ELSEVIER

European Journal of Pharmacology 302 (1996) 109–115

ejp

Induction of guinea pig airway hyperresponsiveness by inactivation of guanylate cyclase

Gudarz Sadeghi-Hashjin, Gert Folkerts *, Paul A.J. Henricks, Peet G.F. Van de Loo, Henk J. Van der Linde, Ilse E.M. Dik, Frans P. Nijkamp

Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80.082, 3508 TB Utrecht, Netherlands

Received 7 August 1995; revised 23 January 1996; accepted 27 January 1996

Abstract

To examine the role of cyclic 3′,5′-guanosine monophosphate (cGMP) in airway responsiveness the effects of substances known to interfere with nitric oxide (NO) or cGMP were investigated on guinea pig airways. Using a perfused organ bath system, it was possible to apply the chemicals from either the serosal or the mucosal side independently. In addition, levels of intracellular cGMP were determined in tissues after various treatments. Sodium nitroprusside (a donor of NO), zaprinast (a specific inhibitor of cGMP phosphodiesterase) and 8-bromo-cGMP (8-Br-cGMP) caused a concentration-dependent relaxation of guinea pig trachea. These results indicate that cGMP is an important second messenger mediating tracheal relaxations. The above mentioned drugs caused a more profound relaxation when applied to the serosal side compared to the mucosal side, suggesting a barrier function of the epithelial layer. Incubation on the mucosal side of the tissues with 100 μM pyrogallol (a generator of superoxide that may inactivate NO) increased the contractile response to histamine at concentrations 0.3–3.2 μM ($P < 0.05$). Treatment of the preparations with 1 mM cystamine (an inactivator of guanylate cyclase) caused a 5-fold increase in the sensitivity to histamine ($P < 0.05$), indicating the involvement of the NO/cGMP pathway in the development of airway hyperresponsiveness. Incubation of the tissues with 100 μM histamine elevated the intracellular cGMP levels 10-fold; this effect was completely prevented by incubation of the tissues with methylene blue (a potent inactivator of guanylate cyclase). Mucosal incubation of the tracheal tubes with 10 μM methylene blue induced an 8-fold increase in sensitivity to histamine ($P < 0.01$) and the E_{max} was slightly increased. 25 min after instillation of 0.4 μmol methylene blue into the airways of anaesthetized guinea pigs, the lung resistance in response to histamine was elevated up to 395 ± 82% ($P < 0.001$). The present study revealed that inactivation of NO or guanylate cyclase enhances the histamine-induced contractions of guinea pig tracheas. Therefore, it is suggested that the NO/cGMP pathway may be implicated in the pathogenesis of airway hyperresponsiveness and that drugs which enhance cGMP levels in airway smooth muscle may be of significance in the treatment of airway obstruction and enhanced reactivity.

Keywords: Airway responsiveness; Nitric oxide (NO); Airway epithelium; Methylene blue; Guanylate cyclase; Zaprinast

1. Introduction

Several nitrogen-containing compounds, such as sodium nitroprusside, and exogenous and endogenous nitric oxide (NO) activate soluble guanylate cyclase, elevate cyclic 3′,5′-guanosine monophosphate (cGMP) and relax airway smooth muscle in vitro (Katsuki and Murad, 1977; Murad et al., 1978; Buga et al., 1989; Gruetter et al., 1989). Inhaled NO reduces or reverses bronchoconstriction in guinea pigs (Dupuy et al., 1992). The relaxations induced by nitrovasodilators in canine and guinea pig trachea correlate well with the cGMP elevation, suggesting a causal role for cGMP in mediating relaxation (Zhou and Torphy, 1991; Suzuki et al., 1986). Inhibition of cGMP-specific phosphodiesterase by xanthine derivatives leads to the relaxation of canine and guinea pig trachea (Polson et al., 1985; Tanaka et al., 1991). A more selective inhibitor of cGMP phosphodiesterase, zaprinast, relaxes bovine tracheal smooth muscle (Shahid et al., 1991), potentiates sodium nitroprusside-induced relaxation and causes cGMP accumulation in canine trachea (Zhou and Torphy, 1991; Torphy et al., 1991). Moreover, 8-bromo-cGMP (8-Br-cGMP), a cell permeable analogue of cGMP, reverses spontaneous tone and carbachol- or methacholine-induced contractions in guinea pig trachea (Suzuki et al., 1986;

* Corresponding author. Tel.: +31-30-2534509; fax: +31-30-2537420; e-mail: g.folkerts@far.ruu.nl

PII S0014-2999(96)00081-7

Heaslip et al., 1987; Zhou and Torphy, 1991). These data support the hypothesis that cGMP is a mediator of relaxation in airway smooth muscle.

Histamine and acetylcholine receptor agonists cause bovine tracheal smooth muscle contraction followed by increased cGMP levels (Katsuki and Murad, 1977). Therefore, the contraction induced by these agonists may be partly reversed as a feedback mechanism. NO may participate in several pathophysiological conditions in airways (Nijkamp and Folkerts, 1994). We have demonstrated that the guinea pig bronchoconstrictor response to histamine or acetylcholine receptor stimulation is enhanced when the formation of endogenous NO is inhibited (Nijkamp et al., 1993). During histamine-induced contraction, simultaneous release of NO is observed in the guinea pig trachea (Folkerts et al., 1995). A diminished NO production, therefore, might be associated with airway hyperresponsiveness in asthmatic patients.

Although cGMP is a known mediator of relaxation in airway smooth muscle (Diamond, 1993), it is unclear if inactivation of guanylate cyclase causes bronchial hyperresponsiveness. The aim of this study was to gain a better insight into the involvement of the NO/cGMP pathway in controlling airway responsiveness.

2. Materials and methods

2.1. Animals

Specified pathogen-free guinea pigs (400–500 g, male Dunkin Hartley, Harlan Olac, UK) were housed under controlled conditions. Water and commercial chow were allowed ad libitum. The guinea pigs were free of respiratory infections as assessed by the health monitoring quality control report of Harlan Porcellus (UK), and by histological examination.

2.2. Airway responsiveness in vitro

Guinea pigs were killed with an overdose of pentobarbitone sodium (Nembutal, 0.6 g/kg body weight, i.p.). Tracheas were dissected free of connective tissue and blood vessels, isolated, divided into two equal parts of 14 rings each and perfused in an organ bath according to a modified method of Pavlovic et al. (1989). Two hooks were inserted through opposite sides of the tracheal wall with the smooth muscle between them (Fig. 1). One hook was attached to a fixed point in the organ bath; the other hook was connected to an isometric transducer (Harvard Bioscience, Kent, UK). Transducers were connected to an analogue-digital convertor (Intelligent Instrumentation PCI System, Burr Brown Company, Tucson, AZ, USA) integrating the organ baths in a semi-automatic set-up. This allowed continuous sampling, on-line equilibrium detection, and real-time display of the responses on a computer

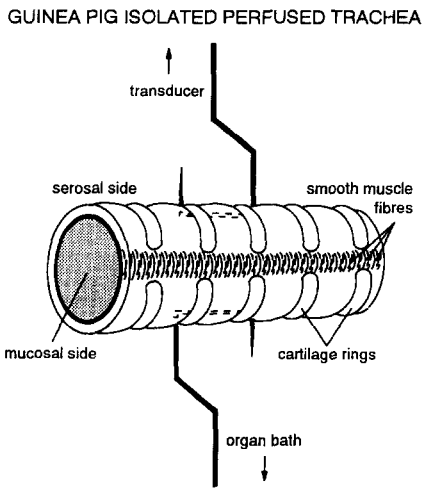

Fig. 1. Schematic representation of guinea pig isolated perfused trachea prepared for isometric recording. Note that the smooth muscle was left intact between upper and lower hooks.

screen of up to 12 baths. The tracheal tension was set at an optimal counter weight of 2 g. The inside of the trachea was perfused (2 ml/min) independently from the outside with the Krebs-bicarbonate solution by means a peristaltic pump. Krebs-bicarbonate solution was continuously gassed with 5% CO_2 in O_2 at 37°C. Every 15 min the buffer was refreshed on both sides until a stable tone was reached (usually within 75 min).

The influences of cumulative concentrations of isoprenaline (a non-specific β-adrenoceptor agonist), sodium nitroprusside, zaprinast and 8-Br-cGMP on tracheal spontaneous tone were studied from the mucosal and the serosal sides. In the experiments where a histamine concentration-response curve was to be made, the tracheas were first incubated mucosally with either control solution or one of the following substances for 25 min: 100 μM pyrogallol (a superoxide generator), 1 mM cystamine or 10 μM methylene blue (inhibitors of guanylate cyclase). Thereafter, histamine concentration-response curves were made for the preparations from the mucosal side. In all experiments the average E_{max} of the corresponding control group was taken as 100% response.

2.3. Measurement of cGMP

Tracheas were divided into four equal segments and placed in Krebs buffer continuously gassed with 5% CO_2 in O_2 at 37°C. The solution was refreshed three times, as it was in the organ bath experiments. Tracheal tubes were treated with methylene blue (10 μM, 25 min), histamine (100 μM, 5 min), or 8-Br-cGMP (10 μM, 15 min). The effect of histamine was investigated after preincubation of the tissues with methylene blue. Thereafter, the reaction was immediately stopped with ice-cold 6% (W/V) trichloroacetic acid, the specimens were purged with liquid nitrogen and kept at −80°C. Tracheal tubes were homoge-

Table 1
Variables [a] derived from concentration-response curves of isoprenaline, sodium nitroprusside, zaprinast, and 8-Br-cGMP in guinea pig trachea perfused from the mucosal vs. serosal side ($n = 4$–6)

	Maximal relaxation (mg)		pD_2	
	Mucosal	Serosal	Mucosal	Serosal
Isoprenaline (0.1 nM–1 μM)	1419 ± 101 [c]	1882 ± 71	7.6 ± 0.2 [c]	8.4 ± 0.1
Sodium nitroprusside (10 nM–100 μM)	1706 ± 86	1701 ± 96	5.7 ± 0.1 [c]	6.4 ± 0.2
Zaprinast (1 μM–1 mM)	1156 ± 147 [b]	1706 ± 107	4.4 ± 0.1	4.4 ± 0.1
8-Br-cGMP (1 μM–1 mM)	1056 ± 204	1481 ± 172	4.3 ± 0.1	4.3 ± 0.1

[a] Maximal relaxation is the maximum decrease in tracheal tension induced by different agents. pD_2 value is the negative logarithm of the molar concentration of each substance leading to the 50% of its maximal relaxant effect. Results are presented as means ± S.E.M. [b,c] $P < 0.05$, $P < 0.01$ (Student's unpaired t test); variables obtained after mucosal application of the substance were significantly different from those obtained after serosal application.

nized in 6% trichloroacetic acid (100 mg/ml) by means of a mechanical homogenizer (RZR 1, Heidolph, Germany). Suspensions were centrifuged at 2000 × g and 4°C for 10 min; supernatant was preserved and pellet was discarded. The lipid phase was extracted four times with a 5-fold excess of water-saturated diethylether and was discarded after each time. Finally, the water phase was completely dried at 60°C under a stream of nitrogen gas and the extracts were kept at −80°C until the assay was performed. For measuring cGMP, the specimens were acetylated and a commercial dual-range enzyme immunoassay kit (RPN 226, Amersham International, Buckinghamshire, UK) was used. The levels of intracellular cGMP were calculated as fmol/mg wet weight of trachea.

2.4. Airway responsiveness in vivo

Guinea pigs were anaesthetized with urethane (2.8 g/kg i.p.) and the airway reactivity was investigated in vivo as described before (Nijkamp et al., 1993). In short, the trachea was cannulated and connected to a pneumotachograph with a flow head. Lung resistance (R_L) was determined breath by breath by a modified method of Amdur and Mead (1958), using a computerized respiratory analyser. Dividing the change in the transpulmonary pressure (ΔTPP) by the change in the airflow (ΔV) at isovolume points (50%) yielded the R_L. 100 μl of saline containing 0.4 μmol methylene blue was instilled into the airways of a group of animals through the tracheal canula. Controls

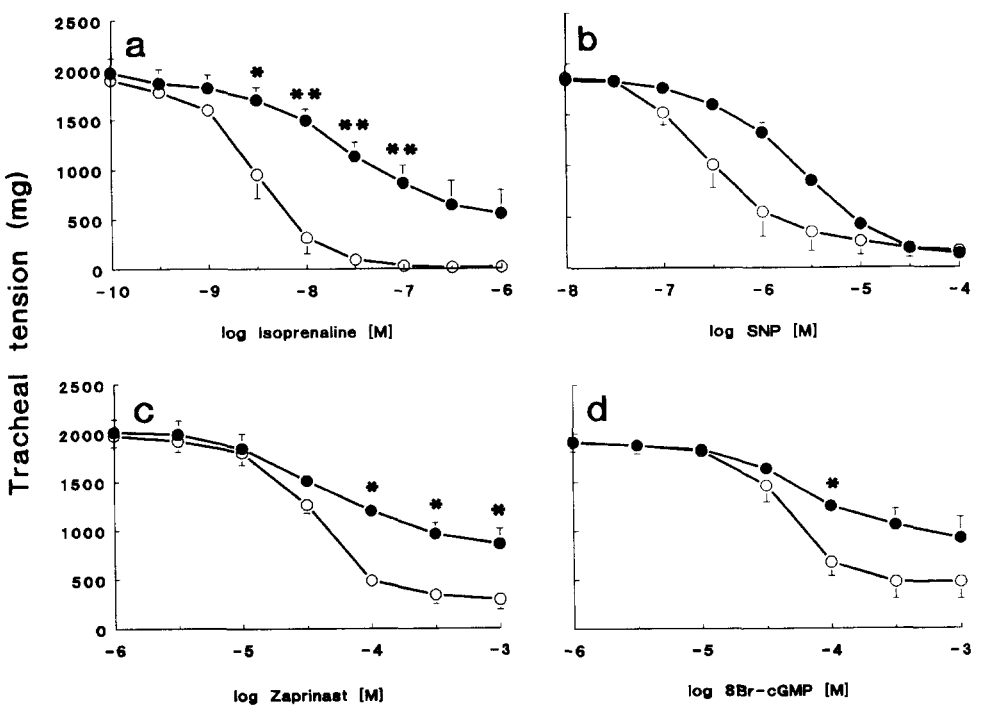

Fig. 2. Concentration-response curves for isoprenaline (*a*), sodium nitroprusside (*b*), zaprinast (*c*) and 8-Br-cGMP (*d*) on guinea pig isolated perfused trachea; drugs were applied to serosal (○) vs. mucosal (●) sides. The results are presented as means ± S.E.M. Pharmacological variables derived from these experiments are presented in Table 1. Maximal relaxations induced by isoprenaline and zaprinast as well as the pD_2 values of isoprenaline and sodium nitroprusside were decreased significantly when the drugs were administered mucosally. * $P < 0.05$, ** $P < 0.01$, significant differences in relaxations at various concentrations between serosal and mucosal application as tested by repeated measures ANOVA.

received saline only. Histamine dose-response curves were made 25 min after methylene blue treatment. Histamine was administered via a polyethylene catheter placed in the right jugular vein. Injections were given at intervals of 5 min by which time R_L had returned to baseline. Responses are presented as increase in R_L above baseline.

2.5. Solutions and drugs

Methylene blue, cystamine dihydrochloride and pyrogallol were purchased from Fluka Chemie (Buchs, Germany). Histamine diphosphate, 8-Br-cGMP and sodium nitroprusside were bought from Sigma Chemical Co. (St. Louis, USA) and isoprenaline sulfate from OPG Groothandel (Utrecht, Netherlands). Zaprinast (M&B 22948), a gift from Rhone-Poulenc Rorer (Essex, UK), was dissolved in ethanol as stock solution, kept at −20°C and diluted in buffer whenever needed. Krebs-bicarbonate buffer was of the following composition (mmol/l): NaCl, 118.1; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; and glucose, 8.3.

2.6. Statistical analysis

Averaged data are presented as means ± standard error of the means (S.E.M.). Differences between the parameters defining the maximal relaxation and E_{max} (maximal contraction or increased lung resistance induced by histamine) as well as the pD_2 value (the negative logarithm of the molar concentration of a substance that leads to a half maximal relaxation or E_{max}) were tested by Student's unpaired t test. Differences in tracheal contractions or relaxations as well as increased lung resistance at various concentrations/doses during a concentration/dose-response curve were examined using repeated measures ANOVA. The cGMP content of the trachea (expressed as fmol/mg) was averaged for each experimental group, statistically evaluated with one way ANOVA, and tested group by group by Bonferroni t test. All P values < 0.05 were considered to reflect a statistically significant difference.

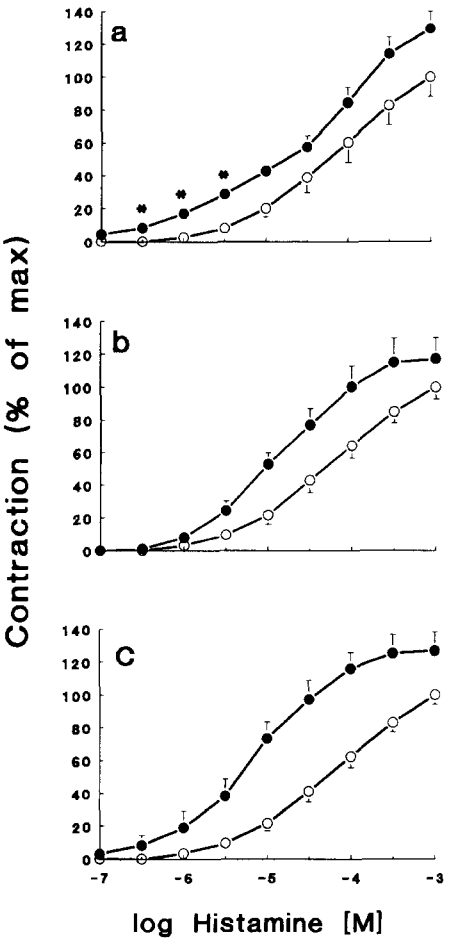

Fig. 3. Histamine concentration-response curves on isolated perfused tracheal tubes from guinea pigs, non-treated (○) or treated (●) with 100 μM pyrogallol (*a*), 1 mM cystamine (*b*) or 10 μM methylene blue (*c*). All substances were applied on the mucosal side. The results are presented as means ± S.E.M. Pharmacological variables derived from these curves are shown in Table 2. Significant differences in contractions between the control and pyrogallol-treated treated groups were observed at concentrations of 0.3–3.2 μM of histamine ($^*P < 0.05$, repeated measures ANOVA). The concentration-response curves were significantly shifted leftwards after incubation with cystamine ($P < 0.05$) and methylene blue ($P < 0.01$) as pD_2 values were tested by Student's unpaired t test.

3. Results

3.1. Tracheal relaxations

Isoprenaline, sodium nitroprusside, zaprinast and 8-Br-cGMP relaxed guinea pig trachea in a concentration-dependent fashion (Table 1, Fig. 2). All agents, except sodium nitroprusside, relaxed the trachea considerably more when applied from the serosal side than from the mucosal side. The tissues were more sensitive to isoprenaline and sodium nitroprusside from the serosal side as the pD_2 values were significantly higher. Sodium nitroprusside, zaprinast and 8-Br-cGMP, respectively, were 80, 1600 and 2000 times less potent from the mucosal side and 100, 10000 and 13000 times less potent from the serosal side than isoprenaline from the corresponding side ($P < 0.001$).

3.2. Tracheal contractions

Incubation on the mucosal side of the tissues with 100 μM pyrogallol increased the contractile response to histamine at concentrations of 0.3–3.2 μM ($P < 0.05$) and the E_{max} was enhanced by 30% (Fig. 3a, Table 2). Preincubation of the preparations with cystamine caused a 5-fold increase in the sensitivity ($P < 0.05$, Fig. 3b, Table 2). Incubation with methylene blue induced an 8-fold

Table 2
Variables [a] derived from histamine concentration-response curves made from the mucosal side of perfused guinea pig trachea, pretreated for 25 min with pyrogallol, cystamine, or methylene blue ($n = 4$–5)

		Control	Treated
Pyrogallol 100 μM	E_{max} (%)	100 ± 12	130 ± 12
	pD_2	4.2 ± 0.2	4.4 ± 0.1
Cystamine 1 mM	E_{max} (%)	100 ± 7	117 ± 13
	pD_2	4.2 ± 0.2	4.9 ± 0.2 [b]
Methylene blue 10 μM	E_{max} (%)	100 ± 6	127 ± 11
	pD_2	4.3 ± 0.1	5.2 ± 0.2 [c]

[a] E_{max} is the maximum increase in tracheal tension induced by histamine. In each case E_{max} in the non-incubated group was taken as 100% response. pD_2 value is the negative logarithm of the molar concentration of histamine leading to the 50% of its maximal contractile effect. Results are presented as means ± S.E.M. [b,c] $P < 0.05$, $P < 0.01$ (Student's unpaired t test): the variable was significantly different from that of the corresponding control group.

increase in sensitivity to histamine ($P < 0.01$) while the E_{max} was not significantly increased (Fig. 3c, Table 2).

3.3. cGMP assay

The basal level of cGMP was 9.3 ± 2.1 fmol/mg tracheal tissue ($n = 6$). Histamine augmented the cGMP content of guinea pig trachea 10-fold ($P < 0.001$). This effect was completely prevented by previous incubation of the preparations with methylene blue. Methylene blue by itself had no significant influence on the basal cGMP content (Fig. 4). 8-Br-cGMP elevated the cGMP level 12-fold ($P < 0.001$), confirming the ability of this analogue to diffuse into the airway tissue (Fig. 4). 8-Br-cGMP (1 nM–1 mM) reacted with the cGMP antibody in a concentration-dependent manner in the absence of tissue.

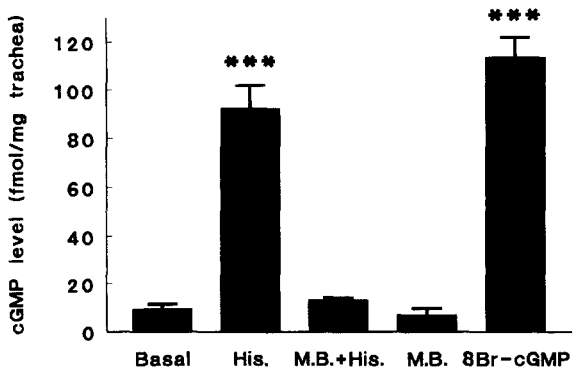

Fig. 4. cGMP levels in guinea pig tracheal tubes ($n = 3$–6) were measured by means of enzyme immunoassay (EIA) under basal conditions or after treatment with histamine (His.; 100 μM for 5 min), methylene blue (M.B.; 10 μM for 25 min), methylene blue followed by histamine, and 8-Br-cGMP (10 μM for 15 min). The results are presented as means ± S.E.M. Histamine and 8-Br-cGMP elevated cGMP content of trachea significantly and methylene blue prevented the effect of histamine entirely. *** $P < 0.001$, significantly different from basal cGMP level as tested by Bonferroni t test after one way ANOVA.

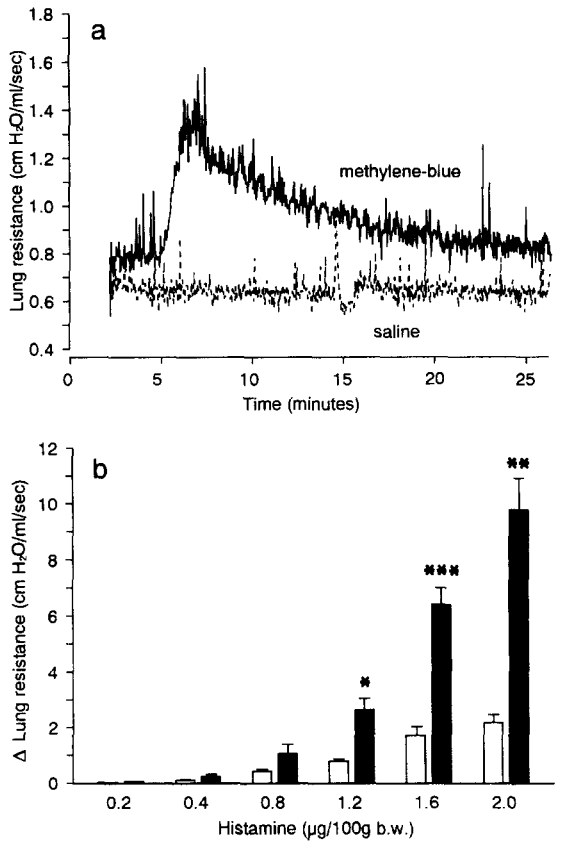

Fig. 5. *a*: A representative tracing of the time course of the effect of 0.4 μmol methylene blue and saline, instilled into the airways of anaesthetized guinea pigs, on the basal lung resistance. Peak increase was observed approximately 5 min after instillation of methylene blue. *b*: Increase in lung resistance after intravenous administration of histamine to guinea pigs 25 min after intratracheal instillation of control solution (open bars) or 0.4 μmol methylene blue (black bars). Results are expressed as means ± S.E.M. for six separate animals. Significant differences in reactivity were observed between two groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significant differences in the histamine-induced lung resistance between the control and treated groups as tested by repeated measures ANOVA.

3.4. Airway responsiveness in vivo

The R_L was enhanced approximately 5 min after instillation of methylene blue by 1.15 ± 0.39 cm H_2O/ml per s and declined and stabilized within 25 min (Fig. 5a). Before histamine dose-response curves were made, no difference in the basal resistance existed between the control and treated groups. Intravenous injections of histamine induced a dose-dependent increase of the R_L. Instillation of methylene blue increased the R_L in response to histamine up to 395 ± 82%, compared to the control group ($P < 0.001$; Fig. 5b).

4. Discussion

This study shows that guinea pig tracheal relaxations can be achieved by exogenous NO and cGMP as well as

by inhibiting the breakdown of endogenous cGMP. All relaxant substances were less potent and/or caused a smaller maximal relaxation when administered from the mucosal side compared to the serosal side of trachea. Airway hyperresponsiveness to histamine was induced when tracheas were treated with a superoxide-generating substance (which inactivates NO) or inhibitors of guanylate cyclase. These functional results were supported by changes in the tracheal cGMP levels when the histamine-induced cGMP increase was prevented by methylene blue. Moreover, guanylate cyclase inhibition in vivo also caused hyperresponsiveness to histamine.

Tracheal reactivity has been reported to be significantly higher when the contractile agents are administered on the serosal side compared to the mucosal side of the guinea pig trachea (Munakata et al., 1989; Nijkamp et al., 1993). In the present study the maximal relaxations and/or potencies were less when isoprenaline, sodium nitroprusside, zaprinast or 8-Br-cGMP were applied on the mucosal side of the trachea compared to the serosal side. This indicates that airway epithelium has a non-specific barrier function for not only contractile but also relaxant substances. Unlike other relaxing substances used in this study, maximal tracheal relaxation was induced by sodium nitroprusside when the agent was added to the mucosal side. It is possible that the epithelial barrier does not function as such in this case, probably because NO, released from sodium nitroprusside, is a small radical which may diffuse from the epithelium and reach smooth muscle easily. Guinea pig tracheal relaxations induced by sodium nitroprusside demonstrate the sensitivity of the airway smooth muscle to exogenous NO (Katsuki and Murad, 1977).

cGMP phosphodiesterase mediates cGMP hydrolysis in canine trachea and is inhibited by zaprinast (Torphy et al., 1991). As demonstrated in the present study, zaprinast relaxes the guinea pig trachea as well, suggesting the presence of cGMP-specific phosphodiesterase and the role of cGMP in basal airway tone in this species. A criterion for the involvement of cGMP in the responsiveness of an organ is to show whether its function is altered by exogenous cGMP (Diamond, 1993). 8-Br-cGMP has been shown to possess the highest potency among analogues of cGMP for activating bovine lung protein kinase G in vitro and for decreasing the carbamylcholine-induced tension of guinea pig trachea (Francis et al., 1988). Guinea pig trachea relaxed when exposed to this nucleotide in the present study. Whether or not the analogue loses the bromine atom when it diffuses into the cells is unclear as 8-Br-cGMP was also found to bind directly with the cGMP antibody in the assay in the absence of tissue.

Histamine-induced contraction of the guinea pig trachea is associated with the release of significant amounts of NO (Folkerts et al., 1995). Inhibition of NO production in the guinea pig trachea also causes a strong increase in the histamine reactivity (Nijkamp et al., 1993). In the present study similar results were obtained with pyrogallol, which generates superoxide in physiological solutions (Moncada et al., 1986). The superoxide production by broncho-alveolar cells of allergic subjects is enhanced after allergen exposure (Calhoun et al., 1992; Sanders et al., 1995) and the interaction between superoxide and NO may lead to the formation of a cytotoxic agent, peroxynitrite (Beckman et al., 1990). Therefore, inactivation of NO by superoxide may impair a braking mechanism against excessive muscle contraction and is implicated in the pathogenesis of bronchial hyperresponsiveness. Cystamine and methylene blue are known to inactivate guanylate cyclase (Conklin and Du, 1992; Martin et al., 1985). Both of these chemicals enhanced guinea pig tracheal responsiveness to histamine. Although it may also affect NO concentrations via alternative pathways (Marshall et al., 1988; Mayer et al., 1993), methylene blue oxidizes a ferrous heme group linked to guanylate cyclase and inactivates it (Martin et al., 1985; Kontons and Wei, 1993). In the present study this was reflected in the complete inhibition of the histamine-induced increase in cGMP. Interestingly, methylene blue increased both basal and histamine-induced lung resistance in the anaesthetized animals. The basal resistance, however, returned to the control level within 25 min. These findings correlate with the in vitro data and confirm the role of guanylate cyclase inactivation in the induction of airway hyperresponsiveness.

In conclusion, *all* pharmacological agents that increase intracellular cGMP concentrations via different mechanisms induced strong tracheal relaxation. On the other hand, *all* drugs that prevented the increase of cGMP after histamine stimulation enhanced tracheal contractions. It is suggested that the NO/cGMP pathway is implicated in the pathogenesis of airway hyperresponsiveness and substances that increase intracellular cGMP level can be considered as new alternatives to prevent excessive airway contractions.

Acknowledgements

The authors would like to thank Dr T.L. Buckley for correction of the English language.

References

Amdur, M.O. and J. Mead, 1958, Mechanics of respiration in unanaesthetized guinea pigs, Am. J. Physiol. 192, 364.

Beckman, J.S., T.W. Beckman, J. Chen, P.A. Marshall and B.A. Freeman, 1990, Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide, Proc. Natl. Acad. Sci. USA 87, 1620.

Buga, G.M., M.E. Gold, K.S. Wood, G. Chaudhuri and L.J. Ignarro, 1989, Endothelium-derived nitric oxide relaxes nonvascular smooth muscle, Eur. J. Pharmacol. 161, 61.

Calhoun, W.J., H.E. Reed, D.R. Moest and C.A. Stevens, 1992, Enhanced superoxide production by alveolar macrophages and air-space cells, airway inflammation, and alveolar macrophage density changes

after segmental antigen bronchoprovocation in allergic subjects, Am. Rev. Respir. Dis. 145, 317.

Conklin, J.L. and C. Du, 1992. Guanylate cyclase inhibitors: effect on inhibitory junction potentials in esophageal smooth muscle, Am. J. Physiol. 263, G87.

Diamond, J., 1993. Role of cyclic GMP in airway smooth muscle relaxation. in: New Drugs in Allergy and Asthma, eds. T.T. Hansel and J. Morley (Birkhäuser Verlag, Basel) p. 13.

Dupuy, P.M., S.A. Shore, J.M. Drazen, C. Frostell, W.A. Hill and W.M. Zapol, 1992. Bronchodilator action of inhaled nitric oxide in guinea pigs, J. Clin. Invest. 90, 421.

Folkerts, G., H. Van der Linde and F.P. Nijkamp, 1995. Virus-induced airway hyperresponsiveness in guinea-pigs is related to a deficiency in nitric oxide, J. Clin. Invest. 95, 26.

Francis, S.H., B.D. Noblett, B.W. Todd, J.N. Wells and J.D. Corbin, 1988. Relaxation of vascular and tracheal smooth muscle by cyclic nucleotide analogs that preferentially activate purified cGMP-dependent protein kinase, Mol. Pharmacol. 34, 506.

Gruetter, C.A., C.E. Childers, M.K. Bosserman, S.M. Lemke, J.G. Ball and M.A. Valentovic, 1989. Comparison of relaxation induced by glyceryl trinitrate, isosorbide dinitrate, and sodium nitroprusside in bovine airways, Am. Rev. Respir. Dis. 139, 1192.

Heaslip, R.J., F.R. Giesa, T.J. Rimele and D. Grimes, 1987. Co-regulation of tracheal tone by cyclic AMP- and cyclic GMP-dependent mechanisms, J. Pharmacol. Exp. Ther. 243, 1018.

Katsuki, S. and F. Murad, 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.

Kontons, H.A. and E.P. Wei, 1993. Hydroxyl radical-dependent inactivation of guanylate cyclase in cerebral arterioles by methylene blue and by LY83583, Stroke 24, 427.

Marshall, J.J., E.P. Wei and H.A. Kontons, 1988. Independent blockade of cerebral vasodilation from acetylcholine and nitric oxide, Am. J. Physiol. 255, H847.

Martin, W., G.M. Villani, D. Jothianadan and R.F. Furchgott, 1985. Selective blockade of endothelium-dependent and glyceryl trinitrite-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta, J. Pharmacol. Exp. Ther. 232, 708.

Mayer, B., F. Brunner and K. Schmidt, 1993. Inhibition of nitric oxide synthesis by methylene blue, Biochem. Pharmacol. 45, 365.

Moncada, S., R.M. Palmer and R.J. Gryglewski, 1986. Mechanism of action of some inhibitors of endothelium-derived relaxing factor. Proc. Natl. Acad. Sci. USA 83, 9164.

Munakata, M., I. Huang, W. Mitzner and H. Menkes, 1989. Protective role of the epithelium in the guinea pig airways, J. Appl. Physiol. 66, 1547.

Murad, F., C.K. Mittal, W.P. Arnold, S. Katsuki and H. Kimura, 1978. Guanylate cyclase: activation by azide, nitrocompounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin, Adv. Cyclic Nucleotide Res. 9, 145.

Nijkamp, F.P. and G. Folkerts, 1994. Nitric oxide and bronchial reactivity, Clin. Exp. Allergy 24, 905.

Nijkamp, F.P., H.J. Van der Linde and G. Folkerts, 1993. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro: role of the epithelium, Am. Rev. Respir. Dis. 148, 727.

Pavlovic, D., M. Fournier, M. Aubier and R. Pariente, 1989. Epithelial vs. serosal stimulation of tracheal muscle: role of epithelium, J. Appl. Physiol. 67, 2522.

Polson, J.B., J.J. Krzandowski and A. Szentivanyi, 1985. Correlation between inhibition of a cyclic GMP phosphodiesterase and relaxation of canine tracheal smooth muscle. Biochem. Pharmacol. 34, 1875.

Sanders, S.P., J.L. Zweier, S.J. Harrison, M.A. Trush, S.J. Rembish and M.C. Liu, 1995. Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects, Am. J. Respir. Crit. Care Med. 151, 1725.

Shahid, M., R.G.M. Van Amsterdam, J. De Boer, R.E. Ten Berge, C.D. Nicholson and J. Zaagsma, 1991. The presence of five cyclic nucleotide phosphodiesterase isoenzyme activities in bovine tracheal smooth muscle and the functional effects of selective inhibitors, Br. J. Pharmacol. 104, 471.

Suzuki, K., K. Takagi, T. Satake, S. Sugiyama and T. Ozawa T, 1986. The relationship between tissue levels of cyclic GMP and tracheal smooth muscle relaxation in the guinea pig. Clin. Exp. Pharmacol. Physiol. 13, 39.

Tanaka, H., K. Ogawa, K. Takagi, T. Satake and H. Hidaka, 1991. Inhibition of cyclic GMP phosphodiesterase by xanthine derivatives relaxes guinea-pig trachealis smooth muscle, Clin. Exp. Pharmacol. Physiol. 18, 163.

Torphy, T.J., H.-L. Zhou, M. Burman and L.B.F. Huang, 1991. Role of cyclic nucleotide phosphodiesterase isoenzymes in intact canine trachealis, Mol. Pharmacol. 39, 376.

Zhou, H.-L. and T.J. Torphy, 1991. Relationship between cyclic guanosine monophosphate accumulation and relaxation of canine trachealis induced by nitrovasodilators, J. Pharmacol. Exp. Ther. 258, 972.