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Nitric oxide inhibition of basal and neurogenic mucus secretion in ferret trachea *in vitro*

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1 In order to examine the role of nitric oxide (NO) on airway mucus secretion we studied the effects of the nitric oxide synthase (NOS) inhibitor L-N^G-monomethyl-L-arginine (L-NMMA), a novel nitric oxide donor, (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide (FK409), and the NO precursor L-arginine on basal mucus secretion in the ferret trachea *in vitro* in Ussing chambers. We also determined the effects of these agents upon secretion induced by electrical stimulation of nerves or by acetylcholine (ACh). We used $^{35}SO_4$ as a mucus marker.

2 L-NMMA (0.01-1 mM) increased basal output of $^{35}SO_4$-labelled macromolecules with a maximal increase above baseline of 248% at 0.1 mM L-NMMA. L-Arginine (1 mM) alone had no significant effect on basal secretion but reversed the potentiating effect of L-NMMA on basal secretion. L-NMMA-induced increases in basal mucus secretion were sustained for at least 30 min in the continuing presence of the NOS inhibitor. In contrast to the potentiating effects of L-NMMA, FK409 (100 nM) reduced basal secretion by 60% (at 1 nM and at 10 nM it was without effect).

3 Electrical stimulation (50 V, 10 Hz, 0.5 ms for 5 min) increased $^{35}SO_4$ output by 174%. L-NMMA (1 and 10 mM) present during stimulation of tracheal segments resulted in significant potentiations of 214% and 116%, respectively, of the neurogenic response. The potentiated response to 10 mM L-NMMA was reversed by L-arginine (1 mM). At this dose L-arginine had no effect itself on basal secretion. In contrast to the potentiating effects of L-NMMA on neurogenic secretion, FK409 at 10 nM and 100 nM inhibited the neurogenic response by 98% and 99%.

4 At all concentrations tested, neither L-NMMA (0.01 mM-1 mM) nor FK409 (1-100 mM) had any significant effect on ACh-induced mucus secretion.

5 These observations lead us to conclude that nitric oxide, derived from constitutive NO synthase, acts as an endogenous inhibitor of both basal and neurogenic mucus secretion in ferret trachea *in vitro*.

Keywords: Nitric oxide (NO); mucus secretion; N^G-monomethyl-L-arginine (L-NMMA); FK409

Introduction

Endogenously-produced nitric oxide (NO) may play an important role in many physiological and pathophysiological processes in the airways (Barnes & Belvisi, 1993). Indeed, NO is the dominant non-adrenergic, non-cholinergic (NANC) neurotransmitter of airway bronchodilator nerves (Lei *et al.*, 1993; Belvisi *et al.*, 1995), as well as being a regulator of pulmonary blood flow (Barnes & Liu, 1995), bronchial plasma exudation (Erjefalt *et al.*, 1994), and airway ciliary beat frequency (Jain *et al.*, 1993). In rat stomach NO donors increase mucus gel thickness (Brown *et al.*, 1992), an effector role that may be linked to cholinergic activation of gastric mucus secretion (Price *et al.*, 1994). However, the role of NO in control of secretion of airway mucus, and in particular on neurogenic mucus secretion, remains unexplored.

In the present study we used the ferret trachea as a model for neurogenic airway mucus secretion because it has a classical cholinergic/adrenergic secretory component and an excitatory NANC secretory component (Ramnarine & Rogers, 1994). The NANC component is mediated principally via capsaicin-sensitive 'sensory-efferent' nerves, with mucus secretion stimulated via tachykinin NK_1 receptors (Ramnarine *et al.*, 1994). Nitric oxide synthase (NOS) has been colocalised with vasoactive intestinal peptide (VIP) in nerves supplying the airways of the ferret (Dey *et al.*, 1993). The aim of the present study was to assess the effects of a nitric oxide synthase (NOS) inhibitor, L-N^G-monomethyl-L-arginine (L-NMMA), and a novel NO donor, (±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide (FK409) (Kita *et al.*, 1994), on basal mucus secretion and on secretion elicited via electrical stimulation or by acetylcholine from ferret tracheal segments *in vitro*.

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Methods

Tissue preparation and mucus collection

Measurement of mucus secretion *in vitro* in the ferret trachea and the induction of neurogenic mucus secretion has been described in detail previously (Ramnarine *et al.*, 1994). Briefly, male ferrets (Regal Rabbits, Great Bookham, Surrey) were terminally anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), bled by cardiac puncture and their tracheae removed and cleared of adventitious tissue. Tracheae were opened longitudinally and four rectangular segments from each trachea were clamped between the two halves of perspex Ussing chambers. The segments were bathed in 9 ml warmed (37°C) aerated (5% CO_2 in O_2) Krebs-Henseleit solution containing 100 μCi $^{35}SO_4$ in the submucosal half chamber to label newly-synthesized mucus macromolecules (mucins). The composition of the Krebs-Henseleit solution was (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_2$ 25.5 and glucose 5.05. Tissue was incubated for 2.75 h to reach a stable secretory baseline, during which time the luminal half-chamber was drained and refilled five times with fresh, warmed, aerated Krebs-Henseleit solution. The luminal fluid collected at the end of the following 15 min incubation was taken as the basal level of secretion. Subsequent to this, tracheal segments were incubated for 15 min with either drug or vehicle (distilled water). Following this incubation period, tissues were either

incubated for a further 15 min with drug or vehicle, or were electrically stimulated at 50 V, 10 Hz, 0.5 ms for the first 5 min of the 15 min incubation period. At these stimulation parameters, the secretory response is blocked by tetrodotoxin indicating that it is neural in origin (Ramnarine *et al.*, 1994). The tissue was stimulated via pins which pierced the tissue and were connected to a pulse generator (model S88, Grass Instruments, Quincy, U.S.A.). Controls for the stimulated periods were collections taken from tissues at the same time-point, but without turning on the stimulator ('sham' stimulation). In experiments involving L-NMMA alone, the electrical stimulation was followed 1 h later by administration of acetylcholine (ACh, 10 μM). We have shown previously using this protocol that there is no significant tachyphylaxis of secretion to consecutive stimulations (Meini *et al.*, 1993; Ramnarine *et al.*, 1994).

Collections of the fluid bathing the luminal surface of the tracheal segments, containing mucus secretions, were made every 15 min. The collections were solubilised in 6 M guanidine hydrochloride and dialysed exhaustively against distilled water containing excess sodium sulphate to remove molecules of $\sim$14 kDa or less, including unbound $^{35}SO_4$. Recovered samples were weighed and duplicate 1 ml samples prepared for liquid scintillation spectrometry. The total radioactivity of each collected sample was estimated by multiplying the radioactivity present in a 1 ml aliquot of that sample by the total weight of the sample (assuming a 1 ml sample weighs 1 g).

Protocols

L-NMMA, L-arginine and FK409 were all prepared fresh in distilled water on the day of experimentation 45 min before administration. A total of eight treatments was carried out: (1) sham stimulation (no stimulation at same time point as electrical stimulation); (2) effects on basal secretion of incubation (0.5 h; i.e. two collection periods) with L-NMMA (0.01 mM – 1 mM), L-arginine (1 mM) or FK409 (1 – 100 nM); (3) effects on basal secretion of incubation with a combination of L-NMMA (10 mM) and L-arginine (1 mM); (4) electrical stimulation in the absence of any drug treatment; (5) electrical stimulation of tracheal segments preincubated separately (0.5 h) with L-NMMA (0.01 mM – 1 mM), L-arginine (1 mM) or FK409 (1 – 100 nM) (6) electrical stimulation of tracheal segments incubated with a combination of L-NMMA (10 mM and L-arginine (1 mM); (7) stimulation of mucus secretion from tracheal segments incubated with ACh alone; (8) stimulation of tracheal segments in the presence of L-NMMA (0.01 – 1 mM) and ACh. Because each ferret trachea generated four Ussing chambers, it was possible to perform a number of different experiments for each animal (4 – 5 experimental treatments, depending upon treatment group). No treatment was repeated in any one ferret and the *n* values given in Results are number of animals.

Data analysis

Comparison of changes in rate of secretion (d.p.m.) pre- and post-stimulation was assessed by use of the Mann-Whitney U-test (two-tailed). The null hypothesis was rejected at $P<0.05$. Inhibition of stimulated secretion was considered complete when the value for the treated stimulation was significantly different from stimulations without treatment and not significantly different to sham stimulation values.

Results

Effect of NOS inhibition on baseline secretion

In this series of experiments, tracheal segments were incubated with L-NMMA for two consecutive 15 min periods, with luminal fluid collected at the end of each period. The first incubation period was at the equivalent time point which in subsequent experiments would be immediately before electrical stimulation. The second incubation period was at the equivalent time point for the incubation period with stimulation.

L-NMMA (0.01 – 1 mM) caused a significant increase in basal secretion which was similar for the two consecutive 15 min incubation periods in the continued presence of the drug (Table 1). At the end of the first incubation period, mean baseline output of $^{35}SO_4$ was increased by 345% with 0.01 mM, by 258% with 0.1 mM, and by 265% with 1 mM L-NMMA. At the end of the second incubation period with L-NMMA, increases in $^{35}SO_4$ output were similar to the previous collections indicating that a maximal increase in secretion had been reached within 15 min and was sustained for a further 15 min (Table 1).

L-Arginine (1 mM) partially reversed the increase in baseline $^{35}SO_4$ output induced by L-NMMA (0.1 mM). Basal output was 711 $\pm$ 212 d.p.m. ($n=6$). This was significantly ($P<0.01$) increased to 2207 $\pm$ 461 d.p.m. by L-NMMA ($n=5$), and this increase was significantly ($P<0.05$) reversed to 1238 $\pm$ 271 d.p.m. by L-arginine ($n=6$). L-Arginine ($n=5$) alone had no significant effect on basal output.

Effect of NOS inhibition on stimulated secretion

In a first series of experiments, administration of L-NMMA (0.1 mM – 1 mM) at the collection period before stimulation (i.e. baseline) dose-dependently increased basal output of $^{35}SO_4$-labelled macromolecules (i.e. mucus). Output of $^{35}SO_4$ was increased by 95% at 0.01 mM (not significant), and by 248% and 202% (significant) at 0.1 mM and 1 mM L-NMMA, respectively (Figure 1). In the same experiments, electrical stimulation of untreated tracheal strips resulted in a 174% increase in $^{35}SO_4$-labelled macromolecule output above sham-stimulated controls (Figure 1). L-NMMA potentiated neurogenic mucus secretion in a dose-dependent manner with increases above stimulated values of 85% at 0.01 mM L-NMMA (not significant) and of 116% and 214% (significant) at 0.1 mM and 1 mM L-NMMA, respectively (Figure 1).

In a second series of experiments, L-arginine reversed L-NMMA-induced potentiation of neurogenic mucus secretion. Electrical stimulation resulted in a significant ($P<0.01$) 300% increase in mucus output from 711 $\pm$ 212 d.p.m. for sham stimulation ($n=6$) to 2847 $\pm$ 746 d.p.m. with stimulation ($n=5$). In the presence of L-arginine (1 mM), L-NMMA (0.1 mM) failed to potentiate neurogenic mucus secretion: the increase in $^{35}SO_4$ output of 383% (3434 d.p.m.; $P<0.05$ compared with basal levels, $n=5$) was not significantly different from electrical stimulation in the absence of L-arginine (above).

ACh (10 μM, $n=6$) resulted in a mean increase in radiolabel output of 328% above sham levels ($n=5$) (data not shown graphically). L-NMMA (0.01 mM – 1 mM) had no significant

Table 1 Effect of inhibition of nitric oxide synthase on basal airway mucus secretion

Incubation period	*Baseline*	*[L-NMMA]* (mM)		
		0.01	0.1	0.1
1	558 $\pm$ 191	2476 $\pm$ 736	1999 $\pm$ 549	2036 $\pm$ 404
2	–	1691 $\pm$ 410	3712 $\pm$ 1211	1507 $\pm$ 275

Data are mean ($\pm$s.e.mean) disintegrations per min (d.p.m.) for $^{35}SO_4$-labelled macro-molecules (representing mucus) collected from ferret trachea *in vitro* after two consecutive 15 min incubation periods with the nitric oxide synthase inhibitor L-NMMA; $n=5$ animals per group. 'Baseline' represents collections taken at the end of a 15 min incubation period immediately before the first incubation with L-NMMA. There are no significant differences in d.p.m. between matched [L-NMMA] for the two incubation periods.

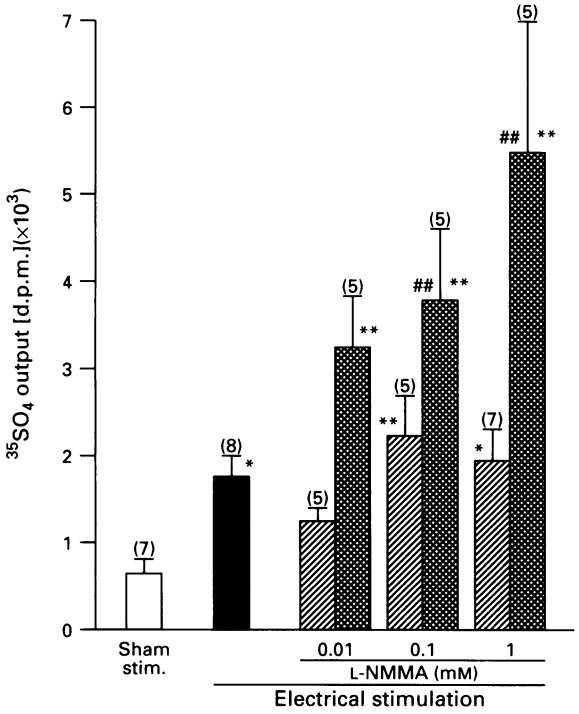

Figure 1 Effect of NO synthase inhibition on basal and neurogenic mucus secretion in ferret trachea *in vitro*. Open column represents sham stimulation; solid column, electrical stimulation (50 V, 10 Hz, 0.5 ms, 5 min); hatched columns, effects of the NO synthase inhibitor L-NMMA (0.01 mM – 1 mM) on basal secretion; cross-hatched, effects of L-NMMA on neurogenic secretion. Data are mean disintegrations per minute (d.p.m.) for secreted macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus) for the number of animals indicated in parentheses for each group, vertical bars show s.e.mean. $*P<0.05$, $**P<0.01$ compared with sham stimulated group (open column); $P<0.05$; $P<0.01$ compared with electrical stimulation (solid column).

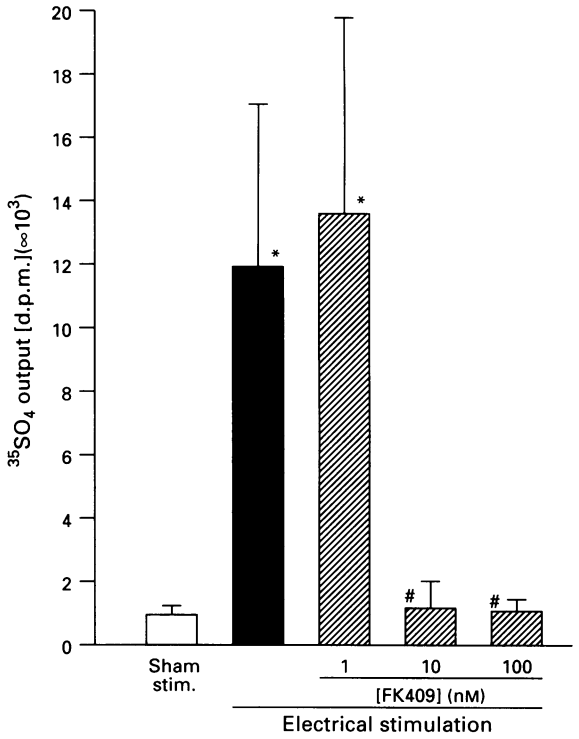

Figure 2 Effect of NO donor on neurogenic mucus secretion in ferret trachea *in vitro*. Open column represents sham stimulation; solid column, electrical stimulation (50 V, 10 Hz, 0.5 ms, 5 min); hatched columns, effects of the NO donor FK409 on neurogenic mucus secretion. Data are mean disintegrations per minute (d.p.m.) for secreted macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus) for $n=5$ animals for each group, vertical bars show s.e.mean. $*P<0.05$ compared with sham stimulated group (open column); $\#P<0.05$ compared with control stimulation (solid column).

effect on ACh-induced secretion given mean increases above basal levels of 252%, 332% and 273% (0.01 mM, 0.1 mM and 1 mM L-NMMA, respectively; $n=6$, for all).

Effect of NO donor on basal and stimulated secretion

FK409 at 100 nM reduced basal output of $^{35}SO_4$ from 941 ± 268 d.p.m. to 348 ± 39 d.p.m. (60% inhibition, $P<0.05$, $n=4$). At 1 nM or 10 nM, FK409 had no discernible effect on basal secretion ($n=2$ and 3 respectively).

In the series of experiments involving FK409, electrical stimulation significantly increased $^{35}SO_4$ output above sham levels (13 fold increase; Figure 2). At 1 nM FK409 had no significant effect on this response, whereas at 10 nM and 100 nM FK409 resulted in significant inhibitions of 98% and 99%, respectively, of evoked mucus output (Figure 2). Thus, inhibition of basal secretion could not completely account for the reduction by FK409 of neurogenic secretion.

In the same series of experiments, ACh caused by 11 fold increase above baseline in $^{35}SO_4$ output ($n=5$, data not shown). FK409, at all of the concentrations tested (1, 10 and 100 nM; $n=5$ for each) had no significant effect on ACh-induced secretion: 12, 11 and 14 fold increases in mucus output, respectively (data not shown).

Discussion

In the present study, the NO synthase inhibitor L-NMMA resulted in a potentiation of both basal and neurogenic mucus secretion which was reversible with L-arginine. Conversely, we found that both basal and neurogenic mucus secretion was inhibited by the novel NO donor FK 409 (Kita *et al.*, 1994). However, mucus secretion induced by exogenous ACh was unaffected in the presence of either the NOS inhibitor of the NO donor. Thus indicates that endogenous NO has two regulatory roles in control of airway mucus secretion, namely (1) regulation of the magnitude of basal secretion and (2) regulation of the magnitude of neurogenic mucus secretion via an effect upon neurotransmission rather than an effect on neurotransmitter-mucus cell interactions. This to our knowledge is the first instance of NO being directly implicated as an inhibitory neurotransmitter to airways mucus secretion.

As the dose of L-NMMA increased, evoked output of mucus due to electrical stimulation increased accordingly over control stimulation levels. Thus, as basal secretion became elevated, mucus secreted in response to electrical stimulation above this new baseline also increased. This indicates either that the magnitude of the actual neurogenic response was unaffected by NOS inhibition and mucus was secreted normally in response to electrical stimulation (as if L-NMMA was not present) or that inhibition of NOS resulted in a potentiation of mucus secretion, causing greater amounts to be secreted in response to electrical stimulation. We postulate that it is the latter possibility since the magnitude of stimulated mucus secretion increased with increasing doses of L-NMMA achieving a more profound level of significance above control stimulation at 0.1 mM and 1 mM than at 0.01 mM. If the first possibility were true and the quantity of mucus secreted in response to electrical stimulation was unaffected by L-NMMA, then the quantity of mucus secreted in response to electrical stimulation would remain static rather than increasing in magnitude and significance above basal levels along with increasing con-

centrations of L-NMMA. In addition, tracheal segments pre-incubated with FK409 inhibited neurogenic mucus secretion further demonstrating the inhibitory influence of NO on mucus secretion. Also, FK409 significantly diminished basal output.

In the present study, we found variability in secretion of $^{35}SO_4$-labelled mucins. Variation in secretion is related to uptake of radiolabel and its incorporation into newly-synthesized mucus macromolecules, to the amount of gland present in different tissue segments, and to the tenacity of the mucus, which affects both its release from the luminal airway surface and its dispersion into the bathing medium. Variation in stimulated secretion is related to the use of Ussing chambers rather than 'traditional' organ baths where all tissue surfaces are bathed in drug-containing solution. In Ussing chambers, drug access to the submucosal glands is influenced by the permeability of the epithelial membrane to the luminally-applied test drug. Variability in $^{35}SO_4$ output by human bronchi mounted in Ussing chambers has also been noted (Baker *et al.*, 1985). Thus, although our conclusions on the actions of NO on ferret tracheal mucus secretion are not unfounded, the potentiating effects of L-NMMA on neurogenic mucus secretion must be viewed in the context of the variability in our experimental system.

The inhibitory action of NO in NANC bronchoconstriction in guinea-pig *in vivo* has been shown to be mediated via activation of soluble guanylyl cyclase (Lei *et al.*, 1993) which leads to production of guanosine 3':5'-cyclic monophosphate (cyclic GMP). However, Boulanger and Vanhoutte (1992) showed that cyclic AMP may also be involved in mediating NO-induced effects. Our findings for the involvement of NO in mucus secretion are different to those of other workers. Adler *et al.* (1995), investigating mucus secretion *in vitro*, from guinea-pig cultured tracheal epithelial cells, evoked by histamine, the cytokine tumour necrosis factor alpha, and reactive oxygen species, observed that increased secretion due to all three inflammatory mediators could be inhibited by a NOS inhibitor and that increased secretion was due to increasing intracellular levels of cyclic GMP. Furthermore, an effector role for NO in mucus secretion was also suggested by Brown *et al.* (1993), who studied the effects of NO donors on mucus secretion from a suspension of rat isolated gastric mucosal cells. The reason for the differing conclusions of our findings, where we find NO to be inhibitory, and these workers, who find NO to stimulate secretion, may be due to species differences, cellular source of mucus, experimental protocol, or enzymatic source of NO. Whereas Adler *et al.* (1995) studied secretion from tracheal epithelial cells in a species, the guinea-pig, noted for its predominance of airway goblet cells, we observed mucus secretion from tracheal submucosal glands. Also, the method of inducing mucus secretion was quite different. Whereas we noted the effects of NO on neurally-stimulated secretion, both aforementioned groups looked at the role of NO released by exogenous agonists. The first two groups mentioned above studied isolated cell preparations making it very difficult to draw parallels between such systems and whole tissue with its surrounding influences as in our system. However, Brown *et al.* (1992) and Price *et al.* (1994) studied NO-induced increases in mucus gel thickness in rat gut *in vivo* and concluded that NO was involved in the pathway by which a muscarinic acetylcholine receptor agonist (carbacol) stimulates mucus secretion. We found L-NMMA and the NO donor FK409 to be without effect on ACh-induced mucus secretion indicating that NO plays no role in muscarinic agonist-induced secretion in ferret trachea. Again, species differences and/or cellular sources of mucus could account for the differences between the findings. Recently, Rand & Li (1995), using the NO-trapping agent carboxy-PTIO, have suggested that the transmitter released from nitrergic nerves is not identical to endothelial-derived relaxing factor, EDRF (free radical NO), but rather may be an NO-adduct. Thus, in our experiments investigating the implications of NO in neurally-innervated mucus secretion, different signalling pathways or neurotransmitter cross-talk due to the nature of the stimulation are all factors that require further elucidation.

The source of the NO involved in mucus secretion is uncertain. Although NOS has been colocalised with VIP in nerves supplying the airways of the ferret (Dey *et al.*, 1993), the fact that NO seems to modulate basal secretion suggests that it may have a cellular source such as epithelial, endothelial or even submucosal gland cells. We also cannot rule out the possibility of a tonic release of NO from nerve endings that might be able to diffuse to the submucosal glands and modulate their secretion of mucus. NO may be derived from constitutive NO synthase (cNOS) in endothelial cells and nerves, or from an inducible enzyme (iNOS) that is only expressed after exposure of cells to endotoxin or proinflammatory cytokines. Inducible NOS produces large amounts of NO which may have different physiological effects compared with the small and transient production of NO by cNOS. In the present study, it is likely that cNOS is involved in the endogenous control of basal mucus secretion and that after nerve stimulation. In inflammatory situations, iNOS may be induced in various cell types, including macrophages and epithelial cells. It is possible that the increased production of NO may then increase mucus secretion, as observed *in vitro* after exposure to tumour necrosis factor-α (Adler *et al.*, 1995). There is a parallel in the role of NO in plasma exudation, where NOS inhibitors increase basal exudation in trachea (Erjefalt *et al.*, 1994), whereas after exposure to endotoxin NOS inhibitors have an inhibitory effect on exudation (Bernareggi *et al.*, 1996).

In conclusion, in ferret trachea *in vitro*, endogenous NO functions to regulate basal (unstimulated) secretion, but also as an inhibitory neurotransmitter to regulate the magnitude of neurogenic mucus secretion. This inhibitory function of NO is in agreement with its respective inhibitory influences on ciliary beat frequency (Jain *et al.*, 1993), bronchial plasma exudation (Erjefalt *et al.*, 1994) and bronchoconstriction (Lei *et al.*, 1993) in the airways. These data have clinical implications. In inflamed airways oxygen-derived free radicals (e.g. superoxide anions) released from activated inflammatory cells may result in degradation of NO and hence the destruction of an endogenous modulator of mucus secretion, which may account for part of the mucus hypersecretion of asthmatic patients. Conversely, it has been shown that in normal human subjects with upper respiratory tract viral infection (Kharitonov *et al.*, 1995) and patients suffering with asthma (Kharitonov *et al.*, 1994), the levels of NO in expired air are significantly greater than in normal control subjects. It may be that in airway diseases such as asthma increased expression of NO is induced which may reach levels of toxicity, thus stimulating the secretion of mucus as a defence and override its function as an endogenous basal modulator.

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References

ADLER, K.B., FISCHER, B.M., LI, H., CHOE, N.H. & WRIGHT, D.T. (1995). Hypersecretion of mucin in response to inflammatory mediators by guinea pig tracheal epithelial cells *in vitro* is blocked by inhibition of nitric oxide synthase. *Am. J. Resp. Cell Mol. Biol.*, **13**, 526–530.

BAKER, B., PEATFIELD, A.C. & RICHARDSON, P.S. (1985). Nervous control of mucin secretion into human bronchi. *J. Physiol.*, **365**, 297–305.

BARNES, P.J. & BELVISI, M.G. (1993). Nitric oxide and lung disease. *Thorax*, **48,** 1034–1043.

BARNES, P.J. & LUI, S.H. (1995). Regulation of pulmonary vascular tone. *Pharmacol. Rev.*, **47,** 87–131.

BELVISI, M.G., WARD, J.K., MITCHELL, J.A. & BARNES, P.J. (1995). Nitric oxide as a neurotransmitter in human airways. *Arch. Intern. Pharmacol. Ther.*, **329,** 97–110.

BERNAREGGI, M., MITCHELL, J.A., BARNES, P.J. & BELVISI, M.G. (1996). Dual action of nitric oxide on airway inflammation: differential effects at different airway levels. *Br. J. Pharmacol.*, **117,** (abstract in press).

BOULANGER, C.M. & VANHOUTTE, P.M. (1992). Cholera toxin augments the release of endothelium-derived relaxing factor evoked by bradykinin and the calcium ionophore A23187. *Gen. Pharmacol.*, **23,** 27–31.

BROWN, J.F., HANSON, P.J. & WHITTLE, B.J.R. (1992). Nitric oxide increases mucus gel thickness in rat stomach. *Eur. J. Pharmacol.*, **223,** 103–104.

BROWN, J.F., KEATES, A.C., HANSON, P.J. & WHITTLE, B.J.R. (1993). Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells. *Am. J. Physiol.*, **265,** G418–G422.

DEY, R.D., MAYER, B. & SAID, S.I. (1993). Colocalisation of vasoactive intestinal peptide and nitric oxide synthase in neurons of the ferret trachea. *Neuroscience*, **4,** 839–843.

ERJEFALT, J.S., ERJEFALT, E., SUNDLER, F. & PERSSON, C.G.A. (1994). Mucosal nitric oxide may tonically supress airways plasma exudation. *Am. J. Crit. Care. Med.*, **150,** 227–232.

JAIN, B., RUBENSTEIN, I., ROBBINS, R.A., LEISE, K.L. & SISSON, J.H. (1993). Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem. Biophys. Res. Commun.*, **191,** 83–88.

KHARITONOV, S.A., YATES, D. & BARNES, P.J. (1995). Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur. Respir. J.*, **8,** 295–297.

KHARITONOV, S.A., YATES, ROBBINS, R.A., LOGAN-SINCLAIR, R., SHINEBOURNE, D. & BARNES, P.J. (1994). Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*, **343,** 133–135.

KITA, Y., HIRASAWA, Y., MAEDA, K., NISHIO, M. & YOSHIDA, K. (1994). Spontaneous nitric oxide release accounts for the potent pharmacological actions of FK409. *Eur. J. Pharmacol.*, **257,** 123–130.

LEI, Y.H., BARNES, P.J. & ROGERS, D.F. (1993). Regulation of NANC neural bronchoconstriction *in vivo* in the guinea-pig: involvement of nitric oxide, vasoactive intestinal peptide and soluble guanylyl cyclase. *Br. J. Pharmacol.*, **108,** 228–235.

MEINI, S., MAK, J.C.W., ROHDE, J.A.L. & ROGERS, D.F. (1993). Tachykinin control of ferret airways: mucus secretion, bronchoconstriction and receptor mapping. *Neuropeptides*, **24,** 81–89.

PRICE, K.J., HANSON, P.J. & WHITTLE, B.J.R. (1994). Stimulation by carbachol of mucus gel thickness in rat stomach involves nitric oxide. *Eur. J. Pharmacol.*, **263,** 199–202.

RAMNARINE, S.I., HIRAYAMA, Y., BARNES, P.J. & ROGERS, D.F. (1994). 'Sensory-efferent' neural control of mucus secretion: characterisation using tachykinin receptor antagonists in ferret trachea *in vitro*. *Br. J. Pharmacol.*, **113,** 1183–1190.

RAMNARINE, S.I. & ROGERS, D.F. (1994). Non-adrenergic, non-cholinergic neural control of mucus secretion in the airways. *Pulm. Pharmacol.*, **7,** 19–33.

RAND, M.J. & LI, C.G. (1995). Discrimination by the NO-trapping agent, carboxy-PTIO, between NO and the nitrergic transmitter but not between NO and EDRF. *Br. J. Pharmacol.*, **116,** 1906–1910.

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