ABSTRACT
Nitric oxide (NO) is produced within the airways and has a variety of actions on the airway function. Nitric oxide is a potent bronchodilator, and NO released from airway epithelial cells and inhibitory nonadrenergic non-cholinergic nerve terminals may attenuate airway hyperresponsiveness. However, a large amount of NO produced by inducible NO synthase may facilitate airway inflammation, which then leads to airway hyperresponsiveness. Although the role of NO remains controversial, the measurement of exhaled NO may well be of value in the clinical management of asthma.

Key words: arginine, epithelial cells, inhibitory nonadrenergic non-cholinergic nerve, L-NAME, peroxynitrate.

INTRODUCTION
Nitric oxide (NO) has recently attracted attention in the pathophysiology of bronchial asthma. Nitric oxide is produced by a variety of cells within the respiratory tract, including nerve cells, endothelial cells, vascular and airway smooth muscle cells, inflammatory cells and airway epithelial cells. Nitric oxide is synthesized from one of the guanidino nitrogens of L-arginine by enzyme nitric oxide synthase (NOS). To date, several isoforms of NOS have been purified and cloned. These proteins represent a novel family of mammalian enzymes that contain both heme and cytochrome P450 reductase domains. The three prototypical forms of NOS are neuronal NOS (nNOS) and endothelial NOS (eNOS), which are constitutive, and inducible NOS (iNOS). They are derived from separate genes and are regulated by diverse signaling pathways. The biological actions of NO are terminated by spontaneous oxidation to NO\textsuperscript{2−} and NO\textsuperscript{3−}. The biological half-life of the very lipophilic NO is only 3–5 s and this allows NO to function locally as an autacoid.

NITRIC OXIDE RELEASED FROM AIRWAY EPITHELIAL CELLS
Nitric oxide is a potent bronchodilator. Nitric oxide and various NO-containing compounds activate cytosolic guanylate cyclase, which catalyzes the formation of the second messenger cyclic guanosine 3′-5′-monophosphate (c-GMP). This cyclic nucleotide is involved in the regulation of certain target cells such as airway smooth muscle. Nitric oxide-containing vasodilators, such as glyceryl trinitrate and sodium nitroprusside, activate guanylate cyclase and raise the c-GMP levels, inducing relaxation of the isolated airway smooth muscle. Tracheal relaxation by bradykinin or potassium chloride is mediated by NO released from the epithelial cells, and it appears to be important in blunting the histamine contractile response of the airway tissue. As a result, in guinea-pigs, inhibition of NO synthesis by N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME) has been shown to enhance airway responsiveness in vivo and in vitro.\textsuperscript{1,2} Due to the fact that epithelial denudation diminished the effects of L-NAME, the investigators concluded that the NO responsible for regulating airway responsiveness may be released from airway epithelial cells. Virus (para-influenza type 3)-induced airway hyperreactivity in guinea-pigs has also been reported to correlate with a deficiency in the endogenous constitutive NO production by the airways and can be blocked by low doses of L-arginine.\textsuperscript{2}
**NITRIC OXIDE AS A NEUROTRANSMITTER OF THE INHIBITORY NONADRENERGIC AND NON-CHOLINERGIC RESPONSE**

Endogenous NO is also likely to account for the bronchodilating effect of the inhibitory nonadrenergic and non-cholinergic (iNANC) response. Although the neurotransmitters responsible for the iNANC mediated relaxation of the airways have not yet been conclusively identified, NO is considered to be a promising candidate.3–7 In humans and other mammalian species, the inhibition of NO has been reported to produce a concentration-dependent inhibition of iNANC mediated relaxation. In the human bronchi especially, L-NAME almost completely abolished iNANC mediated relaxation and the authors thus concluded that NO is entirely responsible for iNANC mediated relaxation in human airways.5 In feline airways, NO has been proposed as the primary mediator of iNANC mediated relaxation.8

We previously investigated the effects of L-NAME on iNANC relaxation evoked by electrical stimulation of the vagus nerves in vivo and in vitro in the cat.9 To that end, we measured the pulmonary resistance (Ṙ) during vagal nerve stimulation (VS) in vivo, and the isometric tension of the small bronchi (1–3 mm outer diameter) during electrical field stimulation (EFS) in vitro. During the infusion of 5-hydroxytryptamine (5-HT), VS transiently decreased Ṙ in the presence of atropine and propranolol, with peak relaxation several seconds after VS and a gradual return to the baseline within 2–3 min. L-NAME abolished the initial peak relaxation and reduced the peak amplitude but did not affect the duration of NANC relaxation (Fig. 1). In the small bronchi obtained from control cats EFS evoked a biphasic NANC relaxation, comprising an initial fast component followed by a second slow component, and L-NAME selectively abolished the first component without affecting the second. However, in the small bronchi obtained from L-NAME pretreated cats, EFS elicited only the slow component of NANC relaxation, which was insensitive to L-NAME but sensitive to tetrodotoxin. These results indicate that the iNANC relaxation induced by VS during the infusion of 5-HT can be classified into two components, and that at least two neurotransmitters, including NO, are involved in the relaxation. However, the role of NO released from the iNANC nerve terminals in airway responsiveness remains to be elucidated.

We next investigated the role of NO in the regulation of airway responsiveness in anesthetized and mechanically ventilated cats. To assess airway responsiveness, we measured the changes in Ṙ produced by delivering 5-HT aerosol to the airways before and after L-NAME, or the blockade of iNANC neurons by hexamethonium. The inhibition of NOS by L-NAME or the blockade of iNANC neurons by hexamethonium significantly increased airway responsiveness (Fig. 2). However, the addition of L-NAME did not further increase airway responsiveness in animals treated with hexamethonium. These results suggest that NO may attenuate airway responsiveness and that NO originates in the iNANC neurons because L-NAME did not cause a further increase in airway responsiveness after the inhibition of iNANC neurons by hexamethonium. To further clarify the mechanism(s) involved, we also determined the effect of inhaled capsaicin in the animals with bronchoconstriction induced by 5-HT after treatment with atropine and propranolol. In the presence of atropine and propranolol, inhaled capsaicin caused a marked bronchodilation during 5-HT-induced bronchoconstriction, suggesting that such bronchodilation was mediated by iNANC. This bronchodilation was significantly suppressed by either hexamethonium or by L-NAME, which suggests that the NO released from the iNANC neurons by a reflex mechanism play an important role in modulating the airway responsiveness of cats in vivo.

The results of our study indicated the importance of iNANC as the source of NO. However, other investigators also suggested NO to be mainly released from airway epithelial cells. The reason for the discrepancy between our study and the previous one by Folkerts et al. is mainly due to species differences.2 Thus, in cats, the airways from the trachea to the bronchiole are innervated with a rich supply of iNANC neurons.10 The activation of these neurons causes a potent bronchodilation of the entire airway in vivo.10–12 In guinea-pigs, however, in vivo studies failed to demonstrate such iNANC-mediated bronchodilation.13–15 This was presumably because the guinea-pig airways beyond the main bronchi are innervated with a rich supply of excitatory NANC neurons.16,17 As a result, iNANC neurons are not considered to play a key role in the regulation of airway responsiveness in guinea-pigs.

**NITRIC OXIDE AS AN INFLAMMATORY MEDIATOR**

In addition to the aforementioned biological activities, NO also acts as an inflammatory mediator in airways. Nitric oxide reacts with superoxide (O₂⁻), forming peroxynitrite (ONO−₂), and this reaction occurs in vivo.
Peroxynitrite is a highly reactive compound with harmful effects on cells and could therefore be an important microbicidal compound. It has recently been reported that peroxynitrate can induce airway hyperresponsive-ness, which suggests the possibility of NO playing a role in the induction of airway hyperresponsiveness.\(^\text{18}\) Inducible NOS has a much greater capacity to produce NO than cNOS and may thus be involved in airway inflammation. Inducible NOS is expressed in epithelial cells after exposure to such cytokines as tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)), interleukin-1\(\beta\) (IL-1\(\beta\)), and interferon-\(\gamma\) (IFN\(\gamma\)).\(^\text{19}\) In addition, exhaled NO has also been shown to be increased in inflammatory airway diseases.\(^\text{20-22}\) Asthmatic patients have higher amounts of NO in their expired air, possibly due to inflammation. This increased production of NO can be inhibited by inhaled corticosteroids. These observations suggest that NO derived from iNOS may facilitate airway inflammation.

We hypothesized that endogenous NO contributes to airway inflammation and hyperresponsiveness, and that interleukin (IL)-8 might also be involved in this mechanism. In human transformed bronchial epithelial cells in vitro, NO donors increased IL-8 production dose-dependently. In addition, TNF\(\alpha\) plus IL-1\(\beta\) plus IFN\(\gamma\) also

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**Fig. 1** Effect of N\(^\text{\textsuperscript{\text{\textwedge}}\text{\textsuperscript{\text{\textn}}}}\)-nitro-L-arginine methyl ester (L-NAME) on inhibitory nonadrenergic and noncholinergic (iNANC) relaxation induced by electrical vagal stimulation in the cats treated with propranolol and atropine. Electrical vagal stimulation caused marked bronchodilatation mediated by iNANC (○), and L-NAME significantly suppressed the magnitude of bronchodilatation immediately after stimulation (●). Electrical vagal stimulation was applied at (a) 10, (b) 20 and (c) 30 pulses with a duration of 1 ms (left) or 4 ms (right). \(R_L\), pulmonary resistance; *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); †\(P < 0.005\).
increased IL-8 production in a culture supernatant of epithelial cells; the combination of NOS inhibitors, aminoguanidine plus L-NAME, attenuated this cytokine-induced IL-8 production. In guinea-pigs in vivo, ozone exposure induced airway hyperresponsiveness to acetylcholine and increased neutrophils in bronchoalveolar lavage fluid, with these changes persisting for at least 5 h. Pretreatment with NOS inhibitors had no effect on either airway hyperresponsiveness or neutrophil accumulation immediately after ozone exposure but significantly inhibited the changes 5 h after ozone exposure. Nitric oxide synthase inhibitors also attenuated the increases in the NO2/NO3 levels in bronchoalveolar lavage fluid and the IL-8 mRNA expression in the epithelial cells and in the neutrophils in guinea-pig airways 5 h after ozone exposure. These results suggest that endogenous NO may play an important role in persistent airway inflammation and hyperresponsiveness after ozone exposure, presumably through, in part, the upregulation of IL-8. Further study is needed in order to elucidate the interaction of NO derived from iNOS with NO released from iNANC neurons and the resultant effect on airway hyperresponsiveness.

**EXHALED NITRIC OXIDE**

Although the role of NO in asthma remains controversial, exhaled NO has been shown to increase in inflammatory airway diseases. These observations suggest, at least in part, the clinical importance of measuring NO in the management of asthma. The evaluation of NO is a non-invasive method which has recently attracted much attention regarding the management of asthma.19,22–27 The exhaled NO level in asthmatics is reported to reflect the amount of NO mainly produced by iNOS in the inflamed airways.26,28–31 Very recently, significant correlations were reported to exist among exhaled NO, sputum eosinophils and airway hyperresponsiveness in patients with mild asthma who were not being treated with inhaled steroids.32

Since bronchial asthma is characterized by eosinophilic airway inflammation, non-invasive methods which can estimate the severity of inflammation are needed for its clinical management. We investigated the correlations among the eosinophil counts in the induced sputum, exhaled NO, diurnal variation of peak expiratory flow (PEF), and airway hyperresponsiveness in order to evaluate their usefulness in predicting deterioration of PEF in patients with mild to moderate asthma. The airway hyperresponsiveness to methacholine, induced sputum and exhaled NO were measured in all patients while daily PEF monitoring was also performed. Peak expiratory flow deterioration was retrospectively assessed by a change in the mean PEF values between the day of the measurements and 1 week after measurements. The exhaled NO significantly correlated with the eosinophils in induced...
sputum and with airway hyperresponsiveness. More importantly, the deterioration of PEF after these measurements significantly correlated with the diurnal variation of PEF, exhaled NO and airway hyperresponsiveness. These results suggest that an evaluation of exhaled NO in combination with sputum eosinophils may be a useful and novel modality in the management of asthma.

Because NO has a variety of effects on the airway, the role of NO in asthma remains uncertain. However, recent studies indicate the clinical usefulness of measuring the exhaled NO level in order to improve the management of asthma.

REFERENCES

11. Irvin CG, Boileau R, Tremblay J, Martin RR, Macklem PT. Bronchodilatation: noncholinergic, nonadrenergic media-


