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Bronchodilatation *in vivo* by carbon monoxide, a cyclic GMP related messenger

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1 Recent studies suggest that gaseous carbon monoxide (CO) is involved in neurotransmission and that this molecule also is an important vasodilator *in vivo*. In the present study we evaluated the effect of inhaled CO on guinea-pig airway smooth muscle tone. The mechanisms involved were characterized by use of a cyclic GMP antagonist, Rp-8Br-cyclic GMPS, and a nitric oxide synthase inhibitor, L-NAME.

2 Anaesthetized, ventilated guinea-pigs were given a bolus injection of histamine (0.12 mg kg^{-1}, i.v.), followed by a continuous infusion of histamine (0.30 μg kg^{-1} min^{-1}) to increase total pulmonary resistance (R_L). Subsequent exposure to 7, 15 or 30 breaths of CO (100%), resulted in a dose-dependent inhibition of the bronchoconstriction. In the highest dose tested (30 breaths), CO inhibited 80% of the histamine-induced increase in R_L.

3 In separate experiments, animals receiving histamine infusions followed by 30 breaths of CO, were pretreated with Rp-8Br-cyclic GMPS (0.05 mg kg^{-1}). This pretreatment abolished >60% of the CO-induced reduction in R_L, but it had no effect on the bronchodilator response induced by salbutamol. In another set of experiments animals were pretreated with L-NAME (1.60 mg kg^{-1}). In contrast to the Rp-8Br-cyclic GMPS pretreatment, the pretreatment with L-NAME did not affect the CO-induced reduction in R_L.

4 The present findings indicate that CO causes bronchodilatation *in vivo* via cyclic GMP.

Keywords: Carbon monoxide; Rp-8Br-cyclic GMPS; airway; *in vivo* pharmacology; trachea; lung; dilatation

Introduction

Nitric oxide (NO) is an endogenous regulator of cell function and communication (Moncada, 1992). Carbon monoxide (CO) is another endogenous gaseous molecule which shares some properties with NO, and several investigators have proposed a physiological role for CO (Marks *et al*., 1991; Schmidt, 1992; Maines, 1993; Verma *et al*., 1993). There are several endogenous sources of CO production, but the degradation of haeme to biliverdin and CO, appear to be the dominating mechanism of CO production (Rodgers *et al*., 1994). CO production has been demonstrated in various peripheral tissues (Maines, 1988; Vreman & Stevenson, 1988), and a role for CO as a peripheral transmitter involved in non-adrenergic non-cholinergic relaxation has been proposed (Rattan & Chakder, 1993). According to recent findings, CO is also produced in vascular smooth muscle and causes vasodilatation via the production of guanasine 3′:5′ cyclic monophosphate (cyclic GMP) (Morita *et al*., 1995). However, CO can also induce the release of NO and NO is another mediator of cyclic GMP (Meilin *et al*., 1996). *In vitro*, exogenous CO induces relaxation of various types of smooth muscle (e.g. arteries, opossum internal anal sphincter, feline lower oesophageal sphincter (Gräser *et al*., 1990; Rattan & Chakder, 1993; Ny *et al*., 1995)). However, there have been no studies on CO in relation to regulation of airway smooth muscle tone. The aim of the present study was therefore to characterize the effects of CO in airways *in vivo*. Changes in R_L were examined in guinea-pigs in response to inhaled CO. The role of cyclic GMP as a mediator in this response was evaluated using a cyclic GMP antagonist (Rp-8Br-cyclic GMPS). A nitric oxide synthase inhibitor (N^G-nitro-L-arginine methyl ester; L-NAME) was used to ensure that the CO bronchodilatation was not mediated via release of NO.

Materials

Total pulmonary resistance (R_L)

Male Hartley guinea-pigs (Simonsen Lab. Inc. Gilroy, CA, U.S.A.), weighing approximately 500 g were anaesthetized with pentobarbitone (45 mg kg^{-1}, i.p.) and ventilated (51×4.80 ml min^{-1}) via a tracheal cannula connected to a constant-volume ventilator (model 683 Harvard Apparatus, South Natick, MA, U.S.A.). Airflow was monitored continuously with a pneumotachygraph (no. 000, Fleisch Medical, Richmond, VA, U.S.A.) connected to a differential pressure transducer (model DP45; Validyne Engineering, Northridge, CA, U.S.A.). The transpulmonary pressure was measured using a differential pressure transducer (model DP7; Validyne Engineering, Northridge, CA, U.S.A.) recording the difference in pressure between a fluid-filled catheter placed in the oesophagus (as an approximation of the pleural pressure) and the intratracheal pressure (recorded via another catheter, connecting the tracheal tube to the pneumotachygraph) (Bertrand *et al*., 1993). The output signals representing the airflow (ml^{-1}) and transpulmonary pressure (cmH_2O) were amplified (model CD19; Validyne Engineering, Northridge, CA, U.S.A.) and recorded on a polygraph recorder (model DASH8, Astro-Med, Inc., West Warwick, RI, U.S.A.). Total pulmonary resistance (R_L) was calculated using the method of Amdur and Mead (1958) after subtracting the resistance of the system (0.079 ± 0.004 cmH_2O ml^{-1} s, $n = 40$). CO (100%) and N_2 (100%) were inhaled through the ventilator, for a brief period of 7, 15 or 30 breaths corresponding to a volume of 0.7,

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1.4 and 2.8 ml of pure CO or N_2, respectively. CO and N_2 were inhaled either during 'basal' conditions, or when a stable 'preincreased' R_L level had been established following an i.v. infusion of histamine (see below). CO and N_2 were replaced by room air in the control experiments. In a separate set of experiments, Rp-8Br-cyclic GMPS or L-NAME were injected i.v. as a bolus, either during stable 'basal' conditions or during bronchoconstriction. In the latter experiments, CO was inhaled 2–6 min after the bolus injection of the antagonists.

Airway smooth muscle tone was increased by a bolus injection of histamine i.v. (0.12 mg kg^{-1}), followed by continuous infusion of histamine, starting at 0.25 mg kg^{-1} min^{-1}. The speed of the infusion was then adjusted to produce an approximate twofold increase in the basal R_L. The final histamine concentration used was 0.30 ± 0.03 mg kg^{-1} min^{-1}, which increased R_L from 0.13 ± 0.01 cmH_2O ml^{-1} s to 0.33 ± 0.02 cmH_2O ml^{-1} s, $n = 27$. Before an aerosol was given, R_L was allowed to stabilize at this level for 20 min.

The heart rate was monitored via an electrocardiogram and the arterial blood pressure was recorded continuously (model P23D, Statham, U.S.A.) via a catheter in the right carotid artery. Drugs were delivered through a catheter in the right jugular or through injections directly into the right femoral vein. In selected animals, multiple blood bases were obtained from a catheter in the right carotid artery. Blood samples were placed on ice and transported to the Neonatal Blood Gas laboratory (University of California, San Francisco, U.S.A.), for immediate analysis.

Drugs

Histamine diphosphate, salbutamol and dibutyryl adenosine cyclic monophosphate and L-NAME (Sigma, St. Louis, MO, U.S.A.), Rp-8Br-cyclic GMPS, (BioLog, La Jolla, CA, U.S.A.). Sodium pentobarbitone (Anpro Pharmaceutical, Arcadia, CA, U.S.A.); pentobarbitone was used in a commercially available buffer solution, all other drugs were dissolved and diluted in PBS. CO and N_2 were obtained as liquid gas (Puritan Bennett, San Ramon, CA, U.S.A.).

Statistical analysis

Results are expressed as mean ± s.e.mean. Statistical analysis was performed by use of Student's *t* test for unpaired data. Differences were accepted as statistically significant at $P < 0.05$; *n* equals the number of guinea-pigs.

Results

Effects of CO on R_L

In animals where R_L had been increased with histamine, inhalation of CO caused a dose-dependent decrease in R_L (Figure 1). This decrease in R_L occurred in less than 30 s after the start of the inhalation of CO, and the peak response was obtained within 1.5–2.5 min, depending on the inhaled dose. After 7–10 min, R_L had returned to the same level as before the inhalation of CO (Figure 1). The procedure was then repeated, using the same protocol, exchanging 30 breaths of CO with 30 breaths of N_2. The inhalation of N_2 caused a slight (<7%) decrease in the levels of R_L (Figure 1), and inhalation of room air induced no change in R_L ($n = 4$, data not shown). When CO and N_2 were inhaled during control conditions, without histamine-induced R_L increase, 30 breaths of CO caused a small reduction in the basal R_L level ($n = 4$, 7 ± 1 cmH_2O ml^{-1} s), while 7–15 breaths of CO and 30 breaths of N_2 caused no reduction in R_L.

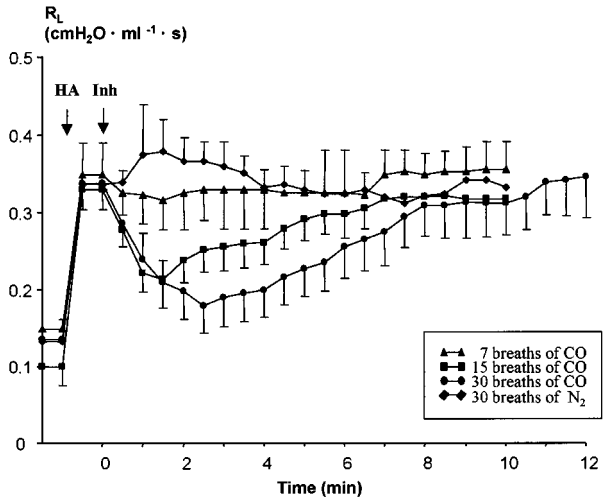

Figure 1 Changes in the total pulmonary resistance (R_L) in anaesthetized, ventilated guinea-pigs exposed to CO and N_2, respectively. R_L was increased by a bolus injection of histamine (0.12 mg kg^{-1}, i.v.) followed by continuous infusion of histamine (0.3 mg kg^{-1} min^{-1}). When a stable level of bronchoconstriction was achieved, the animals were exposed to either 7, 15 or 30 breaths of CO or 30 breaths of N_2. Responses are expressed as R_L, and each point is the mean and vertical lines show s.e.mean of 4–5 experiments. HA = start of histamine infusion. Inh = start of inhalation.

Effects of cyclic GMP antagonist and NO inhibitor on the R_L *response to CO*

In animals with histamine-induced bronchoconstriction, the cyclic GMP-antagonist Rp-8Br-cyclic GMPS (0.05 mg kg^{-1}, i.v.) inhibited >60% of the CO-induced reduction of the R_L (maximal reduction in R_L; 0.06 ± 0.02 cmH_2O ml^{-1} s, and 0.16 ± 0.02 cmH_2O ml^{-1} s, with and without Rp-8Br-cyclic GMPS pretreatment respectively, $n = 4-5$); the duration of the CO-induced (30 breaths) reduction of R_L decreased from 11 to 5 min by Rp-8Br-cyclic GMPS (Figure 2). When the procedure was repeated, using the same protocol, L-NAME was exchanged for Rp-8Br-cyclic GMPS, the CO-induced reduction of the R_L was not affected (R_L; 0.14 ± 0.02 cmH_2O ml^{-1} s, $n = 5$) (Figure 2). The use of Rp-8Br-cyclic GMPS and L-NAME in 100 times higher concentrations (0.05 mg kg^{-1} and 160 mg kg^{-1}, respectively, $n = 2-3$) did not increase the antagonistic effects induced by the substances (data not shown). The addition of Rp-8Br-cyclic GMPS to animals with induced bronchoconstriction resulted in an additional transient increase in R_L, stabilizing at a R_L level slightly above the R_L level recorded before the application of the antagonist (Figure 2). When Rp-8Br-cyclic GMPS was given to animals without a prior increase of R_L, no change in R_L was evident. Rp-8Br-cyclic GMPS did not affect the bronchodilator response induced by salbutamol (maximal reduction in R_L induced by salbutamol (0.003 mg kg^{-1}) 0.19 ± 0.08 cmH_2O ml^{-1} s, and 0.17 ± 0.05 cmH_2O ml^{-1} s in the absence and presence of Rp-8Br-cyclic GMPS, respectively, $n = 3-4$).

Effect of CO on blood gases and cardiovascular parameters

In five animals the levels of arterial PO_2 and PCO_2 and plasma bicarbonate were measured before the start of the CO

inhalation, 2 min after CO inhalation (the time of the maximal decrease in R_L) and 10 min after CO inhalation (when R_L had returned to control level) (Table 1). At the end of the experiment, a slight decrease in the blood pressure was seen, concomitant with a small decrease in the heart rate (Table 2).

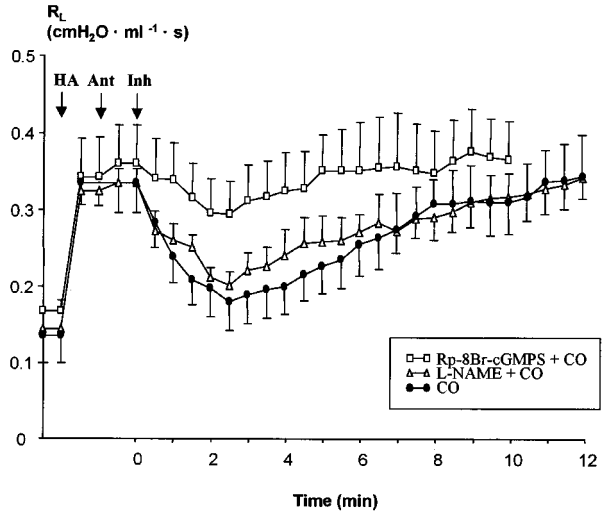

Figure 2 Changes in the total pulmonary resistance (R_L) in anaesthetized, ventilated guinea-pigs exposed to CO with and without Rp-8Br-cyclic GMPS and L-NAME pretreatment. R_L was increased by a bolus injection of histamine (0.12 mg kg^{-1}, i.v.) followed by continuous infusion of histamine (0.3 mg kg^{-1} min^{-1}). When a stable level of bronchoconstriction was achieved, the animals were exposed to 30 breaths of CO. After pretreatment with either Rp-8Br-cyclic GMP (0.05 mg kg^{-1}, L-NAME (1.60 mg kg^{-1}), or vehicle (same data as in Figure 1). Responses are expressed as R_L and each point is the mean and vertical lines show s.e.mean. HA = start of histamine infusion. Ant = addition of Rp-8Br-cyclic GMPS. Inh = start of inhalation.

Table 1 Arterial blood gas analysis in anaesthetized, ventilated guinea-pigs exposed to CO

	Before CO inhalation	*2 min after CO inhalation*	*10 min after CO inhalation*
pH	7.46±0.02	7.39±0.04	7.43±0.02
Pa_{O_2} (mmHg)	80.3±9.3	39.5±3.0	44.3±8.15
Pa_{CO_2} (mmHg)	34.0±1.2	41.7±2.6	36.4±3.3
Sa_{O_2} (%)	96.4±1.0	73.5±9.9	67.4±24.8
HCO_3 (mEq^{-1})	23.6±1.1	24.5±4.8	23.9±2.3

Three samples were taken during each experiment; before the start of the CO inhalation, 2 min after CO inhalation (the time of the maximal decrease in R_L) and 10 min after CO inhalation (when R_L had returned to control level). Pa_{O_2} = arterial tension of oxygen; Pa_{CO_2} = arterial tension of carbondioxide; Sa_{O_2} = arterial oxygen saturation (%); HCO_3 = plasma bicarbonate. The values represent the mean±s.e.mean of 5 guinea-pigs.

Discussion

This study demonstrated a mechanism whereby CO inhibits guinea-pig airway tone: inhaled CO produced a dose-dependent relaxation of guinea-pig precontracted airways, and this dilatation involved cyclic GMP, but not NO.

CO can be generated endogenously from at least two biological sources, fatty acids and haeme, and both processes appear to be enzymatic (Rodgers *et al*., 1994). Although the idea that CO has physiological actions is relatively new, endogenous production of CO during catabolism of haeme by the enzyme haeme oxygenase, has been known for many years (Tenhunen *et al*., 1968). In the present study, exogenous CO induced a dose-dependent reduction of R_L in histamine-precontracted airways. The reduction in R_L lasted for 6 to 12 min (7–30 breaths of CO) with the maximal dilatation occurring in 1.5–2.5 min. This relaxant effect of CO on smooth muscle is also indicated by previous studies on isolated blood vessels, isolated gastrointestinal tissues and rat isolated hearts (Furchgott & Jothianandan, 1991; Utz & Ullrich, 1991; Lefer *et al*., 1993; Rattan & Chakder, 1993; Zygmunt *et al*., 1994; Ny *et al*., 1996). Furthermore, *in vivo* studies have shown that CO produces a marked increase in the cerebral blood flow, presumably via direct dilator effects on the cerebral blood vessels (Koehler *et al*., 1982; Brian *et al*., 1994).

Continuous inhalation of a CO-air mixture during 30 min causes an increase in heart rate concurrent with an increase of the coronary blood flow (Adams *et al*., 1973). Reports of acute lethal and sublethal CO poisoning describes a plethora of effects on different organs including pulmonary oedema, damage to lung parenchyma, reduced respiratory drive and cardiac arrhythmias (Vreman *et al*., 1995). However, these findings were the result of a prolonged CO exposure. In the present study, short bursts of CO (8–35 s), given to guinea-pigs *in vivo*, were not associated with any significant changes in heart rate and blood pressure at the time of the maximal dilatation.

The second messenger molecule cyclic GMP regulates several protein kinases, nucleoside 3′5′-monophosphate phosphodiesterases and ion channels (Walter, 1989), resulting in various types of cellular events, including smooth muscle relaxation (Fostermann *et al*., 1986; Ward *et al*., 1995). The cyclic GMP formation is regulated by cyclic GMP-forming and degrading enzymes, and recent data suggest that one group of cyclic GMP-forming enzymes, the guanyl cyclases, can be stimulated by low molecular weight monoxides of NO, CO and perhaps also hydrogen (Schmidt, 1992). In the present study, the bronchodilator effect of CO was inhibited by RP-8Br-cyclic GMPS, a well-characterized inhibitor of cyclic

Table 2 Changes in arterial blood pressure, heart rate and total pulmonary resistance (R_L) in anaesthetized, ventilated guinea-pigs exposed to CO

	Baseline	*Before CO inhalation*	*2 min after CO inhalation*	*10 min after CO inhalation*
Heart rate	218±11	218±4	208±5	202±2
Blood pressure (mmHg)	67±10	58±7	56±5	47±3
R_L (cmH_2O ml^{-1} s)	0.14±0.04	0.34±0.04	0.18±0.04	0.31±0.04

The results at four timepoints are shown; after the surgical preparation of the guinea-pig was completed (baseline), before the start of the CO inhalation, and after start of histamine, 2 min after CO inhalation (the time of the maximal decrease in R_L) and 10 min after CO inhalation (when R_L had returned to control level). The values represent the mean±s.e.mean of 5 guinea-pigs.

GMP (Zhuo *et al*., 1994), thus indicating the involvement of cyclic GMP in the dilator response. In contrast, Rp-8Br-cyclic GMPS did not affect the dilator response induced by salbutamol, which acts via the production of cyclic AMP. This supports the specificity of the cyclic GMP antagonist Rp-8Br-cyclic GMPS. The hypothesis that CO-induced bronchodilatation is mediated through cyclic GMP formation is supported by findings in other types of smooth muscle as well (Gräser *et al*., 1990; Furchgott & Jothianandan, 1991; Rattan & Chakder, 1993; Ny *et al*., 1995). However, in the present study a significant part of the effect remained in the presence of Rp-8Br-cyclic GMPS, which is compatible with additional intracellular mechanisms being involved. CO can release NO (Meilin *et al*., 1996) and NO can mediate effects caused by cyclic GMP but, in our study, the CO-induced reduction of R_L was not affected by L-NAME, indicating that secondary NO release is not the major pathway for the dilatation.

In conclusion, the present results demonstrate that CO acts as a powerful dilator of guinea-pig airway smooth muscle *in vivo*, and this may reflect a role for CO in the regulation of airway tone.

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