Nitric oxide and airways

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For over 20 yrs it has been recognized that the vasodilator responses to many agents are mediated by the release of a vasodilator substance from endothelial cells [1]. The identity of endothelium-derived relaxant factor (EDRF) remained elusive, largely because of its short half-life, until 1987 when Palmer and co-workers [2] were able to show that EDRF was likely to be nitric oxide (NO). 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 supportive evidence [3]. One of the most important advances has been the discovery of substances which block the production of endogenous NO. Nitric oxide is formed from the semi-essential amino acid L-arginine via the action of an enzyme NO synthase [4]. NO synthase exists in constitutive forms (requiring Ca²⁺ for activation) and inducible forms (which are independent of Ca²⁺), and several NO synthase genes have recently been cloned [3]. Analogues of L-arginine were found, which acted as false substrates for the enzyme and, therefore, blocked the formation of endogenous NO. 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 N⁶-monomethyl-L-arginine (L-NMMA) and N⁰-nitro-L-arginine methyl ester (L-NAME), which have proved to be extremely useful in revealing the role of endogenous NO in a whole variety of processes [5]. Nitric oxide relaxes vascular smooth muscle by activation of soluble guanylyl cyclase, with an increase in concentration of cyclic guanosine 3’5’monophosphate (cGMP). It had long been recognized that directly acting vasodilators, such as glyceryl trinitrate and sodium nitroprusside act as NO donors [6].

The observation that NO is a vasodilator, immediately suggested that it may play a role in the regulation of the pulmonary circulation, and this has been extensively investigated. Nitric oxide mediates the vasodilator action of acetylcholine in animal and human pulmonary vessels [7, 8], and appears to act as a braking mechanism against pulmonary vasoconstriction [8]. Release of NO from endothelial cells in the pulmonary circulation appears to counteract hypoxic vasoconstriction [9, 10], and NO release is apparently decreased in hypoxia [11]. There is circumstantial evidence that NO release from pulmonary vessels may be impaired in patients with chronic obstructive pulmonary disease (COPD) [12]. Since NO is a potent pulmonary vasodilator, inhalation of NO might be effective as a selective pulmonary vasodilator, in view of its short half-life. Inhaled NO has been shown to dose-dependently inhibit pulmonary vasoconstriction induced by an infusion of a thromboxane analogue in lambs [13]. Inhalation of NO has also been shown to cause selective pulmonary vasodilation in patients with pulmonary hypertension [14], and COPD [15].

Nitrovasodilators, such as glyceryl trinitrate and sodium nitroprusside, also relax airway smooth muscle in vitro, resulting from an increase in soluble guanylyl cyclase activity, and an increase in cGMP [16]. It is, therefore, to be expected that NO may act as a bronchodilator and this has been demonstrated in canine airways in vitro [17]. In the present issue of the journal HOGMAN et al. [18] have demonstrated that a relatively high concentration of inhaled NO (80 parts per million) reduced the bronchoconstrictor effect of nebulized methacholine in anaesthetized rabbits. A more detailed study in anaesthetized guinea-pigs has recently demonstrated a concentration-dependent, but transient, reversal of methacholine-induced bronchoconstriction from 5–300 ppm [19]. In addition, a high concentration of NO (300 ppm) caused a baseline bronchodilatation. There was no evidence of tolerance after prolonged administration, and the bronchodilator effect of NO was additive with an β-adrenoceptor agonist. This raises the possibility that NO inhalation, or NO releasing compounds, might have some therapeutic potential as alternative bronchodilators. An advantage of inhaled NO would be its lack of systemic effects, since NO would be rapidly inactivated by haemoglobin. However, there are potential dangers of inhaling NO [20], since in the presence of oxygen it is oxidised to NO₂ and, thence, to nitrous and nitric acids, which may increase airway responsiveness, and in high concentration might cause pulmonary oedema [21, 22]. The interaction between NO and superoxide anions may lead to the formation of peroxynitrite, which may generate tissue damaging hydroxyl radicals [23]. There is also some evidence that high concentrations of NO may have effects on deoxyribonucleic acid (DNA) and be both genotoxic and cytotoxic [24].

There is increasing evidence that NO may function as a neurotransmitter of nonadrenergic noncholinergic (NANC) nerves, and nitrergic neurotransmission has been demonstrated in the gut, bladder and reproductive organs [25]. There is convincing evidence that NO is released from nerves themselves, since a particular form of NO synthase has been localized to peripheral nerves [26], and is activated by calcium entry when the nerve is depolarized. Nitric oxide accounts for approximately half of the inhibitory (bronchodilator) NANC response in guinea-pig trachea.
in vitro [27, 28], and modulates neural bronchoconstriction in vivo [29]. Nitric oxide appears to account for most of the bronchodilator NANC response in human airways in vitro [30, 31], and in contrast to guinea-pig trachea the neuropeptide vasoactive intestinal peptide appears to play little or no role in this response. Endogenous NO appears to modulate cholinergic neurotransmission in both guinea-pig and human airways, by acting as a functional antagonist to acetylcholine at airway smooth muscle [32, 33], but whether it is released from cholinergic nerves in the airways is not yet clear. Since bronchodilator NANC nerves are the only neural bronchodilator pathway in human airways [34], it is possible that there may be a defect in function of these nerves in asthmatic airways. Airway inflammation may be associated with release of superoxide anions from activated inflammatory cells, resulting in increased breakdown of NO [35]. Augmentation of NO release from airway nerves may, therefore, be of benefit in asthmatic patients.

NO is also a neurotransmitter of vasodilator NANC responses in pulmonary vessels and, therefore, may be involved in neural regulation of pulmonary blood flow [36].

Endothelial cells and nerves are not the only source of NO in airways. It is convincing evidence that macrophages, including alveolar macrophages, may synthesize NO after exposure to various cytokines [37], and to endotoxin [38], and that NO is important in host defence [3]. Macrophages express an inducible form of NO synthase, which has recently been cloned [39]. Other cells also have an inducible form of NO synthase, including endothelial cells, neutrophils and vascular smooth muscle cells [3, 40]. Exposure to cytokines such as tumour necrosis factor-α (TNF-α) may result in induction of NO synthase, which may lead to the formation of large amounts of NO. Indeed there is compelling evidence that NO induced in septic shock is a major contributor to the cardiovascular collapse [3]. The amount of NO produced by the inducible enzyme is very much greater than that produced by the constitutive enzyme in endothelial cells and nerves. It is likely that airway epithelial cells may also be a source of NO, and this could be induced by exposure to cytokines such as TNF-α in the airway, although there is no evidence that NO is "epithelium-derived relaxing factor" [41].

Endogenous NO may be a double-edged sword. Nitric oxide may be beneficial in relaxing airway smooth muscle in airways, but may have deleterious effects when produced in high concentrations. It 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 NO production significantly reduces plasma exudation and inflammation, both in skin [42], and in airways [43]. Corticosteroids inhibit the expression of the inducible, but not the constitutive form of NO synthase [38, 44], and this may contribute to their anti-inflammatory action, since massive NO formation may be detrimental, as in the case of endotoxic shock. If NO synthase is induced in airway epithelial cells in asthma, as a result of exposure to cytokines released from inflammatory cells, then inhaled steroids may act to reduce the formation of NO and, thus, to down-regulate the vascular components of the inflammatory response. Steroids would not be expected to affect the release of NO from bronchodilator nerves, since the neural constitutive form of the enzyme is not steroid sensitive.

It is clear that NO may have a very important regulatory role in airway function, and may be implicated in the pathophysiology of airway disease. Interest in NO has revived interest in nitrovasodilators as alternative bronchodilators, although previous studies of such drugs in asthma have not been impressive [45]. New NO donors, such as S-nitrosothiols may have advantages [46]. It is possible, that this may lead to new treatment approaches, both by enhancing the release of neuronal NO and, possibly, by inhibiting the formation of NO by the inducible enzyme. While inhibition of endogenous NO, using arginine analogues which block all forms of the enzyme, is likely to lead to problems, such as hypertension, it is possible that selective inhibitors of the inducible enzyme may be developed in the future. This is an exciting new area of research which has applicability to every branch of pulmonary medicine.

References
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