British Journal of Pharmacology (1999) 127, 391–396
http://www.stockton-press.co.uk/bjp

Hyperosmolarity reduces the relaxing potency of nitric oxide donors in guinea-pig trachea

*,[1]Josephine Hjoberg, [1]Marieann Högman & [1]Göran Hedenstierna

[1]Department of Medical Sciences, Clinical Physiology, Uppsala University, University Hospital, S-751 85 Uppsala, Sweden

1 Non-responders to inhaled nitric oxide treatment have been observed in various patient groups. The bronchodilatory effect of inhaled nitric oxide was attenuated when the airway lumen was rendered hyperosmolar in an *in vivo* study on rabbits. We used a guinea-pig tracheal perfusion model to investigate the effects of increased osmolarity (450 mOsm, NaCl added) on the relaxing potency of the nitric oxide donors sodium nitroprusside (SNP) and (±)-S-nitroso-N-acetylpenicillamine (SNAP).

2 Under iso-osmolar conditions SNP relaxed the carbachol (CCh, 1 μM) contracted trachea by 83±3%. After pretreatment with intraluminal hyperosmolarity SNP relaxed the CCh-contracted trachea by only 31±7% ($P<0.05$). When the trachea was contracted to the same extent under untreated and hyperosmolar conditions, the untreated trachea was completely relaxed by SNP but, after hyperosmolar pretreatment, SNP could no longer relax the trachea.

3 SNAP relaxed the CCh contracted trachea by 27±5%. After pretreatment with intraluminal hyperosmolarity, SNAP relaxed the trachea by 11±4%, which was less than in the iso-osmolar control ($P<0.05$).

4 Extraluminal hyperosmolarity did not affect carbachol elicited contraction, and SNP administered externally during extraluminal hyperosmolarity was able to relax the trachea ($P<0.05$).

5 The cell permeable guanosine 3′5′-cyclic monophosphate analogue 8-Br-cGMP relaxed the CCh contracted trachea in both iso-osmolar ($P<0.05$) and hyperosmolar conditions ($P<0.05$).

6 The relaxant effect of nitric oxide donors on tracheal smooth muscle is markedly reduced when the airway epithelium is exposed to hyperosmolar solution.

Keywords: Nitric oxide donors; sodium nitroprusside; SNAP; 8-Br-cGMP; hyperosmolarity; sodium chloride; airway smooth muscle

Abbreviations: CCh, carbachol; cyclic GMP, guanosine 3′5′-cyclic monophosphate; ΔP, differential pressure; EL, extraluminal; HB, hyperosmolar buffer; IL, intraluminal; mOsm, milliosmolar; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; SNAP, (±)-S-nitroso-N-acetylpenicillamine; SNP, sodium nitroprusside

Introduction

For over 60 years, nitrates have been known to induce bronchial relaxation (Goodman & Gilman, 1941). This effect is thought to be mediated by the release of nitric oxide (NO) which activates the soluble guanylate cyclase, leading in turn to formation of the second messenger guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (Ignarro & Kadowitz, 1985) in the smooth muscle of the airway (Katsuki & Murad, 1977; Buga *et al*., 1989; Ward *et al*., 1995). Inhalation of exogenous nitric oxide is known to attenuate the response to bronchoconstricting agents in both laboratory animals and humans (Dupuy *et al*., 1992; Högman *et al*., 1993a,b).

Considerable variations were seen in the effect when nitric oxide was inhaled by bronchial asthma patients (Högman *et al*., 1993b). In some of these patients, nitric oxide relaxed the airways just as much as a β_2-agonist did, the effect being additive to that of the β_2-agonist, whereas other patients showed no improvement at all. The cause of the non-responder phenomenon is not yet known.

Patients with asthma have varying degrees of airway wall oedema (Jeffery, 1998). Could the oedema be one explanation for the variations in the effect of nitric oxide seen in asthmatics? In a study on mechanically ventilated rabbits we found that increasing the osmolarity of the surface liquid of the rabbit airways leads to a transient airway wall oedema (Högman *et al*., 1997a,b). We have also shown that the prophylactic effect of nitric oxide on methacholine-induced bronchoconstriction was inhibited when the osmolarity on the luminal surface of the airways was increased (Högman *et al*., 1998). This suggests that increased osmolarity of the surface lining fluid could be one event inhibiting the effect of nitric oxide.

The present study was conducted to elucidate the effects of hyperosmolarity on the ability of nitric oxide to relax airway smooth muscle in the guinea-pig trachea. We have used an *in vitro* tracheal perfusion technique to evaluate the effect of luminal sodium chloride hyperosmolarity on the dilating effect of the nitric oxide donors sodium nitroprusside (SNP) and (±)-S-nitroso-N-acetylpenicillamine (SNAP).

Methods

Perfused trachea preparation

Male Dunkin Hartley guinea-pigs weighing 650–850 g, checked for respiratory infections, were used. The experimental protocol was approved by the regional ethics committee on animal experiments. The guinea-pigs were given an overdose of pentobarbital (0.1 mg kg^{-1} body weight intraperitoneally). The tracheas were rapidly dissected free of connective tissue, fat and blood vessels removed and mounted on a stainless steel perfusion holder. The perfusion system used was an improved

*Author for correspondence; E-mail: josephine.hjoberg@klinfys.uu.se

version of Fedan & Frazer's system (Fedan & Frazer, 1992), described earlier by Munakata (Munakata *et al*., 1988). After centrally fixed side-hole catheters were inserted into the lumen of the trachea from each end, the trachea was stretched to its *in situ* length and placed vertically in a 25 ml extraluminal organ bath containing Krebs-Henseleit buffer. The catheter at the inlet of the trachea, the proximal end, was connected to one side of a differential pressure transducer (P300D, Validyne Engineering Cooperation, CA, U.S.A.) and the outlet catheter at the distal end of the trachea was connected to the other side of the transducer. The inside of the trachea was perfused with Krebs-Henseleit buffer from the 25 ml intraluminal bath at 26 ml min^{-1} in a recirculating loop. The Krebs-Henseleit buffer in both the extra- and intraluminal baths was kept at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2 . The transmural pressure was adjusted to correspond to zero at baseline. Responses of the trachea were recorded by a computer (LabView 3.0 software, National Instruments Austin, TX, U.S.A.) using a specially designed program (kindly donated by Astra Draco, Lund, Sweden) adapted to our system. Responses are expressed as the difference in pressure between proximal and distal recording sites (ΔP, cmH_2O).

Solutions and reagents

The isotonic Krebs-Henseleit buffer had an osmolarity of 290 mOsm, and was composed of (in mM): NaCl, 117; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; KCl, 4.7; $CaCl_2$, 2.5 and glucose, 1.03 (pH 7.4, 37°C). The hyperosmolar buffer was formed by increasing the NaCl concentration to 203 mM, which increased the osmolarity to 450 mOsm. Carbachol (Sigma Chemical Co, MO, U.S.A.) was dissolved in saline. The nitric oxide donors sodium nitroprusside (SNP, Sigma), (±)-S-nitroso-N-acetylpenicillamine (SNAP, Calbiochem-Novabiochem Corporation, CA, U.S.A.), and the cyclic GMP analogue 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-Br-cGMP, Sigma) were dissolved in Krebs-Henseleit buffer and protected from light until use. 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, Calbiochem) was dissolved in DMSO (dimethylsulphoxide).

Experimental protocol

All protocols started with an equilibration period of 60–75 min and a wash every 15 min when both intraluminal and extraluminal solutions were replaced for fresh buffer. Thereafter the tracheas were subjected to one of the following protocols.

*Effect of carbachol Reproducibility, (*n = *6)* The trachea was contracted to 50% maximum contraction (as determined previously with carbachol dose-response curves, data not shown) with 1 μM extraluminally applied carbachol, followed by a 60 min washout. A second contraction with 1 μM carbachol was made after the washout.

*Effect of intraluminal hyperosmolar buffer on carbachol contraction, (*n = *6)* The trachea was contracted with carbachol, and after the washout period the intraluminal buffer was replaced for 450 mOsm hyperosmolar buffer 10 min before a second contraction with 1 μM carbachol.

*Effect of intraluminal sodium nitroprusside (SNP) Reproducibility, (*n = *6)* The trachea was contracted with 1 μM extraluminally applied carbachol followed by 3 mM of the nitric oxide donor SNP added to the intraluminal bath. After a 60 min washout period the procedure was repeated.

*Effect of intraluminal NaCl, constant carbachol dose, (*n = *6)* The protocol was as above except that the intraluminal buffer was replaced by hyperosmolar buffer 10 min before the second carbachol contraction.

*Effect of intraluminal NaCl, constant contraction, (*n = *6)* As we found that the contraction by carbachol was reduced after pretreatment with hyperosmolar buffer, the effect of intraluminal SNP was tested on tracheas contracted to the same extent with or without pretreatment with intraluminal hypertonicity. The trachea was first contracted with a low dose of carbachol (0.1 μM) which was shown to cause the same contraction as 0.1 mM carbachol did after pretreating the trachea with hyperosmolarity. The effect of 3 mM SNP was assessed. After 60 min washout, the trachea was pretreated with hypertonic buffer for 10 min and then contracted with 0.1 mM carbachol, thereafter 3 mM SNP was applied.

*Effect of blocking guanylate cyclase, (*n = *7)* To investigate if SNP was mediating its relaxing effect through the release of nitric oxide and activation of soluble guanylate cyclase, we studied the relaxing effect of SNP on CCh contraction before and after pretreating the trachea with the soluble guanylate cyclase inhibitor ODQ (3 μM) given on the extraluminal side 30 min prior to the second CCh contraction.

Effect of intraluminal SNAP and intraluminal hyperosmolarity To ascertain whether the effect of SNP was mediated by nitric oxide, we also tested the nitric oxide donor SNAP ($n = 6$), which is a direct donor of nitric oxide. Protocol (as for Effect of intraluminal NaCl, constant carbachol dose) was repeated with SNAP (1 mM) as the nitric oxide donor instead of SNP.

Effect of extraluminal SNP and intraluminal hyperosmolarity By administering SNP extraluminally ($n = 6$) we tested whether the lack of effect of SNP in hyperosmolar airways could be due to a diffusion barrier, or to inactivation of the nitric oxide molecule somewhere between the airway lumen and the smooth muscle. The effect of SNP was tested according to protocol (as for Effect of intraluminal NaCL, constant carbachol dose), with the exception that 3 mM SNP was given extraluminally.

Effect of extraluminal SNP and extraluminal hyperosmolarity To establish whether contact with the epithelium was a prerequisite for the effect of hyperosmolarity on relaxation by SNP, hyperosmolar buffer was added to the extraluminal bath 10 min before the second challenge with CCh and SNP ($n = 6$). The first challenge was performed according to protocol (as for Effect of extraluminal SNP and intraluminal hyperosmolarity).

Effect of 8-Br-cGMP and intraluminal hyperosmolarity To study if the chain of action of SNP was broken before or after the production of cyclic GMP, the cell membrane permeable cyclic GMP analogue 8-Br-cGMP (2.2 mM) was given to the carbachol contracted trachea on the intraluminal side, ($n = 7$).

Statistics

Statistical analysis was performed with Statistica software (version 5.0, StatSoft. Inc., Tulsa, OK, U.S.A.). The results were analysed with Wilcoxon non-parametric matched pairs test. For analysis within groups, Friedman ANOVA was used.

Results are presented as mean values ± s.e.mean. A statistical result with $P<0.05$ was considered to be statistically significant.

Results

Effect of carbachol

The contractile effect of carbachol was reproducible, with no difference between the first (1.46 ± 0.20 cm H_2O) and second (1.55 ± 0.22 cm H_2O) carbachol contraction. Pretreatment with hyperosmolar buffer relaxed the tissue slightly from baseline (−0.14 ± 0.02 cm H_2O, $P<0.05$). After pretreatment with intraluminal hyperosmolar buffer, the carbachol contraction was reduced to 47 ± 5% of the contraction of the untreated trachea (1.92 ± 0.35 cm H_2O before and 0.84 ± 0.14 cm H_2O after, $P<0.05$).

Effect of intraluminal SNP

SNP relaxed the carbachol-contracted trachea ($P<0.05$). This was a transient relaxation that was maximal after 2 min and levelled off at a higher pressure after 15 min (see Figure 1). The second carbachol contraction was no different from the first (1.49 ± 0.30 cm H_2O and 1.55 ± 0.44 cm H_2O, respectively ns), and the relaxation with SNP at the second stimulation was no different from the first challenge (0.68 ± 0.11 cm H_2O and 0.42 ± 0.10 cm H_2O respectively, ns). Thus, relaxation produced by SNP was reproducible.

In the experiments where a constant carbachol dose was given (protocol as for effect of intraluminal NaCl, constant carbachol dose), SNP relaxed the carbachol contracted trachea by 83 ± 3% ($P<0.05$). After hyperosmolar pretreatment, carbachol contracted the trachea to 47 ± 4% of the contraction in the iso-osmolar control ($P<0.05$). The effect of SNP was markedly attenuated after hyperosmolar pretreatment, with a relaxation of the carbachol contracted trachea by only 31 ± 7%. SNP could not relax the trachea to the same level as in the iso-osmolar control ($P<0.05$, see Figure 2A).

In experiments where the effect of SNP on a constant carbachol contraction was studied (protocol as for effect of intraluminal NaCl, constant contraction), 0.1 μM carbachol contracted the untreated trachea to the same extent as did 0.1 mM carbachol following hyperosmolar pretreatment. Under isotonic conditions, the carbachol-contracted trachea was relaxed ($P<0.05$), whereas after hyperosmolar treatment, SNP could no longer relax the trachea, (see Figure 2B).

Pretreatment with ODQ increased the baseline pressure by 0.23 ± 0.07 cm H_2O ($P<0.05$), but the carbachol contraction was not different before and after pretreatment with ODQ. SNP relaxed the carbachol contracted trachea from 3.30 ± 0.84 cm H_2O to 0.39 ± 0.11 cm H_2O ($P<0.05$). After pretreatment with ODQ, SNP relaxed the trachea from 3.12 ± 0.75 cm H_2O to 2.59 ± 0.57 cm H_2O ($P<0.05$), which was no more than 15 ± 0.1% of the relaxation in the control situation ($P<0.05$).

Effect of intraluminal SNAP and intraluminal hyperosmolarity

SNAP relaxed the carbachol-contracted trachea ($P<0.05$). Pretreatment with hyperosmolar buffer reduced the carbachol contraction ($P<0.05$). SNAP relaxed the pretreated trachea by 11 ± 4%, which was significantly less than relaxation of the untreated trachea (27 ± 5%, $P<0.05$, see Figure 3A).

Effect of extraluminal SNP and intraluminal hyperosmolarity

Extraluminally applied SNP relaxed the carbachol-contracted trachea ($P<0.05$). After hyperosmolar treatment, carbachol produced a smaller contraction ($P<0.05$) but, unlike intraluminal SNP, extraluminally applied SNP was able to dilate the trachea ($P<0.05$), and this to the same level as SNP under isotonic conditions (ns, see Figure 3B).

Effect of extraluminal SNP and extraluminal hyperosmolarity

Extraluminal SNP relaxed the carbachol-contracted trachea ($P<0.05$). Extraluminal hyperosmolarity did not significantly affect the carbachol contraction. SNP applied externally during extraluminal hyperosmolarity was able to relax the trachea ($P<0.05$, see Figure 3C).

Effect of 8-Br-cGMP and intraluminal hyperosmolarity

8-Br-cGMP relaxed the carbachol contracted trachea both in the iso-osmolar control situation (37 ± 0.1%, $P<0.05$), and in the trachea pretreated with hyperosmolar buffer (25 ± 0.1%, $P<0.05$, see Figure 3D).

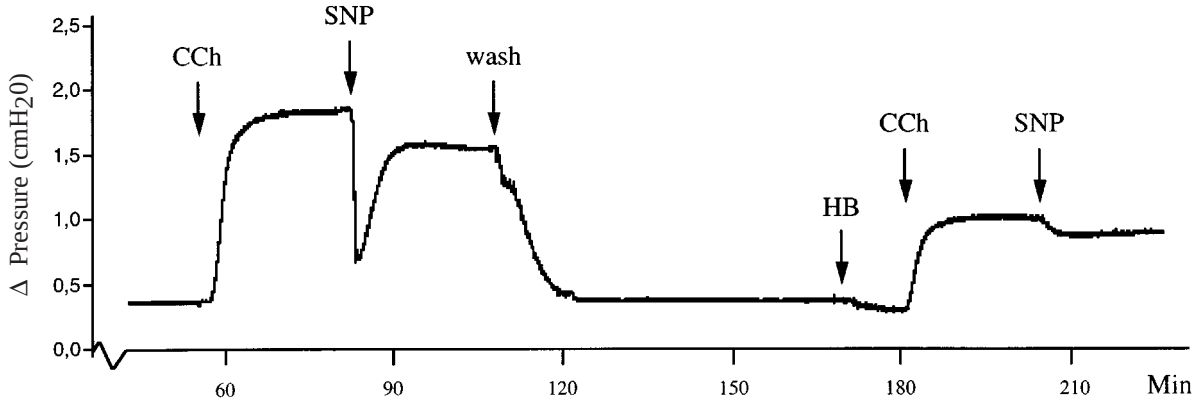

Figure 1 Original tracing from experiment in guinea-pig trachea *in vitro*, performed according to protocol (as for Effect of intraluminal NaCl, constant contraction). Sodium nitroprusside (SNP) relaxes the carbachol (CCh) contracted trachea in a biphasic manner. After washing and recovery to baseline, the buffer at the intraluminal side is replaced by hyperosmolar buffer (HB). After hyperosmolar prereatment the carbachol contraction is lower and the relaxation to SNP is markedly reduced.

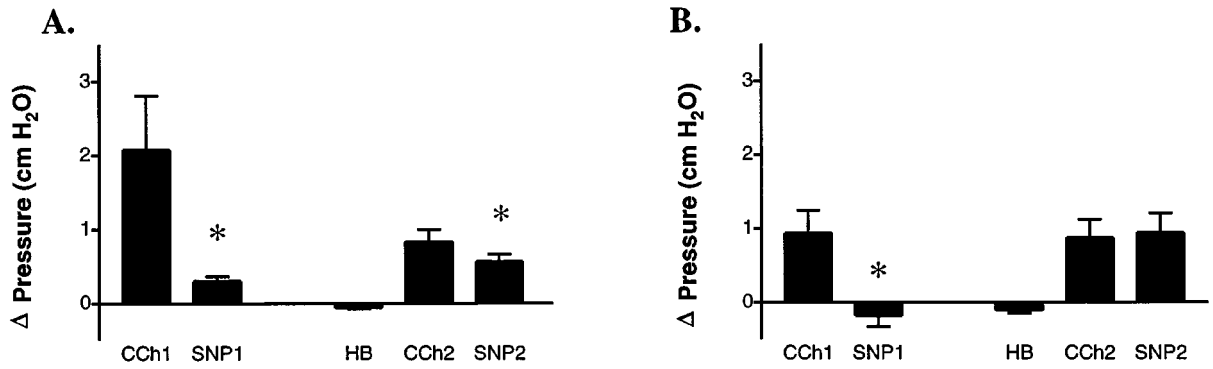

Figure 2 (A) The relaxant effect of sodium nitroprusside (SNP) on contraction induced by carbachol (CCh, 1 μM) is markedly attenuated after applying a hyperosmolar buffer (HB) to the luminal side of guinea-pig trachea (CCh1 and SNP1 indicates iso-osmolar control, and CCh2 and SNP2 hyperosmolar condition). (B) The relaxant effect of SNP was abolished when the trachea was contracted to the same level in iso-osmolar (CCh1, 0.1 μM) and hyperosmolar (CCh2, 0.1 mM) conditions. (*$P<0.05$ vs CCh1 and CCh2 respectively).

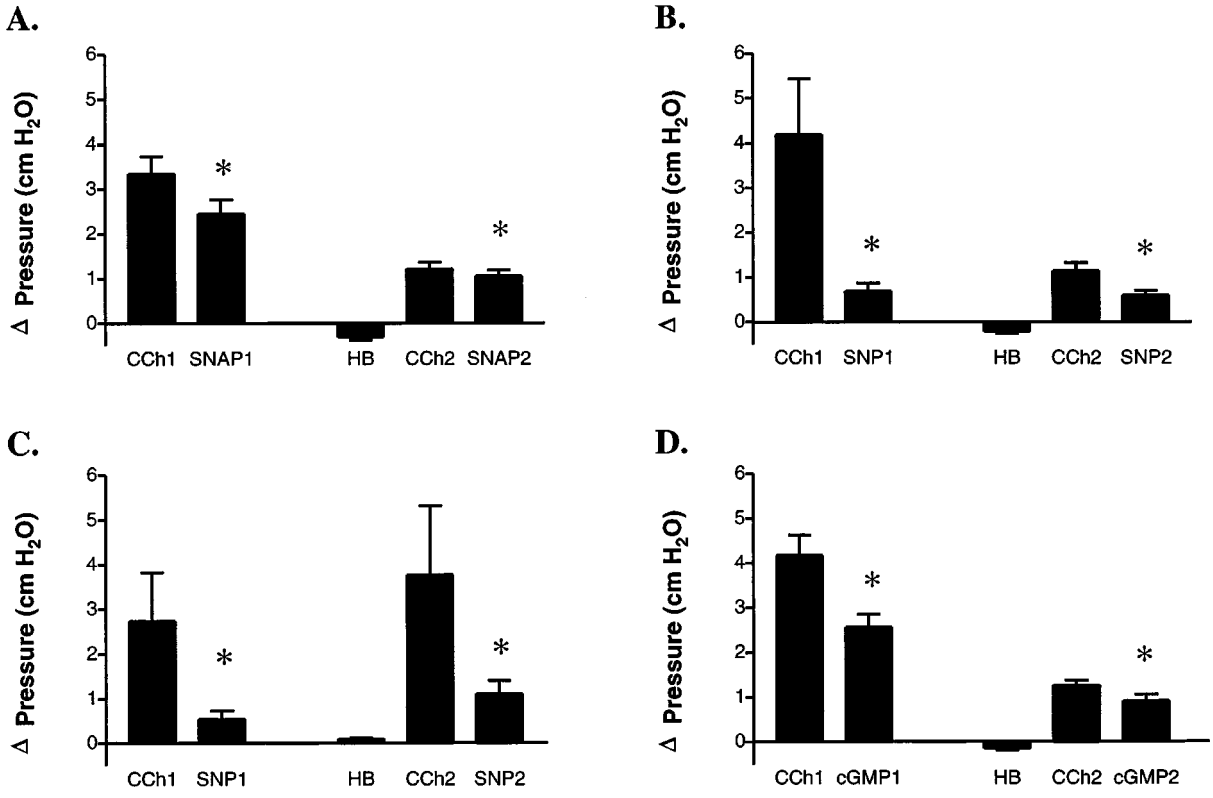

Figure 3 (A) The effect of the nitric oxide donor SNAP on CCh contraction (1 μM) was attenuated after intraluminal hyperosmolarity (HB). (B) When SNP was applied to the extraluminal side it was able to relax the CCh-contracted trachea, even after intraluminal hyperosmolar pretreatment. (C) SNP given to the extraluminal side relaxed the trachea also after applying hyperosmolarity to the extraluminal side. The CCh contraction was not reduced when hyperosmolarity was given on the extraluminal side, which was the case with intraluminal hyperosmolarity (see Figures 1 and 2A, B and D). (D) The cell permeable cyclic GMP analogue 8-Br-cGMP relaxed the trachea in both iso-osmolar and hyperosmolar conditions. (1 and 2 denotes first and second challenge with respective substance; CCh, carbachol; HB, hyperosmolar buffer; cGMP, 8-Br-cGMP; SNP, sodium nitroprusside; SNAP, (±)-S-nitroso-N-acetylpenicillamine). (*$P<0.05$ vs CCh1 and CCh2 respectively).

Discussion

In this study we found that a hyperosmolar airway lumen prevents nitric oxide from relaxing the airway smooth muscle. This is probably because nitric oxide has been prevented from reaching the smooth muscle in an active form or by binding of the molecule.

There are several possible ways that the nitric oxide molecule can be trapped or inactivated on its way from the lumen to the smooth muscle in a hyperosmolar airway. The first hindrance could be a reduced viscosity or a thicker layer of mucus on the airway surface. Dry air hyperventilation leads to evaporation of water from the airways (Högman *et al*., 1997b), and thereby a reduced viscosity of the mucus. It is not known if the viscosity of the mucus layer is reduced after perfusing the trachea with hypertonic buffer, and it is uncertain if this could impede the diffusion of nitric oxide. However, since nitric oxide is a small and highly diffusible molecule (Borland &

Higenbottam, 1989), it is unlikely that mucus would impede the diffusion. Another possible explanation for the lack of effect is that nitric oxide may be inactivated by the hyperosmolarity. However, we found that when both hyperosmolar sodium chloride and SNP were given extraluminally SNP could still relax the tissue. This suggests that the nitric oxide molecule, or SNP, is not directly inactivated by hyperosmolarity. A more likely hindrance preventing the nitric oxide from reaching the smooth muscle is binding of the molecule to cellular or chemical constituents, thereby inactivating the molecule. Nitric oxide can for example react with oxygen radicals to form peroxynitrite ($OONO^-$) (Cross *et al*., 1984; Gaston *et al*., 1994b), which may be stabilized by hydrogen bonding with water, or undergo reactions generating complexes which can have biological effects different from nitric oxide (Royall *et al*., 1997).

We have found that a prerequisite for hyperosmolarity to inhibit the relaxation to nitric oxide is that sodium chloride is supplied to the intraluminal side. This points to an important role for the epithelium in modulating responses to nitric oxide in a hyperosmolar airway, perhaps by release of certain factors. In our preparation we were able to separate the intraluminal side with the epithelium from the extraluminal side. When only the mucosal side was challenged with increased osmolarity, we obtained a model similar to the *in vivo* rabbit experiments (Högman *et al*., 1998). The airway epithelium acts as an osmotic sensor which transduces information about the osmolarity on the surface to the underlying tissues (Willumsen *et al*., 1994). Since the apical membrane of the epithelium is more permeable to water than is the basolateral side, the epithelial cells shrink in response to raised luminal (but not serosal) osmolarity (Willumsen *et al*., 1994). Shrinkage of the epithelial cell leads to release of substances that *in vivo* affect the bronchial circulation by increasing the blood flow (Prazma *et al*., 1994; Baile *et al*., 1987; Deffebach *et al*., 1989) and vascular permeability (Umeno *et al*., 1990), which in turn supplies water to the lumen. Water is drawn by osmotic forces to the shrunken epithelium and to the airway surface in order to counteract the increased osmolarity. Factors released from the shrunken epithelial cells can also affect the smooth muscle of the airways, thereby regulating airway tone (Boucher, 1994; Morrison *et al*., 1990; Rocha *et al*., 1995). A possible mechanism behind the lack of relaxation to nitric oxide could be that hyperosmolarity induces the release of substances that inactivate or bind the molecule, or act on the smooth muscle directly, making the cell resistant to the relaxing stimuli from the nitric oxide. We found that extraluminal SNP could relax the trachea when it was provoked by intraluminal hyperosmolarity. This suggests that the lack of effect of intraluminal nitric oxide donors is probably not due to an inability of the smooth muscle to relax, but rather due to prevention of the nitric oxide from reaching the muscle. This is supported by our finding that the cell membrane permeable cyclic GMP analogue 8-Br-cGMP given to the intraluminal side was able to relax the carbachol contracted trachea even after pretreatment with hyperosmolar buffer.

Nitric oxide donors are generally thought to mediate their relaxant effect by releasing nitric oxide which activates soluble guanylate cyclase and elevates cyclic GMP (Katsuki & Murad, 1977; Murad *et al*., 1978; Ignarro & Kadowitz, 1985; Buga *et al*., 1989; Gruetter *et al*., 1989; Nijkamp & Folkerts, 1994). In the literature, however, there are conflicting results as to whether mechanisms other than activation of soluble guanylate cyclase might contribute to the effect of the nitric oxide donor SNP on airway smooth muscle (Stuart-Smith & Vanhoutte, 1990; Stuart-Smith *et al*., 1994; Zhang *et al*., 1993; Fedan *et al*., 1995; Sadeghi-Hashjin *et al*., 1996; Wong *et al*., 1995; Zhou & Torphy, 1991; Diamond, 1993; Gaston *et al*., 1994a). We found that after pretreating the trachea with the soluble guanylate cyclase inhibitor ODQ, SNP could only relax the trachea to 15% of the relaxation produced in controls. The small relaxation by SNP in presence of ODQ might have been caused by mechanisms other than activation of guanylate cyclase by nitric oxide. We have used two different nitric oxide donors, SNP and SNAP, which generate nitric oxide by different mechanisms (Bauer *et al*., 1995). SNP must be activated before it releases nitric oxide (Bates *et al*., 1991; Marks *et al*., 1991), whereas SNAP is a direct donor of nitric oxide (Kowaluk *et al*., 1992) and has been shown to exert its effect in airways by nitric oxide release (Sadeghi-Hashjin *et al*., 1996). Like SNP, SNAP too was inhibited by pretreatment with luminal hyperosmolarity, which supports that it is in fact the effect of nitric oxide released from the nitric oxide donors that we are investigating.

Our results are in keeping with an *in vivo* study on rabbits (Högman *et al*., 1998) where the effect of inhaled nitric oxide was neutralized after creating a hyperosmolar airway lumen. However, there are differences between the *in vivo* and the *in vitro* situation that remain to be explained. An increased reactivity to the airway constrictors histamine and methacholine was seen *in vivo* after making the airway lumen hyperosmolar by hyperventilation or by nebulization with hypertonic saline (Högman *et al*., 1997b, 1998). *In vitro*, we saw a reduced response to the airway constrictor carbachol after exposing the trachea to luminal hypertonicity. Various species were used in these studies, but the discrepancy was probably due to differences between *in vivo* and *in vitro* models, such as studying only the trachea and lack of neural regulation in the *in vitro* method. Despite certain differences, the *in vitro* method used in this study has proved useful for further investigations into the mechanisms behind the lack of relaxation by nitric oxide in hyperosmolar airways.

In conclusion, the effect of the nitric oxide donors SNP and SNAP is abolished by exposing the luminal side of guinea-pig trachea to hyperosmolarity. It seems probable that the nitric oxide molecule is bound or inactivated on its way from the airway lumen to the smooth muscle, but further investigations are needed to elucidate the exact mechanisms behind this. Further studies are also needed to ascertain if prevention of the nitric oxide molecule is the cause of the non-responder phenomenon to inhaled nitric oxide observed in asthma, and to find a way to overcome this hindrance.

Åke Larsson is thanked for developing the software programs for the tracheal perfusion system and for helpful discussions. This study has been supported by grants from the Swedish Medical Research Council (5315), the Swedish Heart-Lung Foundation and Uppsala University.

References

BAILE, E.M., DAHLBY, R.W., WIGGS, B.R., PARSONS, G.H. & PARÉ, P.D. (1987). Effect of cold and warm dry air hyperventilation on canine airway blood flow. *J. Appl. Physiol.*, **62,** 526–532.

BATES, J.N., BAKER, M.T., GUERRA, R.J. & HARRISON, D.G. (1991). Nitric oxide generation from nitroprusside by vascular tissue. Evidence that reduction of the nitroprusside anion and cyanide loss are required. *Biochem. Pharmacol.*, **42,** S157–S165.

BAUER, J.A., BOOTH, B.P., & FUNG, H.L. (1995). Nitric Oxide. *Biochemistry, Molecular Biology, and Therapeutic Implications,* pp. 361–381, San Diego, California: Academic Press, Inc.

BORLAND, C.D.R. & HIGENBOTTAM, T.W. (1989). A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. *Eur. Respir. J.,* **2,** 56–63.

BOUCHER, R.C. (1994). Human airway ion transport. Part two. *Am. J. Respir. Crit. Care Med.,* **150,** 581–593.

BUGA, G.M., GOLD, M.E., WOOD, K.S., CHAUDHURI, G., & IGNARRO, L.J. (1989). Endothelium-derived nitric oxide relaxes nonvascular smooth muscle. *Eur. J. Pharmacol.,* **161,** 61–72.

CROSS, C.E., HALLIWELL, B. & ALLEN, A. (1984). Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. *Lancet,* **1,** 1328–1330.

DEFFEBACH, M.E., SALONEN, R.O., WEBBER, S.E. & WIDDICOMBE, J.G. (1989). Cold and hyperosmolar fluids in canine trachea: vascular and smooth muscle tone and albumin flux. *J. Appl. Physiol.,* **66,** 1309–1315.

DIAMOND, J. (1993). Role of cyclic GMP in airway smooth muscle relaxation. *Agents Actions Suppl.,* **43,** 13–26.

DUPUY, P.M., SHORE, S.A., DRAZEN, J.M., FROSTELL, C., HILL, W.A. & ZAPOL, W.M. (1992). Bronchodilator action of inhaled nitric oxide in guinea pigs. *J. Clin. Invest.,* **90,** 421–428.

FEDAN, J.S. & FRAZER, D.G. (1992). Influence of epithelium on the reactivity of guinea-pig isolated, perfused trachea to bronchoactive drugs. *J. Pharmacol. Exp. Ther.,* **262,** 741–750.

FEDAN, J.S., WARNER, T.E., YUAN, L.X., ROBINSON, V. & FRAZER, D.G. (1995). Nitric oxide synthase inhibitor and lipopolysaccharide effects on reactivity of airways. *J. Pharmacol. Exp. Ther.,* **272,** 1141–1150.

GASTON, B., DRAZEN, J.M., JANSEN, A., SUGARBAKER, D.A., LOSCALZO, J., RICHARDS, W. & STAMLER, J.S. (1994a). Relaxation of human bronchial smooth muscle by S-nitrosothiols in vitro. *J. Pharmacol. Exp. Ther.,* **268,** 978–984.

GASTON, B., DRAZEN, J.M., LOSCALZO, J. & STAMLER, J.S. (1994b). The biology of nitrogen oxides in the airways. *Am. J. Respir. Crit. Care Med.,* **149,** 538–551.

GOODMAN, L.S. & GILMAN, A. (1941). The nitrites. In *The pharmacological basis of therapeutics*. p. 563. Philadelphia: Lea & Febiger.

GRUETTER, C.A., CHILDERS, C.E., BOSSERMAN, M.K., LEMKE, S.M., BALL, J.G. & VALENTOVIC, M.A. (1989). Comparison of relaxation induced by glyceryl trinitrate, isosorbide dinitrate, and sodium nitroprusside in bovine airways. *Am. Rev. Respir. Dis.,* **139,** 1192–1197.

HÖGMAN, M., ALMIRALL, J., MÖRK, A.C., ROOMANS, G.M., HAGELQVIST, E., LAGERSTRAND, L. & HEDENSTIERNA, G. (1997a). Nebulisation of hypertonic saline causes oedema of the airway wall. *J. Submicrosc. Cytol. Pathol.,* **29,** 59–64.

HÖGMAN, M., FROSTELL, C., ARNBERG, H. & HEDENSTIERNA, G. (1993a). Inhalation of nitric oxide modulates methacholine-induced bronchoconstriction in the rabbit [see comments]. *Eur. Respir. J.,* **6,** 177–180.

HÖGMAN, M., FROSTELL, C.G., HEDENSTRÖM, H. & HEDENSTIERNA, G. (1993b). Inhalation of nitric oxide modulates adult human bronchial tone. *Am. Rev. Respir. Dis.,* **148,** 1474–1478.

HÖGMAN, M., HJOBERG, J. & HEDENSTIERNA, G. (1998). Increased airway osmolarity inhibits the action of nitric oxide in the rabbit. *Eur. Respir. J.,* **12,** 1313–1317.

HÖGMAN, M., HJOBERG, J., MÖRK, A.C., ROOMANS, G.M. & ANDERSON, S.D. (1997b). Dry gas hyperpnea changes airway reactivity & ion content of rabbit tracheal wall. *Respir. Physiol.,* **109,** 65–72.

IGNARRO, L.J. & KADOWITZ, P.J. (1985). The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu. Rev. Pharmacol. Toxicol.,* **25,** 171–191.

JEFFERY, P.K. (1998). Airway pathology in asthma. In *Asthma. Basic mechanisms and clinical management*. eds. Barnes, P.J., Rodger, I.W. & Thomson, N.C. pp. 47–64. San Diego, CA, U.S.A.: Academic press.

KATSUKI, S. & MURAD, F. (1977). Regulation of adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.,* **13,** 330–341.

KOWALUK, E.A., SETH, P. & FUNG, H.L. (1992). Metabolic activation of sodium nitroprusside to nitric oxide in vascular smooth muscle. *J. Pharmacol. Exp. Ther.,* **262,** 916–922.

MARKS, G.S., McLAUGHLIN, B.E., BROWN, L.B., BEATON, D.E., BOOTH, B.P., NAKATSU, K. & BRIEN, J.F. (1991). Interaction of glyceryl trinitrate and sodium nitroprusside with bovine pulmonary vein homogenate and 10,000 × g supernatant: biotransformation and nitric oxide formation. *Can. J. Physiol. Pharmacol.,* **69,** 889–892.

MORRISON, K.J., GAO, Y. & VANHOUTTE, P.M. (1990). Epithelial modulation of airway smooth muscle. *Am. J. Physiol.,* **258,** L254–L262.

MUNAKATA, M., MITZNER, W. & MENKES, H.A. (1988). Osmotic stimuli induce epithelial-dependent relaxation in the guinea-pig trachea. *J. Appl. Physiol.,* **64,** 466–471.

MURAD, F., MITTAL, C.K., ARNOLD, W.P., KATSUKI, S. & KIMURA, H. (1978). Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin. *Adv. Cyclic. Nucleotide. Res.,* **9,** 145–158.

NIJKAMP, F.P. & FOLKERTS, G. (1994). Nitric oxide and bronchial reactivity. *Clin. Exp. Allergy,* **24,** 905–914.

PRAZMA, J., COLEMAN, C.C., SHOCKLEY, W.W. & BOUCHER, R.C. (1994). Tracheal vascular response to hypertonic and hypotonic solutions. *J. Appl. Physiol.,* **76,** 2275–2280.

ROCHA, G., BUCHER, B., TSCHOPL, M. & STOCLET, J.C. (1995). Hyperosmolarity enhances smooth muscle contractile responses to phenylephrine and partially impairs nitric oxide production in the rat tail artery [published erratum appears in J Vasc Res 1995 Mar- Apr;32(2):119]. *J. Vasc. Res.,* **32,** 58–65.

ROYALL, J.A., KOOY, N.W. & BECKMAN, J.S. (1997). Peroxynitrite and other Nitric Oxide-Derived Oxidants. In: *Nitric Oxide and the Lung,* eds Zapol, W.M. & Bloch, K.D., pp.223–246, New York: Marcel Dekker, Inc.

SADEGHI-HASHJIN, G., FOLKERTS, G., HENRICKS, P.A., VAN DE LOO, P.G., DIK, I.E. & NIJKAMP, F.P. (1996). Relaxation of guinea-pig trachea by sodium nitroprusside: cyclic GMP and nitric oxide not involved. *Br. J. Pharmacol.,* **118,** 466–470.

STUART-SMITH, K., BYNOE, T.C., LINDEMAN, K.S. & HIRSHMAN, C.A. (1994). Differential effects of nitrovasodilators and nitric oxide on porcine tracheal and bronchial muscle in vitro. *J. Appl. Physiol.,* **77,** 1142–1147.

STUART-SMITH, K. & VANHOUTTE, P.M. (1990). Epithelium, contractile tone, and responses to relaxing agonists in canine bronchi. *J. Appl. Physiol.,* **69,** 678–685.

UMENO, E., McDONALD, D.M. & NADEL, J.A. (1990). Hypertonic saline increases vascular permeability in the rat trachea by producing neurogenic inflammation. *J. Clin. Invest.,* **85,** 1905–1908.

WARD, J.K., BARNES, P.J., TADJKARIMI, S., YACOUB, M.H. & BELVISI, M.G. (1995). Evidence for the involvement of cGMP in neural bronchodilator responses in humal trachea. *J. Physiol. (Lond.),* **483,** 525–536.

WILLUMSEN, N.J., DAVIS, C.W. & BOUCHER, R.C. (1994). Selective response of human airway epithelia to luminal but not serosal solution hypertonicity. Possible role for proximal airway epithelia as an osmolality transducer. *J. Clin. Invest.,* **94,** 779–787.

WONG, W.S., ROMAN, C.R. & FLEISCH, J.H. (1995). Differential relaxant responses of guinea-pig lung strips and bronchial rings to sodium nitroprusside: a mechanism independent of cGMP formation. *J. Pharm. Pharmacol.,* **47,** 757–761.

ZHANG, Y., PALETTE-PAYS, C., NALINE, E., VAROQUAUX, O. & ADVENIER, C. (1993). Effect of molsidomine and linsidomine on the human isolated bronchus and the guinea-pig isolated trachea. *J. Pharm. Pharmacol.,* **45,** 280–285.

ZHOU, H.L. & TORPHY, T.J. (1991). Relationship between cyclic guanosine monophosphate accumulation and relaxation of canine trachealis induced by nitrovasodilators. *J. Pharmacol. Exp. Ther.,* **258,** 972–978.

(Received November 19, 1998
Revised February 25, 1999
Accepted March 1, 1999)