

CURRENT TOPIC

Nitric oxide and asthma

Bozhena Zoritch

Since nitric oxide (NO) was first described as endothelium derived relaxing factor (EDRF),¹ there has been compelling evidence that NO is involved in very many biological processes.²⁻⁹ It has at least four roles in the lungs.³ These are as a pulmonary vasodilator, bronchodilator, non-adrenergic non-cholinergic transmitter, and inflammatory mediator. There are three types of NO synthase, the enzyme catalysing the formation of NO – endothelial, neuronal (both known as constitutive or cNOs), and macrophage or inducible (iNOs) forms (fig 1).⁸ Agonists activate cNOs by an increase in calcium ion (Ca^{2+}) concentration resulting in release of NO in seconds.¹⁰ iNOs is part of the immune system and is induced by cytokines, endotoxin, and lipopolysaccharide resulting in formation of larger amounts of NO.¹¹ However, this reaction involves gene transcription, and is therefore much slower, resulting in increased production of NO over several hours or even days. It can be blocked by glucocorticosteroids. Once formed, NO activates soluble guanylyl cyclase (sGC) 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).^{12 13} The rise in cGMP results in relaxation of smooth muscle but the mechanism by which this happens is unknown. When produced in large quantities, NO is involved in tissue damage and cell death. Inactivation of enzymes containing transition metals, including mitochondrial enzymes, is one of the proposed toxic effects.¹⁴

Role of NO as vasodilator

NO dependent vasodilator tone is entirely locally regulated and as such is probably one of the simplest and most fundamental of adaptive mechanism in the cardiovascular system. Endothelium dependent relaxation was demonstrated in many vascular preparations, including veins, arteries, and microvessels.¹⁵ However, while the arteries and arterioles are in a continuous state of NO mediated vasodilation, basal release of NO does not control the resting tone of peripheral veins, although it may have an effect in central veins. Endothelial NO synthesis influences the adhesion and aggregation of platelets and leucocytes. Endothelial dysfunction, either genetic or acquired, can lead to cardiovascular disease including atherosclerosis, essential and pregnancy induced hypertension.⁶ In the lung, pulmonary vessels also synthesise NO continuously and this seems to be important for maintaining blood flow within the lungs and matching ventilation to perfusion.¹⁶ Reduced NO release may be the mechanism underlying hypoxic pulmonary vasoconstriction. Inhalation of NO can have a therapeutic effect in diseases associated with pulmonary vasoconstriction.¹⁷⁻¹⁹ NO was first used in adult respiratory distress syndrome as a pulmonary vasodilator.²⁰ In 1992 two groups in North America used NO in treating persistent fetal circulation successfully without causing hypotension or methaemoglobinaemia.^{21 22} It is already recognised that NO is an alternative to extracorporeal membrane oxygenation in selected cases.

NO in higher concentrations can have adverse influences on capillary permeability, causing oedema and plasma leak. In inflammatory lung conditions, such as asthma, induction of NOs in endothelium as well as epithelium may contribute to hyperaemia of mucosa, airway narrowing, and bronchoconstriction.

NO as a bronchodilator

Glyceryl trinitrate and sodium nitroprusside, whose vasodilator activity is partly mediated by NO production, relax airway smooth muscle in vitro, resulting in an increase in sGC activity and increase in cGMP.²³ It has been proposed that the epithelial lining produces an epithelial derived relaxing factor, which is similar to EDRF, but is not certain to be NO.²⁴ There is some evidence that relaxation

University of Southampton, Department of Child Health, Southampton General Hospital, Tremona Road, Southampton SO16 6YD

Correspondence to: Dr Zoritch.

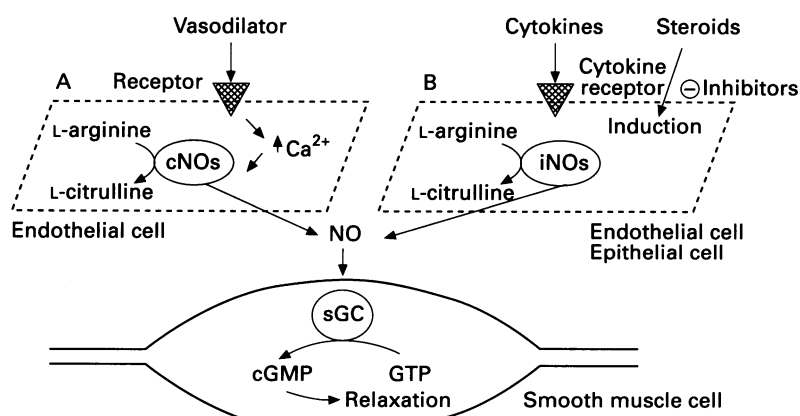


Figure 1 (A) cNOs is activated by a rise in Ca^{2+} in response to activation to a surface receptor. (B) iNOs is induced by cytokines or lipopolysaccharide resulting in larger amounts of NO formation (from Barnes and Belvisi⁵). GTP=guanosine triphosphate.

of precontracted guinea pig trachea is induced by NO.^{25,26} In asthma, the high concentration of NO produced by iNOs in epithelial cells may suppress the activity of cNOs, and cause enhancement in intracellular calcium concentration and airway constriction. The histamine and carbachol induced increase in cGMP production in the respiratory system has been shown to be an L-arginine dependent process.²⁶ It could be speculated that the epithelial layer, by releasing NO, acts as a negative feedback system to histamine induced contractions. In asthma, high concentrations of NO produced by inducible NO synthases in inflammatory or epithelial cells may downregulate the activity of cNOs. This may disturb the breaking mechanism that prevents enhanced airway constrictions. Damage or removal of epithelium increases smooth muscle responsiveness.²⁷ In asthma, epithelium is often damaged which may also affect production of NO by cNOs which would contribute to bronchospasm.²⁸ Airway responsiveness to histamine is increased after intratracheal inoculation of parainfluenza type 3 virus and can be blocked by inhaling L-arginine.²⁹ Therefore, it is likely that the deficiency in endogenous NO production after a viral infection is due to a dysfunction of the cNOs. A challenging hypothesis is that bronchial hyperresponsiveness may be suppressed by blocking the activity of the inducible form of NOs by using glucocorticosteroids. This would prevent downregulation of cNOs by iNOs. The effect of NO as a bronchodilator in humans has been disappointing. High concentrations of inhaled NO (>20 000 parts per billion) caused an increase in airway resistance in healthy subjects.³⁰ An alternative might be to manipulate release of neuronal NO which should give selective bronchodilatation. This might be particularly effective in wheezy infants who respond poorly to β agonists or in chronic obstructive pulmonary disease. However, there are potential dangers of inhaling NO in the presence of oxygen because of the formation of nitrate and nitrous and nitric acid which may increase airway responsiveness and in high concentration may cause pulmonary oedema.³¹ Peroxynitrite may generate tissue damaging

hydroxyl radicals.³² High concentration of NO may have effects on DNA and be genotoxic and cytotoxic.³³

Role of NO as neurotransmitter

There is increasing evidence that NO may function as a neurotransmitter of inhibitory non-adrenergic non-cholinergic (NANC) nerves or purinergic pathways as they were previously described³⁴ (fig 2). NO appears to account for the bronchodilator NANC response in vitro in human central and peripheral airways. Nerves containing NOs appear to supply bronchial vessels, airway smooth muscle, and submucosal glands. Parasympathetic, sympathetic, and sensory ganglia supplying the airways also contain NOs. NOs inhibitors potentiate the cholinergic neural response by acting as a functional antagonist to acetylcholine in airway smooth muscle. There is evidence that NO modulates reflex bronchoconstriction.³⁵ Bronchodilator NANC nerves are the only neural bronchodilator pathway in the human airway.

The question for research is whether there is any defect in those nerves in diseased airways. There is some evidence for this. Although NANC responses in tissues from patients with mild asthma were not altered, responses were significantly reduced from patients with cystic fibrosis.³⁶ Further information is needed on the NANC neural bronchodilator pathway to unravel whether it has a part to play in the pathophysiology of early asthma in children.

Persson *et al* demonstrated that NO in exhaled air increased with exercise in healthy volunteers.³⁷ It would be of value to study the change of NO concentration in exercise induced asthma to establish if there is any abnormality in the modulation of bronchoconstriction in response to exercise in asthmatics. Perhaps the bronchoconstriction produced during exercise in subjects with asthma and cystic fibrosis is due to the increased NO release.

Active smoking decreases the amount of NO in exhaled air in healthy people.³⁸ The regulation of NO synthesis by neuronal cNOs may be impaired in children whose mothers smoke in pregnancy and who were exposed to passive smoking in infancy which may predispose them to wheezing illnesses.

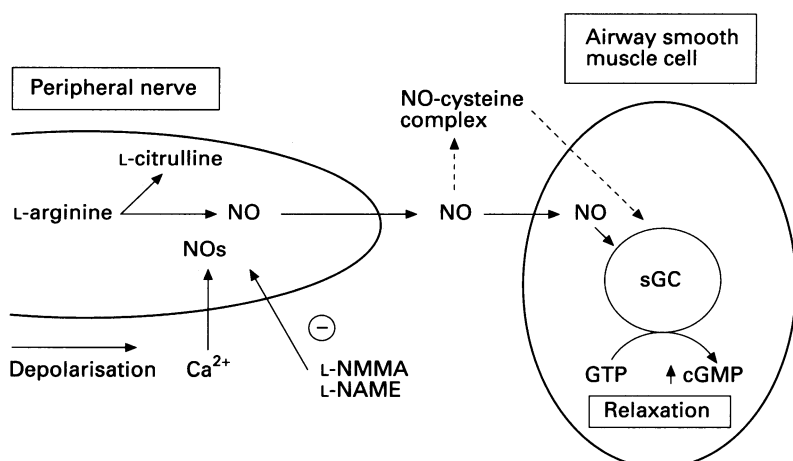


Figure 2 Inhibitory NANC nerves in airways utilise NO as a neurotransmitter (from Barnes and Belvisi³⁵). GTP=guanosine triphosphate; L-NMMA=N^G-monomethyl-L-arginine; L-NAME=N^G-nitro-L-arginine methylester.

Role of NO as an inflammatory mediator

Macrophages express iNOs after exposure to cytokines, in particular tumour necrosis factor- α , interferon γ , and interleukin-1 β as well as endotoxins. Thus NO contributes to their cytotoxic effect.¹¹ The cytotoxic activity of macrophages as well as nitrite and nitrate formation are blocked by inhibitors of iNOs, such as glucocorticoids.³⁹

Epithelial cells are also capable of producing NO after induction of iNOs by cytokines. Recent evidence elegantly demonstrated how iNOs can be induced by tumour necrosis factor- α in cell lines from human bronchial mucosa.⁴⁰ The same paper described using

specific antisera to stain for iNOs on lung biopsy specimens from a group of people with asthma. There was increased iNOs expression on the samples from asthmatics who were not on inhaled steroids compared with samples from patients with lung cancer. In asthma there is upregulation of many cytokines including tumour necrosis factor- α , interferon γ , and interleukin- 1β .⁴¹ It is, therefore, easy to understand why iNOs is upregulated.

NO has an immunomodulatory role in asthma and most likely in other inflammatory conditions via effects on T lymphocytes,⁴² neutrophils,⁴³ and macrophages.¹¹ Furthermore, mast cells and fibroblasts are also capable of producing NO⁴⁴ and mast cell degranulation causing release of many bronchoconstriction mediators is a feature of asthma. It is likely that in the early stages of asthma NO acts as an endogenous bronchodilator to counteract the bronchoconstriction caused by mast cell degranulation. However, when greater quantities of NO are produced in established asthma, its production is a double edged sword. It causes plasma leak as a result of vasodilatation and has a cytotoxic effect contributing to epithelial shedding.

Thus NO is involved in chronic lung injury in asthma and probably also in cystic fibrosis. It mediates the effect of ozone in damaging the epithelium,⁴⁵ which may be significant in view of the increased incidence of asthma in recent times. Further evidence of its importance in asthma comes from a study by Kharitonov *et al* looking at NO concentration in expired air from asthmatic patients.⁴⁶ Measured by chemiluminescence, it was significantly raised in subjects with asthma who were not on inhaled or oral steroids, compared with controls and subjects on steroids. The concentration of NO in exhaled air was reduced by inhaling an arginine analogue, L-NMMA (*N*^G-monomethyl-L-arginine), which inhibits NO synthesis. The increased level of NO in asthmatics has been confirmed by other authors.^{37 47 48} Further development of this technique may make it possible to monitor lung inflammation by measuring NO in exhaled air and this may be a valuable tool in directing anti-inflammatory treatment.⁴⁹ Asthma is an obvious clinical situation where it can be used, although it might be of value in children with cystic fibrosis and bronchopulmonary dysplasia.

There is some evidence that in healthy people the major part of NO in exhaled air originates in the nasal airways with only a minor contribution from lower airways.^{47 48} This relatively large production should be taken into account when measuring NO as an indicator of inflammation in lower airways. In subjects with seasonal rhinitis, however, the nasal levels of NO were much higher than in controls but the level of NO was also increased with oral sampling, suggesting generalised inflammation of airways in patients with rhinitis but no obvious evidence of clinical asthma (U Martin, personal communication). By comparison, the study by Alving *et al* suggested that the level of NO was not signifi-

cantly increased in asthmatics if measured nasally but became 2–3 fold higher if measured orally compared to controls.⁴⁷ This suggests that the major source of NO in normal airway is bronchial epithelium but when this is shed, as in inflammation, it is fibroblasts and macrophages that produce NO.

The majority of studies measuring NO in exhaled air involve adult patients. However, Lundberg *et al* have used a direct nasal sampling technique to measure NO in exhaled air from children.⁴⁸ It would be of particular interest to look at NO production in infants and young children in order to unravel the role of inflammation in early asthma. This could then lead to development of early intervention strategies. Whether inhibiting of the NO inflammatory pathway will have any direct therapeutic effect in asthma remains to be seen. Considering the information already available regarding the site of production of NO in asthma and atopic rhinitis, and the technical difficulties associated with collecting samples in children, careful subject selection will be needed for future studies.

Finally the presence of NO in car exhaust, gas cookers and cigarette smoke, may in part explain the increased incidence of asthma over the last few decades.

I am grateful to Professor J O Warner for his helpful comments and advice.

- Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524–6.
- Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; 43: 109–41.
- Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993; 48: 1034–43.
- Barnes PJ. Nitric oxide and airways. *Eur Respir J* 1993; 6: 163–5.
- Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; 329: 2002–12.
- Anggard E. Nitric oxide: mediator, murderer and medicine. *Lancet* 1994; 343: 1199–206.
- Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airway. *American Journal of Respiratory Critical Care Medicine* 1994; 149: 538–51.
- Vallance P, Collier J. Biology and clinical relevance of nitric oxide. *BMJ* 1994; 309: 454–7.
- Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J* 1994; 298: 249–58.
- Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 1990; 87: 682–5.
- Hibbs JB, Taintor RR, Vaurin Z. Macrophage cytotoxicity: role of L-arginine deiminase activity and imino nitrogen oxidation to nitrite. *Science* 1987; 235: 473–6.
- Waldman SA, Murad F. Biochemical mechanisms underlying vascular smooth muscle relaxation: the guanylate cyclase-cyclic GMP system. *J Cardiovasc Pharmacol* 1988; 12 (suppl 5): S115–8.
- Liu SF, Crawley DE, Rohde JAL, Evans TW, Barnes PJ. Role of nitric oxide and guanosine 3'5' cyclic monophosphate in mediating non-adrenergic, non-cholinergic relaxation in guinea-pig pulmonary arteries. *Br J Pharmacol* 1992; 107: 861–6.
- Hibbs JB, Vaurin 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.
- Calver A, Collier J, Vallance P. Nitric oxide and cardiovascular control. *Exp Physiol* 1993; 78: 303–26.
- 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.
- Higenbottam TW, Pepke Zaba J, Woolman P, Wallwork J. Inhaled endothelium derived relaxing factor (EDRF) in primary pulmonary hypertension (PPH). *Am Rev Respir Dis* 1988; 137: A107.
- Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J. Inhaled nitric oxide causes selective pulmonary vasodilatation in patients with pulmonary hypertension. *Lancet* 1991; 338: 1173–4.

- 19 Frostell C, Fratacci MD, Warin JC, Jones R, Zapol WM. Inhaled nitric oxide, a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991; **83**: 2038-47.
- 20 Rossiant R, Falke KJ, 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.
- 21 Roberts JD, Palaner DM, Long P, Zapol WM. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992; **340**: 818-9.
- 22 Kinsella JP, Neish SR, Shaffer E, Abman SH. Low dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992; **340**: 819-20.
- 23 Dupuy PM, Shore SA, Drazen JM, et al. Bronchodilator action of inhaled nitric oxide in guinea-pigs. *J Clin Invest* 1992; **90**: 421-8.
- 24 Munakata M, Masaki Y, Sakuma I, et al. Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. *J Appl Physiol* 1990; **69**: 665-70.
- 25 Filep JG, Battistini B, Sirois P. Induction of endothelin-1 of epithelium-dependent relaxation of guinea-pig trachea in vitro: role for nitric oxide. *Br J Pharmacol* 1993; **109**: 637-44.
- 26 Leurs R, Broziuum MM, Hansen W, Bast A, Timmerman H. Histamine H1-receptor-mediator cyclic GMP production in guinea pig lung tissue is an L-arginine-dependent process. *Biochem Pharmacol* 1991; **42**: 271-7.
- 27 Vanhoutte PM. Epithelium-derived relaxing factor(s) and reactivity. *J Allergy Clin Immunol* 1989; **83**: 855-61.
- 28 Djukanovic R, Roche WR, Wilson JW, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; **142**: 434-57.
- 29 Nijkamp FP, van der Linde HJ, Folkerts G. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium. *Am Rev Respir Dis* 1993; **148**: 727-34.
- 30 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**: A515.
- 31 Rasmussen TR, Kjaergaard SK, Tarp U, Pedersen OF. Delayed effects of NO₂ exposure on alveolar permeability and glutathione peroxidase in healthy humans. *Am Rev Respir Dis* 1992; **146**: 654-9.
- 32 Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 1990; **87**: 1620-4.
- 33 Wink DA, Kasprzak KS, Marangos CM. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; **259**: 1000-3.
- 34 Belvisi MG, Stretton CD, Barnes PJ. Nitric oxide is the endogenous neurotransmitter or bronchodilator nerves in human airway. *Eur J Pharmacol* 1992; **210**: 221-2.
- 35 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.
- 36 Tuan Dinh-Xuan A, Higenbottam TW, Clelland CA, et al. Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; **324**: 1539-47.
- 37 Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 1994; **343**: 146-7.
- 38 Schilling J, Holzer P, Guggenbach M, Gyurech D, Marathia K, Geroulanos S. Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives. *Eur Respir J* 1994; **7**: 467-71.
- 39 Radomski MW, Palmer RMJ, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not constitutive nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; **87**: 10043-9.
- 40 Hamid Q, Springall DR, Riveros-Moreno V, et al. Induction of nitric oxide synthase in asthma. *Lancet* 1993; **342**: 1510-3.
- 41 Holgate S. Mediator and cytokine mechanisms in asthma. *Thorax* 1993; **48**: 103-9.
- 42 Liew FY, Li Y, Severn A, et al. A possible novel pathway of regulation by murine T helper type-2 cells (Th2) of Th1 cell activity via the modulation of the induction of nitric oxide synthase in macrophage. *Eur J Immunol* 1993; **21**: 2489-94.
- 43 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.
- 44 Salverini 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.
- 45 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.
- 46 Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; **343**: 133-5.
- 47 Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993; **6**: 1368-70.
- 48 Lundberg JON, Weitzberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K. Primary nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. *Eur Respir J* 1994; **7**: 1501-4.
- 49 Borland C, Cox Y, Higenbottam T. Measurement of exhaled nitric oxide in man. *Thorax* 1993; **48**: 1160-2.