pulmonary hypertension nitric oxide produces a significant improvement in oxygen exchange and arterial oxygen tension.[54] Inhalation of nitric oxide has also been reported to cause selective pulmonary vasodilatation in patients with COPD.[55]

Inhaled nitric oxide (18 ppm) has recently been reported to reduce pulmonary artery pressure in patients with severe adult respiratory distress syndrome (ARDS) and increase oxygenation by improving ventilation perfusion matching without systemic vasodilatation.[56] Continuous nitric oxide inhalation was effective over a period of 3–53 days without evidence of tachyphylaxis, indicating that this might be an effective therapy for this condition although controlled trials are now required.

Nitric oxide is also a potent bronchial vasodilator in animal airways.[57] Cigarette smoke contains a high concentration of nitric oxide[58] which may account for the increase in airway blood flow after exposure of airways to cigarette smoke.[57]

Role as a bronchodilator

Nitrovasodilators such as glyceryl trinitrate and sodium nitroprusside also relax airway smooth muscle in vitro, resulting in an increase in soluble guanylyl cyclase activity and an increase in cGMP.[59] It is therefore to be expected that nitric oxide may act as a bronchodilator and this has been shown in canine airways in vitro.[60] The effect of nitric oxide on isolated epithelium intact guinea pig tracheal strips contracted with carbachol was, however, considerably less than on epithelium denuded strips.[61] The mechanism by which the epithelial layer reduces the effects of nitric oxide is unclear, but the epithelium may act as a metabolic or diffusional barrier for nitric oxide. High concentrations of inhaled nitric oxide (80 ppm) reduce the bronchoconstrictor effect of nebulised methacholine in anaesthetised rabbits.[62] In anaesthetised guinea pigs methacholine induced bronchoconstriction is reduced, albeit transiently, by inhaled nitric oxide in a concentration dependent manner from 5 ppm to 300 ppm and by the nitric oxide releasing compound *S*-nitroso-*N*-acetylpenicillamine (SNAP).[63] In addition, a high concentration of nitric oxide (300 ppm) causes a small degree of baseline bronchodilatation. There is no evidence for tolerance after prolonged administration and the bronchodilator effect of nitric oxide is additive with a β adrenoceptor agonist. Histamine release from bovine lung is also reduced by nitrovasodilators, probably by alterations in guanylyl cyclase activity,[64] suggesting that nitric oxide may have mast cell stabilising effects.

Nitric oxide inhalation (at estimated doses of 0·5 mg and 10 mg) has no effect on airway resistance in normal subjects, although it is associated with a small fall in oxygen saturation, presumably as a result of changes in ventilation perfusion ratio.[65] Inhaled nitric oxide at a concentration of 80 ppm similarly has no effect in normal individuals, but has a

small and inconsistent bronchodilator effect in asthmatic patients.[66]

This raises the possibility that nitric oxide inhalation or nitric oxide releasing compounds might have some therapeutic potential as alternative bronchodilators in obstructive airways diseases. An advantage of inhaled nitric oxide would be its lack of systemic effects since it would be rapidly inactivated by haemoglobin. There are, however, potential dangers of inhaling nitric oxide[67] since, in the presence of oxygen, it is oxidised to nitrate and thence to nitrous and nitric acids[68] which may increase airway responsiveness and, in high concentration, might cause pulmonary oedema.[69 70] The interaction between nitric oxide and superoxide anions may lead to the formation of peroxynitrite ($ONOO^-$) that may generate tissue damaging hydroxyl radicals.[68 71] There is also some evidence that high concentrations of nitric oxide may have effects on DNA and be both genotoxic and cytotoxic.[72] Nitric oxide has been detected in the exhaled air of humans and is presumably derived from pulmonary capillary endothelial cells.[13]

Role as a non-adrenergic non-cholinergic (NANC) transmitter

There is increasing evidence that nitric oxide may function as a neurotransmitter of inhibitory non-adrenergic non-cholinergic (iNANC) nerves, and "nitrergic" or "nitroxidergic" neurotransmission has been shown in the gut, bladder, and reproductive organs (fig 3).[73] There is convincing evidence that nitric oxide is released from nerves themselves, since a particular form of cNOS has been localised to peripheral nerves[74] and is activated by calcium entry when the nerve is depolarised. Nitric oxide accounts for approximately half of the iNANC (bronchodilator) response in guinea pig trachea in vitro.[75 76] In pig tracheal smooth muscle there is a prominent iNANC response which is completely inhibited by NOS inhibition and reversed by L-arginine in a stereospecific manner.[77 78] Similar results have been reported in cat and horse airways[79 80] although a previous report in feline airways found no effect of NOS inhibitors.[81] This indicates that the NANC response is evoked by release of nitric oxide and provides evidence that it is the principal iNANC neurotransmitter. Nitric oxide also appears to account for the bronchodilator NANC response in human airways in vitro in central and peripheral airways[82–85] and, in contrast to guinea pig trachea, the neuropeptide vasoactive intestinal peptide (VIP) appears to play little or no part in this response.[83] A similar mechanism seems to be responsible for iNANC responses in central and peripheral airways, as evidenced by similar kinetics and frequency dependency.[84] The cellular source of nitric oxide is not completely certain, but it is most likely to be derived from intrinsic nerves within the airway rather than another transmitter substance inducing the release of nitric oxide from endothelial, epithelial, or smooth muscle cells. Epithelial removal has no effect on the iNANC response in guinea pig airways.[86 87] However, nitric oxide is not stored in vesicles and electron microscopy studies have shown that NOS, at least in the myenteric plexus, is located in the cytoplasm. Immunocytochemical staining for neuronal cNOS and NADPH diaphorase shows localisation to nerves of guinea pig, ferret, and human airways.[88–90] NOS immunoreactive nerves appear to supply airway vessels, airway smooth muscle, and submucosal glands. NOS is colocalised with VIP in these species. NOS immunoreactive neurones are found in parasympathetic ganglia of human but not guinea pig airways, and also in sympathetic and sensory ganglia supplying the airways.[88 89] NOS immunoreactive nerves are more prominent in proximal than in distal airways.

Nitric oxide is probably synthesised on demand in a neurone and its release may involve simple diffusion. Alternatively, it can spontaneously combine with naturally occurring thiols such as cysteine in aqueous media to form nitrosothiols such as L-cysteine-NO at acidic pH.[91] Once formed, nitrosothiols may be stabilised at the low pH in secretory vesicles. If nitrosothiols are stored in secretory vesicles then release would expose these compounds to the higher pH of the extracellular fluid, with rapid breakdown to release nitric oxide. Another possibility is that nitric oxide is released by electrical field stimulation from glial cells that have been shown to contain L-arginine[92] and voltage gated sodium channels, which could account for a tetrodotoxin sensitive iNANC response.[93] In the gastrointestinal tract there is some evidence that VIP stimulates the release of nitric oxide from smooth muscle cells so that it acts as an indirect transmitter of relaxation,[94 95] but this is unlikely in airways since VIP induced bronchodilatation is not reduced by NOS inhibitors.[76 84 85]

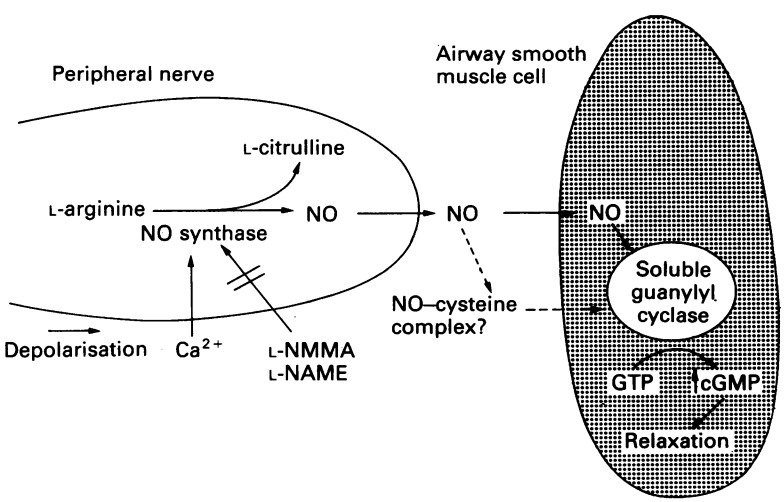

Figure 3 Inhibitory non-adrenergic non-cholinergic nerves in airways utilise nitric oxide (NO) as a neurotransmitter. Depolarisation of the nerve results in influx of calcium ions (Ca^{2+}) and activation of nitric oxide synthase. Nitric oxide diffuses from the nerve to activate soluble guanylyl cyclase in airway smooth muscle cells, resulting in increased cyclic GMP (cGMP) and relaxation. Nitric oxide may form an intermediate and more stable complex with cysteine.

NOS inhibition markedly potentiates cholinergic neural responses in both human and guinea pig airways,[96-98] but NOS inhibitors do not affect neural acetylcholine release in either guinea pig or human airways,[96 98] suggesting that endogenous nitric oxide modulates cholinergic neural responses by acting as a functional antagonist to acetylcholine at airway smooth muscle. However, this effect is absent in human airways when the pulse width of the electrical field stimulation is reduced to 0·1 ms which suggests that iNANC responses are less evident under these conditions, so the chance of functional interactions between the NANC relaxation response and the cholinergic contractile response is reduced.[84] There is also evidence to suggest that nitric oxide may modulate reflex bronchoconstriction in vivo. Endogenous nitric oxide modulates the atropine sensitive component of bradykinin induced bronchoconstriction in the guinea pig.[99] In addition, endogenous nitric oxide released in association with vagal nerve stimulation regulates the magnitude of NANC neurogenic bronchoconstriction (due to tachykinin release from sensory nerves) in guinea pigs in vivo.[100]

Nitric oxide may be released from parasympathetic nerves as a cotransmitter with acetylcholine, although there is no direct evidence for this yet. In the gastrointestinal tract NOS immunoreactivity is colocalised with VIP, which may also be localised to cholinergic nerves,[101] and NOS is colocalised with VIP in vascular nerves of guinea pigs.[102] Some evidence that argues against the colocalisation of NOS in human airway cholinergic nerves is the observation that bronchodilator neural responses are virtually absent in extrinsically denervated airways obtained from heart-lung transplant recipients undergoing a second transplantation,[103] while cholinergic neural responses are preserved.[104] In an innervated guinea pig tracheal tube preparation a bronchodilator response to postganglionic nerve stimulation (via electrical field stimulation) is present which is reduced by NOS inhibitors as expected, but no bronchodilator response is observed after stimulation of the vagus nerve although both types of neural stimulation elicit a cholinergic bronchoconstrictor response.[87] This suggests that the neurones which release nitric oxide are separate from cholinergic pathways and may arise from outside the airways. There is some evidence that the nerve cell bodies may be localised in the vicinity of the oesophagus.[105]

Since bronchodilator NANC nerves are the only neural bronchodilator pathway in human airways,[106] it is possible that there may be a defect in the function of these nerves in diseased airways. iNANC responses in tissues from patients with mild asthma were not altered, but the responses were significantly reduced in tissues from patients with cystic fibrosis compared with iNANC responses in donor tissue.[103] This defect in iNANC function may be because nitric oxide released from either nerves or cells is degraded by inflammatory mediators. Airway inflammation may be associated with release of superoxide anions from activated inflammatory cells resulting in increased breakdown of nitric oxide.[107] Alternatively, this could be the result of a malfunction at the level of the NOS or guanylyl cyclase activation.

Nitric oxide may also be involved in neurogenic vasodilator responses in the pulmonary circulation. Electrical field stimulation causes a vasodilator response in pulmonary vessels in vitro which is partly due to the release of nitric oxide from endothelial cells[108 109] via ATP release from sympathetic nerves.[108] Nitric oxide may also act as a neurotransmitter of vasodilator NANC responses in guinea pig pulmonary vessels.[110] Selective destruction of adrenergic nerves with 6-hydroxydopamine has no effect on the NANC vasodilator response, suggesting that nitric oxide is likely to be released from some other type of nerve. Endothelial removal is similarly ineffective, indicating that nitric oxide is not of endothelial cell origin.[110] Interestingly, superoxide anions appear to inhibit the NANC vasodilator response[110] which may suggest that in inflammation neurogenic vasodilatation may be impaired.

NOS inhibition enhances adrenergic neural vasoconstrictor responses in pulmonary vessels.[40 111] This indicates that, as in the airways, endogenous nitric oxide appears to act as a functional antagonist or "braking" mechanism to vasoconstrictor responses in pulmonary vessels.

Although the evidence is now convincing that endogenous nitric oxide mediates NANC relaxant effects,[73] there are still some doubts as to whether nitric oxide itself acts as the transmitter or whether some more stable intermediate complex is formed. As for endothelial responses, nitrocysteine has been suggested as an intermediary compound and closely mimics NANC responses in the gut,[112] and several *S*-nitrosothiols relax smooth muscle directly.[113] Hydroquinone blocks relaxation induced by nitric oxide but not NANC nerve stimulation or other nitrovasodilators in guinea pig trachea in vitro, and the mechanism of action appears to be by free radical scavenging rather than superoxide anion generation.[114] This differential effect may indicate that the NANC transmitter is not free nitric oxide but a nitric oxide containing or nitric oxide generating substance.[115]

Role as an inflammatory mediator

Endothelial cells and nerves are not the only source of nitric oxide in airways and several types of inflammatory and structural cells have now been found to produce nitric oxide.

MACROPHAGES

There is convincing evidence that macrophages, including alveolar macrophages of some species, may synthesise nitric oxide after exposure to various cytokines[116 117] and to endotoxin,[26] and that nitric oxide is

important in host defence.[1] Macrophages can perform many cytotoxic activities which include inhibition of mitochondrial respiration, aconitase activity and DNA synthesis, and are thought to be due to the inhibition of iron containing enzymes in target cells. Those activities, as well as NO_2^- and NO_3^- generation, are blocked by inhibitors of NOS.[118] Macrophages express an inducible form of NOS that has recently been cloned.[24 25] The induction of iNOS in alveolar macrophages can be blocked by glucocorticosteroids.[117] Interleukin 10 also inhibits iNOS induced by interferon γ in murine macrophages.[119] Nitric oxide itself may act as a feedback inhibitor of iNOS in these cells, providing a mechanism to limit induced nitric oxide generation.[120] Human alveolar macrophages, in contrast to rat and murine macrophages, do not appear to produce nitric oxide in vitro under the same experimental conditions,[121] but it is possible that a suitable combination of cytokines and cofactors would allow the production of nitric oxide from these cells.

MAST CELLS

Rat serosal mast cells have been reported to synthesise nitric oxide from L-arginine,[122] but whether nitric oxide is produced by activated human lung mast cells is not yet certain.

SMOOTH MUSCLE CELLS

Other cells also have an inducible form of NOS, including endothelial cells, neutrophils and vascular smooth muscle cells.[1 27 123] A combination of cytokines (IL-1β, TNFα, and IFNγ) and lipopolysaccharide potently induce iNOS in cultured rat pulmonary artery smooth muscle and this effect is blocked by an inhibitor of protein synthesis.[124] Treatment of rats with in vivo lipopolysaccharide induced iNOS after six hours in the lung[125] and induced the expression of mRNA for iNOS, but not cNOS, in rat lung.[126] Induction of NOS by cytokines and lipopolysaccharide appears to result in much greater amounts of nitric oxide than is possible with cNOS. There is compelling evidence that nitric oxide in septic shock is a major contributor to the cardiovascular collapse.[1]

EPITHELIAL CELLS

Airway epithelial cells are also a source of nitric oxide and this could be induced by exposure to cytokines such as TNFα and IFNγ in the airway, although nitric oxide does not appear to be "epithelium derived relaxing factor".[61] In guinea pigs inhaled L-NAME increases the bronchoconstrictor response to histamine in vivo and intraluminal application in a tracheal tube preparation also enhances the contractile response to histamine in vitro. This effect is mimicked by removal of airway epithelium, suggesting that airway epithelium releases nitric oxide which counteracts the bronchoconstrictor effect of spasmogens.[127] Cultured bovine epithelial cells metabolise L-arginine to L-citrulline, an effect blocked by NOS inhibitors, indicating that airway epithelial cells have the capacity to produce nitric oxide.[128] A cultured human epithelial cell line also produces nitrite when incubated with L-arginine, indicating the capacity of human airway epithelial cells to produce nitric oxide.[129] Immunocytochemical staining has demonstrated the expression of iNOS in human and rat airway epithelial cells[130] and there is evidence for increased iNOS expression in epithelial cells of bronchial biopsy specimens from asthmatic patients compared with normal controls, whereas cNOS is not expressed in either group.[131] This suggests that iNOS may be induced by cytokines produced in asthmatic inflammation, including IFNγ, TNFα, and IL-1β. Endogenous nitric oxide may be an important modulator of mucociliary clearance and is reported to increase ciliary beat frequency in bovine ciliated epithelial cells.[132]

FIBROBLASTS

Rat lung fibroblasts are capable of producing nitric oxide after stimulation with IFNγ, and this effect is enhanced by lipopolysaccharide and IL-1β.[133] The role of endogenous nitric oxide in fibrogenesis is not yet certain, however.

IMMUNOLOGICAL EFFECTS

Alveolar macrophages have a suppressive effect on T lymphocyte proliferation both in rats[134] and humans.[135] There is some evidence that at least part of this immunosuppressive effect is mediated by nitric oxide in rats.[136] This suggests that endogeneous nitric oxide may have an immunomodulatory role in the airways.

ROLE IN LUNG INFLAMMATION

Endogenous nitric oxide may be a double edged sword. Nitric oxide produced in small amounts locally by cNOS activation may be beneficial in relaxing airway smooth muscle in airways, but may have deleterious effects when produced in much higher concentrations from iNOS. Nitric oxide is a potent vasodilator and might contribute to the hyperaemia of asthmatic airways. This may also increase exudation of plasma from leaky post capillary venules in the airways. Indeed, inhibition of endogenous nitric oxide production significantly reduces plasma exudation and inflammation, both in skin[137] and in airways.[138] High concentrations of nitric oxide may have cytotoxic effects in the airway and could conceivably contribute to the epithelial shedding observed in asthmatic patients. Ozone inhalation increases nitric oxide release from rat alveolar macrophages and this could contribute to the damaging effect of ozone on airway epithelial cells.[139]

Nitric oxide may be involved in acute and chronic lung injury. L-NAME protects rats against lung injury induced by injection of immune complexes and the high levels of nitrite recovered from bronchoalveolar lavage fluid of these animals[140] provide further evidence for the involvement of nitric oxide in this process.

EFFECT OF CORTICOSTEROIDS

Corticosteroids potently inhibit the expression of the inducible, but not the constitutive, form of NOS[26 27] and this may contribute to their anti-inflammatory action since massive nitric oxide formation may be detrimental as in the case of endotoxin shock. If NOS is induced in airway epithelial cells in asthma as a result of exposure to cytokines released from inflammatory cells, inhaled steroids may act to reduce the formation of nitric oxide and thus downregulate the vascular components of the inflammatory response. Steroids would not be expected to affect the release of nitric oxide from bronchodilator nerves since the neural constitutive form of the enzyme is not steroid sensitive.

CLINICAL PROSPECTS

It is clear that nitric oxide may have a very important regulatory role in pulmonary function and may be implicated in the pathophysiology of several lung diseases.

PULMONARY VASCULAR DISEASE

Inhaled nitric oxide is clearly effective in counteracting hypoxic pulmonary vasoconstriction and has the major advantage that there are no systemic vasodilator effects, which are the major limitation to the use of other vasodilators (such as prostacyclin) for the treatment of pulmonary hypertension. Furthermore, inhaled nitric oxide selectively dilates the pulmonary vasculature to which it is delivered thereby improving ventilation perfusion matching, whereas systemically administered vasodilators may increase ventilation perfusion mismatch. Inhaled nitric oxide is also effective in improving the oxygenation in ARDS.[56] These preliminary clinical studies suggest that nitric oxide may be useful as an acute selective pulmonary vasodilator in critical care and may be administered safely over a period of several days.

BRONCHODILATATION

Recent studies with nitric oxide have revived interest in nitrovasodilators as alternative bronchodilators which work by a different molecular pathway from β agonists and theophylline. Previous studies of such drugs in asthma have not been impressive[141] but new nitric oxide donors such as *S*-nitrosothiols may have advantages.[142] In particular, inhalation of nitric oxide gas or nitric oxide releasing compounds may present a useful approach in the treatment of diseases such as asthma, and rapid inactivation of inhaled nitric oxide by red blood cells will prevent any systemic haemodynamic side effects. Nitric oxide itself, however, may not reach airway smooth muscle in adequate concentrations unless large amounts are inhaled when the risk of vasodilator effects is increased. Inhaled nitric oxide would probably be suitable only as an acute bronchodilator in severe asthma, but there is likely to be no advantage (and several disadvantages) compared with nebulised β agonists. Studies of the effects of inhaled nitric oxide in human airway are limited, but inhalation of >15 000 ppb in healthy subjects causes a small fall in arterial oxygen tension and an *increase* in airways resistance at concentrations of >20 000 ppb.[143] Another approach would be to enhance the release of neuronal nitric oxide which should give selective bronchodilatation.

TOXICITY

High concentrations of nitric oxide are likely to have toxic effects resulting from the formation of nitrites and peroxynitrite radicals which may have cytotoxic and genotoxic effects.[71 72] Formation of nitrite may lead to methaemoglobinaemia. Nitric oxide is a recognised air pollutant derived from car exhaust and from domestic gas cookers, but little is known about its effects on airway function after long term exposure. It is a major constituent of the gaseous phase of cigarette smoke, derived from nitrates in tobacco, and concentrations of >50 ppm are inhaled in mainstream smoke.[58] The nitric oxide content is particularly high in French and American blends because of the high nitrate content.

L-ARGININE

Since L-arginine is the necessary precursor for nitric oxide formation, it is possible that this amino acid may increase endogenous nitric oxide production, particularly when there is a possibility that endogenous L-arginine is the limiting factor. L-Arginine infusion has been studied in patients with primary pulmonary hypertension, without any effect, however.[144]

NOS INHIBITORS

The production of large amounts of nitric oxide by induction of NOS in response to cytokines may be deleterious and contribute to the inflammatory response in both airways and in lung parenchyma. This has suggested that NOS inhibitors might have therapeutic potential. However, non-selective NOS inhibitors such as L-NMMA and L-NAME lead to hypertension by blocking cNOS in endothelial cells.[145] What is required is a selective inhibitor of iNOS and this is achieved by corticosteroids, as discussed above. A selective iNOS inhibitor might have the beneficial anti-inflammatory effects of steroids, without the systemic side effects which limit their usefulness when systemic administration is required. There is evidence that some NOS inhibitors may have selectivity for particular types of NOS; aminoguanidine appears to have a 10–100-fold selectivity for inhibition of iNOS compared with cNOS[33] indicating that selective inhibition is a realistic possibility in the future. Cloning of NOS has revealed several distinct enzyme genes, each of which may have subtypes resulting from differential splicing, and this

raises the prospect that even more selective NOS inhibitors may be developed.

Conclusions

Endogenous nitric oxide may have a key role in many physiological and pathophysiological events in the lung. It appears to be important in neural bronchodilator and vasodilator mechanisms, in the regulation of airway and pulmonary blood flow, and in immune defence. When produced in low concentrations by constitutive NOS it appears to have a generally beneficial role, but when produced in large amounts by inducible NOS it may result in increased inflammatory responses and tissue damage, suggesting that nitric oxide may be involved in the pathophysiology of several pulmonary diseases. This raises the prospects for new approaches to the treatment of airway inflammation, ARDS, and pulmonary vascular disease in the future.

1 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;**43**:109–41.
2 Nathan C. Nitric oxide as a secretory product of mammalian cells. *FASEB J* 1992;**6**:3051–64.
3 Barnes PJ. Nitric oxide and airways. *Eur Respir J* 1993;**6**: 163–5.
4 Jorens PG, Vermeire PA, Herman AG. L-arginine-dependent nitric oxide synthase: a new metabolic pathway in lung and airways. *Eur Respir J* 1993;**6**:258–66.
5 Furchgott RF. The role of endothelium in the responses of vascular smooth muscle to drugs. *Ann Rev Pharmacol Toxicol* 1984;**24**:175–7.
6 Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;**327**:524–6.
7 Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein in nitric oxide. *Proc Natl Acad Sci USA* 1987;**84**:9265–9.
8 Hibbs JB, Taintor RR, Vaurin Z. Macrophage cytotoxicity: role of L-arginine deimunase activity and imino nitrogen oxidation to nitrite. *Science* 1987;**235**:473–6.
9 Iyengar R, Stuehr DJ, Marletta MA. Macrophage synthesis of nitrite, nitrate and *N*-nitrosamines: precursors and role of the respiratory burst. *Proc Natl Acad Sci USA* 1987;**84**:6369–73.
10 Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988;**333**:664–6.
11 Moncada S, Higgs EA, Hodson HF, Knowles RG. Lopez-Jaramillo P, McCall T, *et al.* The L-arginine: nitric oxide pathway. *Cardiovasc Pharmacol* 1991; **17**:51–9.
12 Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci USA* 1989;**86**:9030–3.
13 Gustafsson LE, Leone AM, Persson M-G, 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**:852–7.
14 Gaston B, Drazen JM, Reilly J, Sugarbaker DJ, Mullins M, Ramdev P, *et al.* The identification of endogenous bronchodilator *S*-nitrothiols in human airways. *Am Rev Respir Dis* 1988;**147**:A514.
15 Lowenstein CJ, Snyder SH. Nitric oxide: a novel biological messenger. *Cell* 1992;**70**:705–7.
16 Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 1990;**87**:682–5.
17 Hope BT, Michael GJ, Knige KM, Vincent SR. Neuronal NADPH-diaphorase is a nitric oxide synthase. *Proc Natl Acad Sci USA* 1991;**88**:2811–4.
18 Hope BT, Vincent SR. Histochemical characterisation of neuronal NADPH-diaphorase. *J Histochem Cytochem* 1989;**37**:653–61.
19 Lamas S, Marsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. *Proc Natl Acad Sci USA* 1992;**89**:6348–52.
20 Janssens SP, Shimouchi A, Quertermous T, Bloch DB, Block KD. Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase. *J Biol Chem* 1992;**267**:14519–22.
21 Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Synder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991;**351**:714–8.
22 Schmidt HHHW, Murad F. Purification and characterization of a human NO synthase. *Biochem Biophys Res Commun* 1991;**181**:1372–7.
23 Kwon NS, Nathan CF, Stuehr DS. Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. *J Biol Chem* 1989;**264**: 20496–501.
24 Xie Q-W, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, *et al.* Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 1992;**256**:225–8.
25 Lyons CR, Orloff GJ, Cunningham JM. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J Biol Chem* 1992;**267**:6370–4.
26 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**:1246–52.
27 Radomski MW, Palmer RMJ, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitute nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990;**87**: 10043–9.
28 Ding A, Nathan CF, Gracar J, Derynck R, Stvehr DJ, Spimal S. Macrophage deactivating factor and transforming growth factors β-1, β-2 and β-3 inhibit induction of macrophage nitrogen oxide synthesis by interferon-gamma. *J Immunol* 1990;**145**:940–4.
29 Rees DD, Palmer RMJ, Schulz R, Hodson MF, Moncada S. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br J Pharmacol* 1990;**101**:746–52.
30 Bogle RG, Moncada S, Pearson JD, Mann GE. Identification of inhibitors of nitric oxide synthase that do not interact with the endothelial cell L-arginine transporter. *Br J Pharmacol* 1992;**105**:768–70.
31 Rajanayagam MAS, Li C-G, Rand MJ. Differential effects of hydroxocobalamin on NO-mediated relaxations in rat aorta and anococcygeus muscle. *Br J Pharmacol* 1993;**108**:3–5.
32 Babbedge RC, Moore PK, Gathen Z, Hart SL. L-N^G-nitroarginine *p*-nitroanilide (L-NAPNA): a selective inhibitor of nitric oxide synthase in the brain? *Br J Pharmacol* 1992;**107**:194P.
33 Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, *et al.* Selective inhibition of inducible nitric oxide synthase by aminoguanidine. *Eur J Pharmacol* 1993;**233**:119–25.
34 Lincoln TM, Cyclic GMP and mechanism of vasodilatation. *Pharmacol Ther* 1989;**41**:479–502.
35 Garg UC, Hassid A, Nitric oxide decreases cytosolic free calcium in Balb/c3T3 fibroblasts by a cyclic GMP-independent mechanism. *J Biol Chem* 1991;**266**:9-12.
36 Gruetter CA, Barry BK, McNamara DB, Gruetter DY, Kadowitz PJ, Ignaro LJ. Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside and a carcinogenic nitrosamine. *Nature* 1979;**5**:211–44.
37 Myers PR, Minor RL, Guerra R, Bates JN, Harrison DB. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resembles *S*-nitrosocysteine than nitric oxide. *Nature* 1990;**345**: 161–3.
38 Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 1993;**259**:381–4.
39 Dinh-Xuan AT. Endothelial modulation of pulmonary vascular tone. *Eur Respir J* 1992;**5**:757–62.
40 Crawley DF, Liu SF, Evans TW, Barnes PJ. Inhibitory role of endothelium-derived nitric oxide in rat and human pulmonary arteries. *Br J Pharmacol* 1990;**101**: 166–70.
41 Altiere RJ, Thompson DC. Modulation of cholinergic response by N^W-nitro-L-arginine in rabbit intrapulmonary arteries. *Pulmonol Pharmacol* 1992;**5**:149–51.
42 Crawley DE, Liu S-F, Barnes PJ, Evans TW. Endothelin-3 is a potent pulmonary vasodilator in the rat. *J Appl Physiol* 1992;**72**:1425–31.
43 Liu SF, Crawley DE, Barnes PJ, Evans TW. Endothelium-derived nitric oxide inhibits pulmonary vasoconstriction in isolated blood perfused rat lungs. *Am Rev Respir Dis* 1991;**143**:32–7.
44 Persson MG, Gustafsson LE, Wiklund NP, Moncada S, Hedqvist P. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol Scand* 1990:**140**: 449–57.
45 Adnot S, Raffestin B, Eddamibi S, Braquet P, Chabrier PE. Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia: *J Clin Invest* 1991;**87**:155–62.
46 Crawley DE, Zhao L, Giembycz MA, Liu SF, Barnes PJ,

Winter R, *et al.* Chronic hypoxia impairs soluble guanylyl cyclase-mediated pulmonary arterial relaxation in the rat. *Am J Physiol* 1992;**262**:L325-32.
47 Dinh-Xuan AT, Higenbottam TW, Clelland CA, Pepke-Zaba J, Cremona G, Butt AY, *et al.* Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; **324**:1539–47.
48 Dinh-Xuan AT, Higenbottam TW, Pepke-Zaba J, Clelland CA, Wallwork J. Reduced endothelium-dependent relaxation of cystic fibrosis pulmonary arteries. *Eur J Pharmacol* 1989;**163**:401–3.
49 Cremona G, Dinh-Xuan AT, Higenbottam T. Endothelium-derived relaxing factor and the pulmonary circulation. *Lung* 1991;**169**:185–202.
50 Frostell CG, Fratacci M-D, Wain JC, Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991;**83**:2038–47.
51 Frostell CG, Blomqvist M, Hedenstierna G, Lundberg J, Zapol WM. Inhaled nitric oxide selectively reverses human hypoxic vasoconstriction without causing systemic vasodilation. *Anaesthesiology* 1993;**78**:427–35.
52 Pison U, Lopez FA, Heidelmeyer CF, Russaint R, Falke KJ. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing gas exchange. *J Appl Physiol* 1993;**74**:1287–92.
53 Pepke-Zaba J, Higenbottam T, Dinh-Xuan A, Stone D, Wallwork J. Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet* 1991;**338**:1173-4.
54 Roberts JD, Polaner DH, Lang P, Zapol WM. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992;**340**:818–9.
55 Moinard J, Pillet O, Castaing Y, Guenard H. Inhaled nitric oxide: effects on pulmonary circulation and gas exchange in man. *Am Rev Respir Dis* 1992;**145**:A207.
56 Rossaint R, Falk RJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled nitric oxide for the adult respiratory distress syndrome. *N Engl J Med* 1993;**328**:399-405.
57 Alving K, Fornhem C, Weitzberg E, Lundberg JM. Nitric oxide mediates cigarette-smoke-induced vasodilatory responses in the lung. *Acta Physiol Scand* 1992;**146**:407–8.
58 Norman V, Keith CM. Nitrogen oxides in tobacco smoke. *Nature* 1965;**205**:915–6.
59 Gruetter CA, Childers CC, Bosserman MK, Lemke SM, Ball JG, Valentovic MA. Comparison of relaxation induced by glyceryl trinitrate, isosorbide dinitrate and sodium nitroprusside in bovine airways. *Am Rev Respir Dis* 1989;**139**:1192–7.
60 Masaki Y, Munakata M, Ukita H, Houma Y, Kawakami Y. Nitric oxide (NO) can relax canine airway smooth muscle. *Am Rev Respir Dis* 1989;**139**:A350.
61 Munakata M, Masaki Y, Saxuma I, Ukita H, Obuka Y, Homma Y, *et al.* Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. *J Appl Physiol* 1990;**69**:665-70.
62 Högman M, Frostell C, Arnberg H, Hedenstierna G. Inhalation of nitric oxide modulates methacholine-induced bronchoconstriction in the rabbit. *Eur Respir J* 1993;**6**:177–80.
63 Dupuy PM, Shore SA, Drazen JM, Frostell C, Hill WA, Zapol WM. Bronchodilator action of inhaled nitric oxide in guinea pigs. *J Clin Invest* 1992;**90**:421-8.
64 Valentovic MA, Ball JG, Morenas M, Szarek JL, Gruetter CA. Influence of nitrovasodilators on bovine pulmonary histamine release. *Pulmonol Pharmacol* 1992;**5**:97-102.
65 Hulks G, Warren PM, Douglas NJ. The effect of inhaled nitric oxide on bronchomotor tone in the normal human airway. *Am Rev Respir Dis* 1993;**147**:A287.
66 Frostell C, Högman M, Hedenström H, Hedenstierna G. Is nitric oxide inhalation beneficial to the asthmatic patient? *Am Rev Respir Dis* 1993;**147**:A515.
67 Foubert L, Fleming B, Latimer R, Jonas M, Odura A, Borland C, *et al.* Safety guidelines for use of nitric oxide. *Lancet* 1992;**339**:1615–6.
68 Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox activated forms. *Science* 1992;**258**:1898–902.
69 Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. *J Clin Invest* 1976;**57**:301–7.
70 Rasmussen TR, Kjaergaard SK, Tarp U, Pedersen OF. Delayed effects of NO_2 exposure on alveolar permeability and glutathione peroxidase in healthy humans. *Am Rev Respir Dis* 1992;**146**:654–9.
71 Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 1990;**87**:1620–4.
72 Wink DA, Kasprzak KS, Marangos CM. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991;**259**:1000–3.
73 Rand MJ, Nitrergic transmission: nitric oxide as a mediator of non-adrenergic, non-cholinergic neuro-effector transmission. *Clin Exp Pharmacol Physiol* 1992;**19**: 147–69.
74 Bredt DS, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 1990;**347**:768–70.
75 Tucker JF, Brane SR, Charalambons L, Hobbs AJ, Gibson A. L-N^G-nitroarginine inhibits non-adrenergic, non-cholinergic relaxations of guinea pig isolated tracheal smooth-muscle. *Br J Pharmacol* 1990;**100**: 663–4.
76 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**:91–4.
77 Kannan MS, Johnson DE. Functional innervation of pig tracheal smooth muscle: neural and non-neural mechanisms of relaxation. *J Pharmacol Exp Ther* 1992; **260**:1180–4.
78 Kannan MS, Johnson DE. Nitric oxide mediates the neural nonadrenergic, noncholinergic relaxation of pig tracheal smooth muscle. *Am J Physiol* 1993; **262**:L511–4.
79 Fisher JT, Anderson JW, Waldron MA. Nonadrenergic noncholinergic neurotransmitter of feline trachealis: VIP or nitric oxide? *J Appl Physiol* 1993;**74**:31–9.
80 Yu M, Robinson E, Wang Z. Regional distribution of nitroxidergic and adrenergic nerves in equine airway smooth muscle. *Am Rev Respir Dis* 1993;**147**:A286.
81 Diamond L, Lantta J, Thompson DC, Altiere RJ. Nitric oxide synthase inhibitors fail to affect cat airway non-adrenergic non-cholinergic (NANC1) responses. *Am Rev Respir Dis* 1992;**145**:A382.
82 Belvisi MG, Stretton CD, Barnes PJ. Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in human airways. *Eur J Pharmacol* 1992;**210**:221–2.
83 Belvisi MG, Stretton CD, Miura M, Verleden GM, Tadjarimi S, Yacoub MH, *et al.* Inhibitory NANC nerves in human tracheal smooth muscle: a quest for the neurotransmitter. *J Appl Physiol* 1992;**73**:2505–10.
84 Ellis JL, Undem BJ. Inhibition by L-N^G-nitro-L-arginine of nonadrenergic noncholinergic mediated relaxations of human isolated central and peripheral airways. *Am Rev Respir Dis* 1992;**146**:1543–7.
85 Bai TR, Bramley AM. Effect of an inhibitor of nitric oxide synthase on neural relaxation in human bronchi. *Am J Physiol* 1993;**8**:425–30.
86 Rhoden K, Barnes PJ. Epithelial modulation of NANC and VIP-induced responses: role of neutralendopeptidase. *Eur J Pharmacol* 1989;**171**:247–50.
87 Watson N, Maclagan J, Barnes PJ. Vagal control of guinea pig tracheal smooth muscle:lack of involvement of VIP and nitric oxide. *J Appl Physiol* 1993;**74**:1964–71.
88 Fischer A, Hoffman B, Hauser-Kronberger C, Mayer B, Kummer W. Nitric oxide synthase in the innervation of the human respiratory tract. *Am Rev Respir Dis* 1993;**147**:A662.
89 Fischer A, Mundel P, Mayer B, Preissler U, Philippin B, Kummer W. Nitric oxide synthase in guinea-pig lower airway innervation. *Neurosci Lett* 1993;**149**:157–60.
90 Dey RD, Dalal G, Pinkstaff CA, Mayer B, Kummer W, Said SI. Nitric oxide synthase and vasoactive intestinal peptide are colocalized in neurons of the ferret tracheal plexus. *Am Rev Respir Dis* 1993;**147**:A288.
91 Ignarro LJ, Lipton H, Edwards JC, Baricos WU, Hyman AL, Kaddowitz PJ, *et al.* Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of *S*-nitrosothiols as active intermediates. *J Pharmacol Exp Ther* 1981;**218**:739–49.
92 Aoki E, Sembra R, Kashiwamata S. Evidence for the presence of L-arginine in the glial component of the peripheral nervous system. *Brain Res* 1991;**559**: 159–62.
93 Chiu SY. Functions and distribution of voltage-gated sodium and potassium channels in mammalian Schwann cells. *Glia* 1991;**4**:541–8.
94 Grider JR, Murthy KS, Jin J-G, Makhlouf GM. Stimulation of nitric oxide from muscle cells by VIP: prejunctional enhancement of VIP release. *Am J Physiol* 1992;**262**:774–8.
95 Li CG, Rand MJ. Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur J Pharmacol* 1990;**191**:303–9.
96 Ward JR, Belvisi M, Fox AJ, Miura M, Tadjirarimi S, Yacoub MH, *et al.* Modulation of cholinergic neurotransmission by nitric oxide in human airway smooth muscle. *J Clin Invest* 1993;**92**:736–42.
97 Belvisi MG, Stretton CD, Barnes PJ. Nitric oxide as an endogenous modulator of cholinergic neurotransmission in guinea pig airways. *Eur J Pharmacol* 1991;**198**: 219–21.
98 Brave SR, Hobbs AJ, Gibson A, Tucker JF. The influence of L-N^G-nitro arginine on field stimulation induced contractions and acetylcholine release in guinea pig isolated tracheal smooth muscle. *Biochem*

Biophys Res Commun 1991;**179**:1017–22.
99 Belvisi MG, Miura M, Peters MJ, Barnes PJ. Nitric oxide modulates bradykinin-induced bronchoconstriction in guinea-pig airways in vivo. *Am Rev Respir Dis* 1992;**145**:A384.
100 Lei Y-Y, Barnes PJ, Rogers DF. Regulation of NANC bronchoconstriction in vivo in guinea pig: involvement of NO, VIP and soluble guanylyl cyclase. *Br J Pharmacol* 1993;**108**:228–35.
101 Costa M, Furness JB, Brookes SJH, Bredt DS, Snyder SH. Presence and chemical coding of neurons with nitric oxide synthase immunoreactivity in the guinea-pig small intestine. *Proc Aust Physiol Soc* 1991;**22**:98P.
102 Kummer W, Fischer A, Mundel P, Mayer B, Hora B, Philippin B, *et al.* Nitric oxide synthase in VIP-containing vasodilator nerve fibres in the guinea pig. *Neuro Rep* 1992;**3**:653–5.
103 Belvisi MG, Ward JK, Tadjarimi S, Yacoub MH, Barnes PJ. Inhibitory NANC nerves in human airways: differences in disease and after extrinsic denervation. *Am Rev Respir Dis* 1993;**147**:A286.
104 Stretton CD, Mak JCW, Belvisi MG, Yacoub MH, Barnes PJ. Cholinergic control of human airways in vitro following extrinsic denervation of the respiratory tract by heart-lung transplantation: *Am Rev Respir Dis* 1990;**142**:1030–3.
105 Canning BJ, Undem BJ. Relaxant innervation of the guinea-pig trachealis: demonstration of capsaicin-sensitive and insensitive vagal pathways. *J Physiol* 1992;**460**:719–39.
106 Lammers JWJ, Barnes PJ, Chung KF, Non-adrenergic, non-cholinergic airway inhibitory nerves. *Eur Respir J* 1992;**5**:239–46.
107 Rubanyi GM, Vanhoutte PM. Oxygen derived free radicals, endothelium and responsiveness of vascular smooth muscle. *Am J Physiol* 1986;**250**:H815–21.
108 Liu SF, Crawley DE, Evans TW, Barnes PJ. Endothelium-dependent non-adrenergic non-cholinergic neural relaxation in guinea pig pulmonary artery. *J Pharmacol Exp Ther* 1992;**260**:541–8.
109 Buga GM, Ignarro LJ. Electrical field stimulation causes endothelium-dependent and nitric oxide-mediated relaxation of pulmonary artery. *Am J Physiol* 1992; **262**:973–9.
110 Liu SF, Crawley DE, Rohde JAL, Evans TW, Barnes PJ. Role of nitric oxide and guanosine 3'-,5'-cyclic monophosphate in mediating nonadrenergic noncholinergic neural relaxation in guinea pig pulmonary arteries. *Br J Pharmacol* 1992;**107**:861–6.
111 Liu SF, Crawley DE, Evans TW, Barnes PJ. Endogenous nitric oxide modulates adrenergic neural vasoconstriction in guinea pig pulmonary artery. *Br J Pharmacol* 1991;**104**:565–9.
112 Thornbury KD, Ward SM, Dalziel HH, Carl A, Westfall DP, Sanders RM. Nitric oxide and nitrocysteine mimic nonadrenergic noncholinergic hyperpolarization in canine proximal colon. *Am J Physiol* 1991;**261**: G553–7.
113 Konaluk EA, Fung HL. Spontaneous liberation of nitric oxide cannot account for in vitro vascular relaxation by *S*-nitrosothiol. *J Pharmacol Exp Ther* 1990;**255**: 1256–64.
114 Hobbs AJ, Tucker JP, Gibson A. Differentiation by hydroquinone of relaxations induced by exogenous nitrates in non-vascular smooth muscle: role of superoxide anions. *Br J Pharmacol* 1991;**104**:654–50.
115 Gillespie JS, Sheng H. A comparison of haemoglobin and erythrocytes as inhibitors of smooth muscle relaxation by the NANC transmitter in the BRP and rat anococcygeus and by EDRF in the rabbit aortic strip. *Br J Pharmacol* 1989;**98**:445–50.
116 Tayeh MA, Marietta MA. Macrophage oxidation of L-arginine to nitric oxide, nitrite and nitrate. *J Biol Chem* 1989;**264**:19654–8.
117 Jorens PG, van Overveld FJ, Bult H, Vermeira PA, Herman AG. L-arginine-dependent production of nitrogen oxides by pulmonary macrophages. *Eur J Pharmacol* 1991;**200**:205–9.
118 Hibbs JB, Vavrin Z, Taintor RR. L-arginine is required post expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J Immunol* 1987;**138**:550–65.
119 Cunha FQ, Moncada S, Liew FY. Interleukin-10 (IL-10) inhibits the induction of nitric oxide synthase by interferon-γ in murine macrophages. *Biochem Biophys Res Commun* 1992;**186**:1155–9.
120 Assrevy J, Cunha FQ, Liew FY, Moncada S. Feedback inhibition of nitric oxide synthase by nitric oxide. *Br J Pharmacol* 1993;**108**:833–7.
121 Hite RD, Smith RM. Inducibility of human and murine mononuclear phagocyte nitric oxide synthase by lipolysaccharide and g-interferon. *Am Rev Respir Dis* 1992;**145**:A284.
122 Salvemini D, Masini C, Anggard E, Mannaioni PF, Vane J. Synthesis of 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.
123 McCall TB, Broughton-Smith NK, Palmer RMJ, Whittle BJR, Moncada S. Synthesis of nitric oxide from L-arginine by neutrophils. Release and interaction with superoxide anions. *Biochem J* 1989;**261**:293–8.
124 Nakayama DR, Geller DA, Lowenstein CJ, Davies P, Pitt BR, Simmons RL, *et al.* Cytokines and lipopolysaccharide induce nitric oxide synthase in cultured rat pulmonary artery smooth muscle. *Am J Respir Cell Mol Biol* 1992;7:471–6.
125 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**:833–6.
126 Liu SF, Brown CR, Evans T, Barnes PJ, Adcock IM. Endotoxin effects on inducible and constitutive nitric oxide synthase on RNA expression in rat lung and other tissues. *Am Rev Respir Dis* 1993;**147**:A246.
127 Nijkamp FP, van der Linde HJ, Folkerts G. Nitric oxide synthesis inhibition induces airway hyperresponsiveness: role of the epithelium. *Am Rev Respir Dis* 1993;**147**:A287.
128 Robbins RA, Hamel FG, Floreani AA, Gossman GL, Nelson KJ, Belenky NS, *et al.* Bovine bronchial epithelial cells metabolize L-arginine to L-citrulline: possible role of nitric oxide synthase. *Life Sci* 1993;**52**:709–16.
129 Chee C, Gaston B, Garard C, Loscalzo J, Kobzik L, Drazen JM, *et al.* Nitric oxide is produced by a human epithelial cell line. *Am Rev Respir Dis* 1993;**147**:A433.
130 Kobzik L, Drazen J, Bredt D, Lowenstein C, Snyder S, Gaston B, *et al.* Nitric oxide synthase (NOS) in the lung: immunologic and histochemical localization in human and rat tissue. *Am Rev Respir Dis* 1993; **147**:A515.
131 Springall DR, Hamid QA, Buttery LKD, Chanez P, Howarth P, Bousquet J, *et al.* Nitric oxide synthase induction in asthmatic human lung. *Am Rev Respir Dis* 1993;**147**:A515.
132 Jain B, Lubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun* 1993;**191**:83–8.
133 Jorens PG, van Overveld FJ, Vermeire PA, Bult H, Herman AG. Synergism between interleukin-1β and the nitric oxide synthase inducer interferon-γ in rat lung fibroblasts. *Eur J Pharmacol* 1992;**224**:7–12.
134 Holt PG. Regulation of antigen-presenting cell function(s) in lung and airway tissues. *Eur Respir J* 1993;**6**:120–9.
135 Spiteri MA, Knight RA, Wordsell M, Barnes PJ, Chung KF. Alveolar macrophage-induced suppression of T-cell hyperresponsiveness in asthma is reversed following allergen exposure in vitro. *Am Rev Respir Dis* 1993; in press.
136 Kawabe T, Isobe KI, Hasegawa Y, Nakashima I, Shikomota K. Immunosuppression actively induced by nitric oxide in culture supernatants of activated rat alveolar macrophages. *Immunology* 1992;**76**:72–8.
137 Ialenti A, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. *Eur J Pharmacol* 1992;**221**:177–82.
138 Kuo H-P, Liu S, Barnes PJ. The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea pig airways. *Eur J Pharmacol* 1992;**221**:385–8.
139 Pendino K, Punjabi C, Lavnikova N. Inhalation of ozone stimulates nitric oxide production by pulmonary alveolar and interstitial macrophages. *Am Rev Respir Dis* 1992;**145**:A650.
140 Mulligan MS, Hevel JM, Marletta MA, Ward PA. Tissue injury caused by deposition of immune complexes in L-arginine-dependent. *Proc Natl Acad Sci USA* 1991;**88**:6338–42.
141 Hirschleiter L, Arora Y. Nitrates in the treatment of bronchial asthma. *Br J Dis Chest* 1991;**39**:275–83.
142 Jansen A, Drazen J, Osborne JA, Brown R, Loscalzo J, Stamler JS. The relaxant properties in guinea pig airways of *S*-nitrosothiols. *J Pharmacol Exp Ther* 1992; **261**:154–60.
143 van Snick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990;**8**:253–78.
144 Baudouin SV, Bath P, Martin JF, du Bois R, Evans TW. L-Arginine infusion has no effect on systemic haemodynamics in normal volunteers or systemic and pulmonary haemodynamics in patients with elevated pulmonary vascular resistance. *Br J Clin Pharmacol* 1993;**36**:45–51.
145 Rees DD, Palmer RMJ, Moncada S, Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989;**86**:3375–8.