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Low-dose carbon monoxide reduces airway hyperresponsiveness in mice

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Ameredes, Bill T., Leo E. Otterbein, Lauryn K. Kohut, Amber L. Gligonic, William J. Calhoun, and Augustine M. K. Choi. Low-dose carbon monoxide reduces airway hyperresponsiveness in mice. *Am J Physiol Lung Cell Mol Physiol* 285: L1270–L1276, 2003. First published August 1, 2003; 10.1152/ajplung.00145.2003.—Carbon monoxide (CO) in expired gas has been shown to be elevated with asthma; however, its function is not known, and there is some potential that it may serve a bronchoprotective role to decrease airway hyperresponsiveness (AHR). Thus the ability of CO to reverse methacholine (MCh)-induced bronchoconstriction was evaluated in C57BL/6 (C57) and A/J mice with and without airway inflammation produced by ovalbumin (OVA). Acutely administered CO (1% in air, 10 min) reduced MCh-driven increases in lung resistance in OVA-challenged C57 mice by an average of 50% (from 14.5 to 7.1 $cmH_2O \cdot ml^{-1} \cdot s^{-1}$), whereas no effect was observed in naïve C57 mice or OVA-challenged C57 mice inhaling air alone. Acutely inhaled CO (500 ppm = 0.05%, for 10 min) reduced MCh-induced airway reactivity (AR) by 20–60% in airway hyperresponsive naïve A/J mice, whereas repeated 10-min administrations of 500 ppm CO over a 5-day period decreased AR by 50%. Repeated administration of low-dose CO [250 (0.025%) and (0.05%) 500 ppm, 1 h/day, 5 days] to A/J mice with airway inflammation likewise resulted in a drop of AR by 50%, compared with those not receiving CO. Inhibition of guanylyl cyclase/guanosine 3′,5′-cyclic monophosphothioate (cGMP) using 1H-[1,2,4] oxydiazolo[4,3-a]quinoxalin-1-one or a competitive inhibitor, *Rp* diastereomers of 8-bromo-cGMP, resulted in inhibition of the effect of CO on AHR, suggesting that the effects of CO were mediated through this mechanism. These results indicate that low-dose CO can effectively reverse AHR in the presence and absence of airway inflammation in mice and suggest a potential role for CO in the modulation of AHR.

airway inflammation; airway resistance; airway smooth muscle; bronchoconstriction

CARBON MONOXIDE (CO) is a small diatomic gaseous molecule formed by the bonding of one atom of carbon with one atom of O_2. CO has been known for many years to be toxic and lethal to living things in high doses (1, 6). It is also known to be an industrial pollutant, resulting in chronic hypoxia at high levels (22). However, given the relatively recent discovery of nitric oxide (NO) in 1987 (11, 18), which introduced the idea that a small, ubiquitous diatomic gas molecule other than O_2 could be critical to so many cellular processes, there has been recent interest in the investigation of CO from this perspective.

Although CO was used in classic experiments to characterize hemoglobin (Hb) structure and function, it is now known that the formation of CO in the body can occur through the action of heme oxygenase metabolizing heme to biliverdin and, in the process, generating iron and CO (23). Furthermore, similar to NO (16), CO has been shown to increase generation of guanosine 3′,5′-cyclic monophosphothioate (cGMP) through the activation of guanylyl cyclase (15). Therefore, the potential exists that CO, when formed in small amounts endogenously in the body, may act as a cellular signal or physiological agent, similar to NO. It is well known that one of the actions of NO is to produce dilation in the cardiovascular vessels (14). We hypothesized that CO might produce dilation, or perhaps an inhibition of constriction, in the airways, similar to the dilatory action of NO in the cardiovascular system. This property of CO could be potentially important in diseases such as asthma, in which the airways are known to be hyperresponsive and prone toward closure.

Airway hyperresponsiveness (AHR) can be defined as an increase in the airway constrictive response to a cholinergic stimulant, such as methacholine (MCh). AHR is known to occur in association with airway inflammation (19); however, the nature of this association remains poorly understood. In the setting of airway inflammation, a bronchodilatory property of CO could ameliorate bronchoconstriction, thereby preventing increases in airway resistance. Certain strains of mice, such as the A/J, are highly predisposed toward AHR, whereas others such as the C57BL/6 (C57) are less responsive (13). Induction of airway inflammation in these strains can enhance AHR (24) and is a method that we utilized to increase AHR in our studies below.

Therefore, we performed experiments with moderate concentrations of CO (1% in air) in an effort to deter-

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mine whether applied CO could reduce AHR in a murine model. Furthermore, no data exist about the effect of lower levels of CO mixtures (≤0.05%) on AHR, particularly in the presence of airway inflammation. Thus we tested the hypothesis that exogenously administered, low-dose CO would decrease MCh-induced AHR in mice, in the presence and absence of airway inflammation. We also tested whether the mechanism of CO effects on AHR in the presence and absence of airway inflammation was associated with cGMP, by inhibiting both production of cGMP and preventing the action of cGMP by use of a competitive inhibitory analog. The results indicate that inhaled CO at low concentrations was effective in reducing AHR in the presence and absence of airway inflammation and that its effect in both cases was associated with cGMP production.

METHODS

General. Male mice of the C57 and A/J strains, at 8–12 wk of age, were obtained from Jackson Laboratories and housed in a specific pathogen-free-barrier facility in the Division of Laboratory Animal Resources Central Animal Facility at the University of Pittsburgh. Mice had constant access to Purina mouse chow and water ad libitum. All procedures and protocols utilized in these studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee, which conforms to guidelines recommended by the National Institutes of Health and the United States Department of Agriculture.

Induction of airway inflammation. To enhance AHR, some mice were sensitized and aerosol challenged with ovalbumin (OVA) by a protocol described previously (2) that reliably increases total cell numbers and eosinophils in recovered bronchoalveolar lavage fluid. Briefly, sensitization was achieved by using 0.5-ml injections of OVA (50 μg/ml ip, grade VI, Sigma) and alum (1 mg/ml) as a mild adjuvant, dissolved in normal saline. All mice were injected once per day, over a period of 5 days. Forty-eight hours after the last injection, this set of mice was subjected to a 10-min airway allergen challenge using OVA/saline aerosol (5 mg/ml), which was repeated once per day, over a period of 5 days.

Lung resistance measurement. Mice were anesthetized with pentobarbital sodium (65 mg/mg ip), tracheostomized, intubated, placed supine in a sealed whole body plethysmograph, and connected to a positive pressure ventilator (frequency = 110 stroke/min, tidal volume = 0.21–0.25 ml/stroke) with ambient air. Transducers connected to the ventilatory circuit provided voltage signals of pressure and flow, which were amplified and transmitted to an analog/digital (A/D) card (National Instruments) in a microcomputer running a program (BioSystem XA, Buxco) that in turn calculated lung resistance (R_L) from the digitized pressure and flow signals. A small catheter (PE 10) placed in the left jugular vein provided an injection site for MCh (Sigma), dissolved in phosphate-buffered saline, used to provoke airway constriction in situ. Typical injection volume was 20 μl, delivered over 3–4 s. R_L responses to MCh were monitored over 10- to 15-min periods, allowing reestablishment of the baseline R_L before proceeding. Typical baseline R_L before MCh was 1.8–3.5 $cmH_2O \cdot ml^{-1} \cdot s^{-1}$. In other experiments, mice were set up for R_L assessment in the same way, with the exception that MCh was delivered as an inhaled aerosol through the ventilator with a DeVilbiss nebulizer attached to the inspiratory delivery line. The nebulizer was switched on for 15 s, and the R_L response was monitored over this period and over a subsequent interval of 3–5 min. The MCh aerosol was measured to reach the endotracheal tube within 5–7 s of initiation of the nebulizer, and the aerosol was visibly present within the ventilation system for up to 1 min afterward. The R_L response was continually monitored throughout these times, with return of R_L to the pre-MCh level before initiation of the next MCh administration.

Airway reactivity measurement. Unanesthetized mice were placed within small-volume (~600 ml) Plexiglas chambers specially designed for mice, which allowed for free movement and had transducers that monitored chamber pressure alterations as a function of mouse breathing patterns (Buxco). The signals were amplified and transmitted to an A/D board in a microcomputer running a computer program (BioSystem XA, Buxco) that calculated values from the digitized pressure signals. The enhanced pause (Penh) variable was calculated in the conventional way (10), in which changes in the amplitude and duration of the expiratory pressure signal determined the alterations in the Penh value, as a function of MCh-induced airway responses. MCh dissolved in phosphate-buffered saline (pH 7.4) was administered as an aerosol to the mice within the chambers, by using a DeVilbiss ultrasonic nebulizer (aerosol droplet size = 1–5 μm) connected to an aerosol driver and pump apparatus (Buxco). The administration duration of each MCh concentration was 2 min, followed by a 3-min observation and continued data collection period. The measured response was taken as the highest Penh value achieved during the administration and observation periods.

Acute CO with airway inflammation. CO was administered through the endotracheal tube over a period of 10 min to anesthetized C57 mice in situ in the supine whole body plethysmograph, via the ventilator circuit from a tank containing 1% CO in air [10,000 ppm = 0.01 fractional gas content (fgc)]. The administration period was begun after recovery from a prior 40-μg/g MCh injection, which served to define the maximal pre-CO administration R_L response. After return of R_L to the baseline level, 1% CO was administered as described above, and the same dose of MCh was again injected after the CO exposure period. These studies were conducted in both naïve and OVA-challenged C57 mice. Simple calculations, assuming ideal gas behavior at room temperature with 1 atm of ambient pressure and the set values of tidal volume and frequency due to positive pressure ventilation, indicated administration of 114 μmol of CO during these experiments.

Acute/Repeated CO without airway inflammation. CO was administered to unanesthetized, naïve A/J mice while they were in the whole body chambers. The CO level was titrated to 500 ppm (0.05% = 0.0005 fgc) with a mixture of compressed air and 1% CO in air, measured with a sensitive monitor (Interscan). The Penh response to 50 mg/ml inhaled MCh was measured on 3 sequential days, separated by 1 day each, to reproducibly establish the baseline airway response to this concentration of MCh. Similarly, in three trials, 500 ppm CO was administered into the ambient mouse chamber mixture for an initial period of 5 min, after a prior 50-mg/ml MCh Penh assessment. After this 5-min period, the CO was continued, the 50-mg/ml MCh aerosol was simultaneously administered to the mice, and the Penh response was recorded. Thus the mice were administered 500 ppm for a total time of 10 min. This approach was followed on 2 additional days, separated by 1-day intervals, and Penh was compared across all trials. Simple calculations, as described above and using assumed normal average mouse ventilatory rate and tidal volume (163 breaths/min, 0.15 ml; Ref. 1), indicated

administration of 5 μmol of CO gas per mouse per exposure session and a cumulative administration of 15 μmol per mouse over the three CO trials, which is <1/5 that administered in the acute 1% CO experiments described above. Although there would be expected some fluctuation above and below this level with alterations in breathing pattern with MCh treatment, this value served as an approximate benchmark for comparison with other experiments.

Repeated CO with airway inflammation. CO was administered to unanesthetized, OVA-challenged A/J mice in large (3 × 2 ft), closed Plexiglas chambers for a period of 1 h/day, over 5 days. In this case, the level of CO was titrated to 250 ppm (0.025% = 0.00025 fgc) by mixture of compressed air with 1% CO in air. After the week of repeated administrations, the Penh response to 25 mg/ml inhaled MCh aerosol was measured and compared with OVA-challenged A/J mice that had not been exposed to CO. Due to the lowering of the administered CO concentration, we chose to examine a maximal dose of 25 mg/ml for this portion of the study, and also because it represents the maximal dose typically given clinically in a MCh-challenge test in humans (9). Simple calculations, as described above, indicated administration of 15 μmol of CO per mouse per 1-h exposure per day and a cumulative total of 75 μmol of CO per mouse over the 5-day period.

Inhibition of cGMP production. Naïve A/J mice were injected intraperitoneally with either 1H-[1,2,4] oxydiazolo[4,3-ea]quinoxalin-1-one (ODQ; 20 mg/kg, 3× over 1.5 wk) dissolved in dimethyl sulfoxide (DMSO) to inhibit cGMP production or an equivalent volume of DMSO as a sham vehicle control. Forty-eight hours later, airway reactivity was assessed by measurement of Penh. In another series of experiments, *Rp* diastereomer of 8-bromo-cGMP (*Rp*-8-BrcGMP), another inhibitor of cGMP production, was administered by miniosmotic pumps over a period of 10 days (0.5 mg/kg per day, Alzet) during the development of airway inflammation, and with repeated administration of CO (500 ppm, 1 h/day, 5 days; 150 μmol of total CO administered) as described above. *Rp*-8-BrcGMP was used instead of ODQ, due to the requirement of the minipumps for aqueous-based vehicle for proper function, which precluded the use of ODQ in DMSO. In these experiments, airway reactivity with the presence of airway inflammation was assessed 72 h after the final OVA provocation. Finally, a series of experiments was performed to assess the effect of acute cGMP inhibition on chronic CO-induced reductions in R_L in the presence of airway inflammation induced by OVA. In these experiments, *Rp*-8-BrcGMP dissolved in saline was infused into a jugular vein catheter between repeated assessments of AHR with 25 mg/ml inhaled MCh aerosol.

Carboxyhemoglobin. Carboxyhemoglobin (COHb) levels were measured in separate experiments that replicated the low-dose CO exposure protocols, using blood obtained from the retroorbital sinus. The mice were withdrawn from the CO exposure chamber one at a time, and specimens were obtained within 1 min of sinus puncture in heparinized Natelson tubes. Specimens were immediately placed on ice and subsequently assessed by using an OSM3 Heme-Oximeter (Radiometer, Copenhagen, Denmark). Levels were expressed as percentages of total Hb and compared across treatments.

Statistics. Statistical analysis of COHb levels was performed by independent sample *t*-tests. Changes in R_L with administration of moderate-dose CO in C57 mice were evaluated by a paired *t*-test, comparing R_L with and without CO. Changes in Penh with administration of low-dose CO (500 ppm) to A/J mice with and without inflammation were evaluated by ANOVA, with post hoc testing of discreet data by Student-Newman-Keuls test. Differences in R_L induced by low-dose CO (500 ppm) in A/J mice were assessed by two-factor ANOVA, with the main factors of airway inflammation (+OVA) and CO. Changes in Penh with 250 ppm CO were evaluated by using an independent sample *t*-test, comparing Penh between groups with and without CO. All statistical tests were performed using Sigma Stat (Jandel), in which a value of $P < 0.05$ was considered significant, at an alpha level of 0.05.

RESULTS

COHb levels with low-dose CO administration. Average total Hb levels were 14.4 ± 1.8% in the blood samples tested. Average levels of COHb in naïve mice were low (~5%), rising significantly as a function of inhaled CO concentration (Table 1). Inhalation of 250 ppm CO over 1 h resulted in an average COHb of 31%, whereas inhalation of 500 ppm CO for this same time interval yielded levels of 43%. Interestingly, inhalation of 500 ppm CO for 10 min (0.2 h) likewise resulted in high COHb levels (38%), implying that inhalation of 500 ppm CO results in achievement of a near-plateau quickly, changing only slightly over the subsequent 50 min of administration.

Effect of CO with airway inflammation. All mice with airway inflammation displayed the expected increase in R_L compared with the naïve controls (average = 15 vs. 5 $cmH_2O \cdot ml^{-1} \cdot s^{-1}$, $P < 0.05$, Fig. 1*A*). In mice with airway inflammation, acutely inhaled CO significantly reduced subsequent MCh-driven R_L by 5–10 $cmH_2O \cdot ml^{-1} \cdot s^{-1}$, whereas R_L remained elevated in mice given no CO. The magnitude and direction of these changes are shown for individuals in each treatment group, illustrating the large decrease in each mouse with airway inflammation that was administered CO (Fig. 1*B*). As shown, R_L in mice with no inflammation was not significantly altered by CO administration. These results demonstrated a significant reduction of AHR associated with airway inflammation, by an acute inhalation of CO.

Effect of brief/repeated CO in naïve mice. The airway reactivity response to 50 mg/ml inhaled MCh was stable over three sequential trials in naïve A/J mice without CO, with Penh averaging 9–10. Airway reactivity to MCh was consistently lowered by 10 min of acute CO inhalation, falling to values from 80%, down to 40%, of non-CO values, by the third sequential trial (Fig. 2*A*). With repeated CO administration (Fig. 2*B*) Penh was decreased compared with the control group, having dropped to an average of 5 by the third CO trial, achieving statistical significance compared with

Table 1. *Carboxyhemoglobin levels in mice with inhaled low-dose CO*

CO, ppm	0	250	500	500
Duration, min	0	60	10	60
COHb, %	4.9 ± 0.2	31.4 ± 0.8*	37.5 ± 1.5*†	42.5 ± 0.9*†‡

Values are means ± SE; $n = 6$ / group. COHb, carboxyhemoglobin. *$P < 0.05$ compared with 0 ppm CO; †$P < 0.05$ compared with 250 ppm, ‡$P < 0.05$ compared with 500 ppm for 10 min.

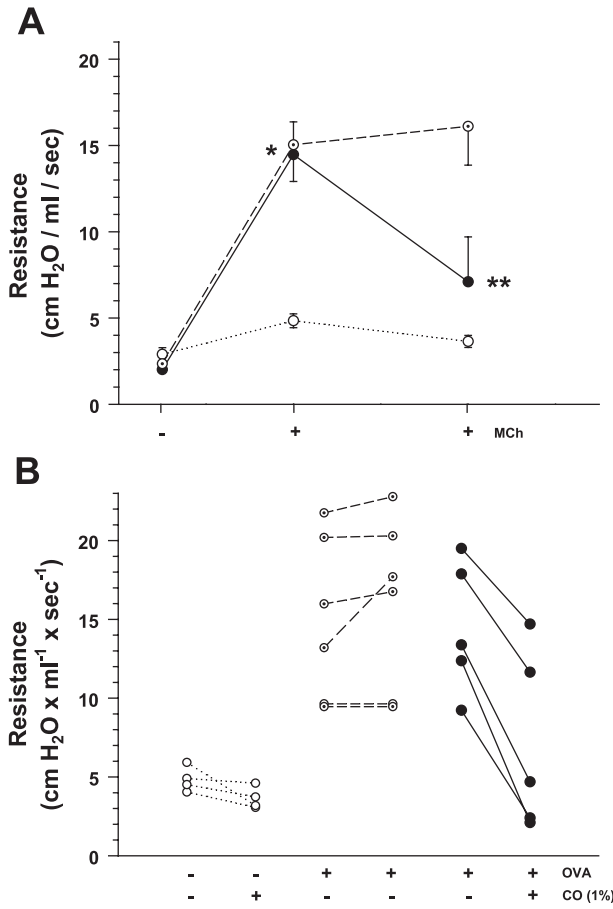

Fig. 1. *A*: lung resistance changes in C57BL/6 mice with airway inflammation [+ ovalbumin (OVA)] either with acute application of CO (1% = 10,000 ppm; ●, solid line, $n = 5$), or no CO (dotted circles, dashed line, $n = 6$), in response to methacholine (+MCh). A naïve case (○, dotted line, $n = 4$) is also shown to illustrate increased airway hyperresponsiveness with induction of airway inflammation. Resistance with sham PBS injection (−MCh) averaged 2.5 $H_2O \cdot ml^{-1} \cdot s^{-1}$ for all groups (not significant) and was not different from baseline resistance before PBS injection. *B*: individual responses to sequential applications of MCh with and without CO, indicating uniform decrements in resistance with CO applied to mice with airway inflammation (+OVA). *$P < 0.05$, for increased resistance vs. both sham PBS injection (−MCh) and naïve case +MCh; **$P < 0.05$, +OVA/+CO decreased vs. +OVA/−CO; +OVA/+CO was not significantly different from −OVA/+CO.

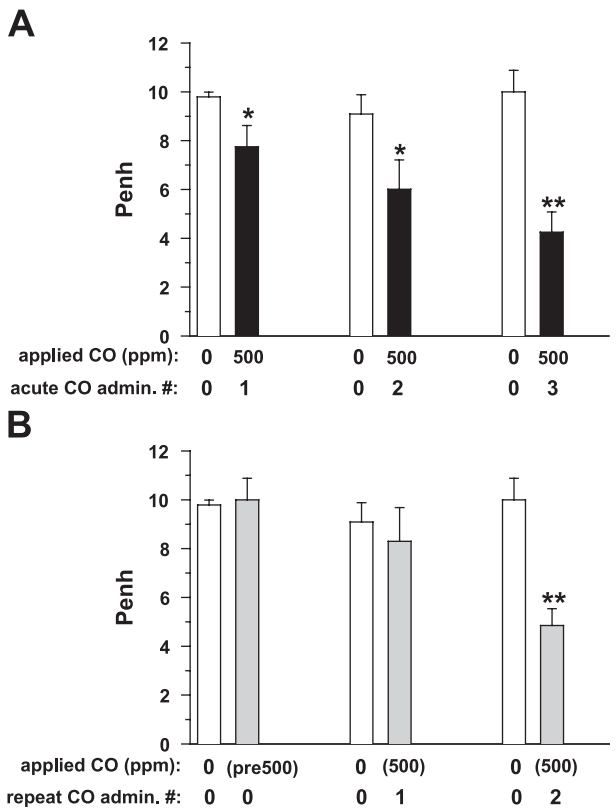

Fig. 2. *A*: airway reactivity [enhanced pause (Penh)] responses to 50 mg/ml inhaled MCh and either 0 ppm (open bars, repetition control) or 10 min of 500 ppm inhaled CO (solid bars) in A/J mice. *$P < 0.05$ compared with respective repetition control; **$P < 0.05$ acute CO administration *#3* compared with both respective repetition control and initial acute post-CO value (CO administration *#1*). *B*: airway reactivity 48 h after the prior acute 10-min 500-ppm CO inhalation, denoted by 500 in parentheses (gray bars), but before the subsequent 10-min inhalation of 500 ppm depicted by the solid bars in *A*. CO administration *#0* and pre-500 (*leftmost* gray bar) refers to response to MCh before acute CO administration *#1* in *A*. **$P < 0.05$ repeat CO administration *#2* compared with both respective repetition control and initial value (repeat CO administration *#0*); $n = 4$/bar.

non-CO values. This final level of responsiveness in the CO repetition group was not different from the final value achieved in the acute CO administration group. This finding suggests that repeated CO administration had decreased airway reactivity to the same degree as that observed with the acute administration of CO.

Effect of repeated CO with airway inflammation. In contrast to the levels of airway reactivity observed in naïve A/J mice (Penh ~10, Fig. 2), induction of enhanced AHR with airway inflammation was evident in the OVA-challenged A/J mice (Penh ~20, Fig. 3). In the OVA-challenged A/J mice, airway reactivity was significantly reduced by repeated 1-h administrations of 250 ppm CO. The magnitude of the decrease was approximately to 50% of the OVA-challenged A/J mice not exposed to CO (Penh = 19.2 ± 2.1 vs. 8.9 ± 0.2, $P <$ 0.05). These findings indicate that 250 ppm CO, administered for regular intervals over 1 wk, reduced the MCh-induced airway reactivity associated with airway inflammation.

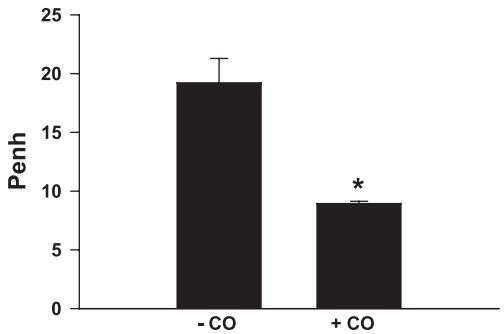

Fig. 3. Airway reactivity (Penh) after 1 wk of repeated administration of 250 ppm CO for 1 h/day in A/J mice with airway inflammation given 25 mg/ml MCh aerosol. *$P < 0.05$ +CO vs. −CO, values are mean ± SE, $n = 4$/bar.

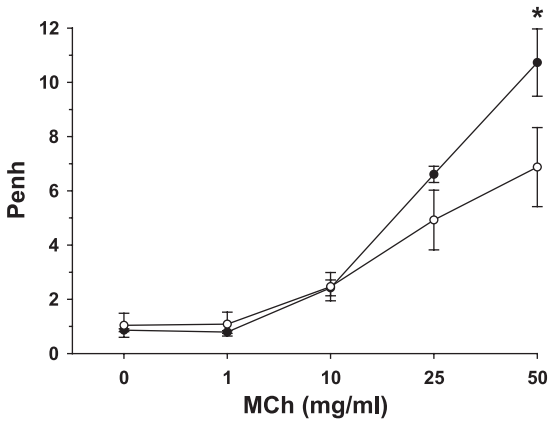

Fig. 4. Airway reactivity (Penh) in naïve A/J mice with inhibition of cGMP by 1H-[1,2,4] oxydiazolo[4,3-a]quinoxalin-1-one (●). Dimethyl sulfoxide (○) vehicle control serves as reference control. *$P < 0.05$ vs. control; $n = 4$/group.

Effect of inhibition of cGMP. In naïve A/J mice, cGMP inhibition via administration of ODQ resulted in an increase in airway reactivity vs. vehicle controls (Fig. 4), suggesting an association between cGMP and baseline AHR in this strain. Furthermore, chronic administration of the inactive cGMP analog *Rp*-8-BrcGMP during the development of airway inflammation and repeated treatment with CO resulted in increased airway reactivity (Fig. 5), suggestive of a cGMP-dependent effect of CO on AHR. Although repeated inhalation of CO was effective at reducing airway reactivity in A/J mice with airway inflammation (Fig. 3), we found that acutely administered *Rp*-8-BrcGMP was ineffective at negating the effect of repeated CO administration ($R_L = 6.3 \pm 1.4$ vs. 6.4 ± 0.4 $cmH_2O \cdot ml^{-1} \cdot s^{-1}$; $n = 6$/group; data not shown), suggesting these longer term effects were not ameliorated by interfering with the effects of cGMP.

DISCUSSION

The main findings of this study are as follows: *1*) acutely inhaled moderate levels of CO (1%) reduced AHR in the presence of airway inflammation, *2*) acutely inhaled low levels of CO (0.05%) reduced AHR in naïve hyperresponder mice (A/J), *3*) repeated brief periods (10 min) of inhalation of CO reduced AHR of naïve hyperresponder mice, *4*) repeated administration of very low levels of CO (0.025%) over a 1-wk period decreased the AHR in the presence of airway inflammation, and *5*) the influence of low-dose CO on AHR was associated with a cGMP mechanism both in the presence and absence of airway inflammation. These findings suggest that low levels of inhaled CO can significantly decrease AHR and that this effect is modulated through cGMP.

Exogenous application of CO. Our acute CO studies in situ (Fig. 1) were performed as competitive, pharmacological, near-maximal cholinergically driven airway constriction and dilation experiments in the presence and absence of airway inflammation. They are consistent with prior studies in naïve guinea pigs, in which pure CO gas (1,000,000 ppm = 1.0 fgc), given with 7–30 ventilator breaths (0.7, 1.4, 2.8 ml of pure CO), decreased histamine-induced bronchoconstriction (4, 5). We calculated that the total amount of CO administered at the highest level in that study was 157 μmol, which is of similar magnitude but numerically greater than the amount of CO we delivered in our acute moderate-dose (1% CO) experiments (114 μmol) and similar to the cumulative total we administered in chronic low-dose experiments over 5 days (500 ppm, 150 μmol). We have extended these findings to demonstrate a basis for the airway dilatory effect of inhaled CO in reversing the effect of a strong cholinergic bronchconstrictor stimulus, in a setting known to potentiate AHR, i.e., airway inflammation. These findings led us to reduce the concentration of inhaled CO, to determine whether low-dose CO would retain these properties when tested in A/J mice, known for AHR and a strong airway inflammatory response.

Administration of low concentrations of CO in humans has been performed for many years, in the form of clinical diffusing capacity studies to assess lung function (17). Typically, 0.3% (3,000 ppm) CO is utilized in single-breath diffusing capacity studies, resulting in administration of ~10 ml of CO (8), or 413 μmol, assuming ideal gas behavior at room temperature with 1 atm of ambient pressure. In our initial experiments (Figs. 2 and 3), repeated administration of 250 and 500 ppm CO resulted in calculated cumulative administration of 15 and 75 μmol of CO over 5 days, respectively, which is much less than the maximum typically used in one diffusing capacity test administration. Its application was well tolerated, with no outward ill effects or lethality in any of the mice tested. These experiments indicate that CO could be administered at low, sublethal levels that had no outwardly untoward effects but retained physiological effects on AHR.

Specifically, these experiments showed two important effects of low-dose CO on AHR. One was that low

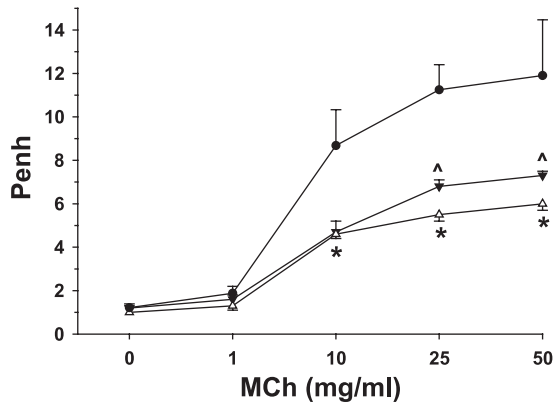

Fig. 5. Airway reactivity (Penh) with simultaneous chronic administration of inactive cGMP analog *Rp* diastereomer of 8-bromo-cGMP (▼) and repeated CO administrations (500 ppm, 1 h/day, 5 days), in the presence of airway inflammation, compared with CO-treated saline vehicle control (△) and non-CO-treated controls (●). ^$P < 0.05$ vs. vehicle control, and *$P < 0.05$ vs. non-CO-treated controls at corresponding MCh concentrations, $n = 6$/group.

concentrations of CO had a measurable effect of AHR reduction in naïve hyperresponsive A/J mice. The other was that, in this same strain of mice, low levels of administered CO also could reduce AHR in the setting of airway inflammation, which typically potentiates, or occurs concurrently with, AHR. These effects were observed with both chronic and acute CO administration schemes and may have relevance toward a potential bronchoprotective mechanism of low-dose CO effects on AHR. We did not measure whether indexes of airway inflammation were reduced in our mice due to the difficulty of performing lavage on lungs previously exposed to MCh. However, a prior study by Chapman et al. (7) has suggested that inhaled CO (250 ppm) reduces airway inflammation in BALB/c mice and, therefore, may represent a mechanism through which repeated low-dose CO inhalation can modulate AHR. In summary, low-dose CO reduced AHR, suggesting its potential role as a gaseous molecule that can modulate AHR, both through direct, acute mechanisms and through mechanisms that may be associated with downregulation of airway inflammation.

CO as a modulator of AHR. Similar to NO, CO can act as a neurotransmitter in carotid body chemoreception (20) and in parasympathetic ganglia of both human and guinea pig airways (3). Therefore, there is a potential for CO to modulate AHR indirectly through effects on respiratory neurons and perhaps directly through influence on airway smooth muscle. One mechanism through which CO may regulate airway smooth muscle contractility is through its effects on cGMP. Studies conducted in guinea pigs have shown a link between bronchodilation, neurotransmission, and CO release by airway smooth muscle (12). It has been reported that the effects of adenylate cyclase activating peptides, which induce bronchodilation in guinea pigs, occur through a mechanism involving CO (12, 21). Interestingly, this effect has been reported to occur through elevations of cGMP and can be abolished through application of guanylyl cyclase inhibitors (12, 21). Our experiments confirm and extend these findings to lower concentrations of CO (250–500 ppm) for the case of mice and specifically in the presence and absence of airway inflammation. In the absence of inflammation, inhibition of cGMP in naïve hyperresponder A/J mice was effective in reversing the effect of low-dose CO, particularly at the higher levels of MCh stimulation (Fig. 4). Acute application of the inactive cGMP analog did not alter the longer term effect of repeated low-dose CO in reducing AHR, suggesting that modifications had occurred over time that were not easily reversible. However, concomitant inhibition of cGMP during repeated low-dose CO administration did show modulation of the CO effect on AHR, in that airway reactivity was increased with inhibition of cGMP (Fig. 5). Furthermore, it is important to note that these effects are likely to be independent of the nonadrenergic, noncholinergic neural system, as applications of NO inhibitors have been without effect on these CO-mediated responses (5, 12, 21). Thus it appears that there may exist a distinct mechanistic pathway for modulation of AHR by CO; however, further studies are necessary to determine the relationship of these mechanisms through which CO has its effects on AHR.

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DISCLOSURES

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