Inhaled carbon monoxide does not cause pulmonary vasodilation in the late-gestation fetal lamb

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Inhaled carbon monoxide does not cause pulmonary vasodilation in the late-gestation fetal lamb. Am J Physiol Lung Cell Mol Physiol 278: L779–L784, 2000.—As observed with nitric oxide (NO), carbon monoxide (CO) binds and may activate soluble guanylate cyclase and increase cGMP levels in smooth muscle cells in vitro. Because inhaled NO causes potent and sustained pulmonary vasodilation, we hypothesized that inhaled CO may have similar effects on the perinatal lung. To determine whether CO can lower pulmonary vascular resistance (PVR) during the perinatal period, we studied the effects of CO on late-gestation fetal lambs. Catheters were placed in the main pulmonary artery, left pulmonary artery (LPA), aorta, and left atrium to measure pressure. An ultrasonic flow transducer was placed on the LPA to measure blood flow to the left lung. After baseline measurements, fetal lambs were mechanically ventilated with a hypoxic gas mixture (inspired O2 fraction < 0.10) to maintain a constant fetal arterial P02. After 60 min (baseline), the lambs were treated with CO (5–2,500 parts/million (ppm)). Comparisons were made with NO (5 and 20 ppm) and combined NO and CO (100 and 2,500 ppm). We found that CO did not alter left lung blood flow or PVR at any of the study doses. In contrast, low-dose NO increased PVR by 47% (P < 0.005). The combination of NO and CO did not enhance the vasodilator response to NO. To determine whether endogenous CO contributes to vascular tone in the fetal lung, zinc protoporphyrin IX, an inhibitor of heme oxygenase, was infused into the LPA in three lambs. Zinc protoporphyrin IX had no effect on baseline PVR, aortic pressure, or the pressure gradient across the ductus arteriosus. We conclude that CO does not cause vasodilation in the near-term ovine transitional circulation, and endogenous CO does not contribute significantly to baseline pulmonary vascular tone or ductus arteriosus tone in the late-gestation ovine fetus.

Recent studies (24, 26, 33) have suggested that carbon monoxide (CO) may play an important role in the regulation of vascular tone. Similar to nitric oxide (NO), CO is an endogenously produced gas molecule that inhibits platelet aggregation (25) and relaxes isolated blood vessels in vitro (33). Both NO and CO avidly bind the heme moiety of soluble guanylate cyclase (sGC). NO activates sGC after binding to the heme moiety of sGC, converting GTP to cGMP and causing vasodilation. CO also binds in the heme moiety of sGC, and in vitro and in vivo studies (3, 30, 33) have demonstrated the ability of CO to activate sGC. This activation of sGC by CO increases intracellular levels of cGMP in vascular smooth muscle cells and isolated vessels (7, 27, 28) and has been demonstrated to cause vasorelaxation in experimental settings (13, 33, 35). However, the affinity of CO for the heme moiety of guanylate cyclase is lower than that of NO (30, 33). In addition, some investigators (4) have reported that despite CO binding to sGC, no production of cGMP was seen. The direct effects of CO on the pulmonary circulation in vivo have not been studied.

CO is produced by the degradation of heme by heme oxygenase (HO) (32). HO is a ubiquitous protein that exists in two isoforms: HO-1, an inducible form, and HO-2, a constitutive form (23). HO-2 is the predominant isof orm present in endothelial cells, smooth muscle cells, connective tissue, and epithelial cells (15, 24, 29). HO-1 is also present in endothelial cells (11) and is upregulated by many stimuli including shear stress, hypoxia, hyperoxia, metals, and heat shock (6, 20, 21). In addition, putative inhibitors of HO, including zinc protoporphyrin IX and tin protoporphyrin IX, are capable of producing a pressor effect in vivo, causing a rise in systemic blood pressure and peripheral vascular resistance (5, 12, 17). Whether CO contributes to pulmonary vasoregulation in the developing fetus is unknown.

Recent studies (9, 11) have also demonstrated that HO activity can produce CO in the ductus arteriosus (DA) and that CO may mediate vascular tone in the DA under certain conditions. Several other stimuli have been implicated in the direct regulation of DA tone, including PGE2, NO, and endothelin (ET)-1 (8), although CO does not appear to affect cyclooxygenase activity. Whether CO interacts with these other mediators of vascular tone to maintain basal tone in the DA is unknown. In addition, the effects of inhibition of CO production on DA tone in vivo have not been studied.

Therefore, we hypothesized that exogenous CO would cause potent and sustained pulmonary vasodilation in the developing lung as observed with inhaled NO (1, 19). We further hypothesized that blockade of endog-
enous CO production in the lung with an inhibitor of HO, zinc protoporphyrin IX (36), would increase basal pulmonary hemodynamics and constrict the DA. To test these hypotheses, we performed a series of experiments on the effects of inhaled CO (ICO) and zinc protoporphyrin IX in late-gestation fetal lambs.

METHODS

Surgical Preparation

All procedures were reviewed and approved by the Animal Care and Use Committee at University of Colorado Health Sciences Center (Denver, CO). Eight mixed-breed (Columbia-Rambouillet) pregnant ewes between 138 and 144 days of gestation (term 147 days; protocol 1) or between 128 and 135 days of gestation (protocol 2) were fasted for 24 h before surgery. Ewes were sedated with intravenous pentobarbital chloride (3 mg) by lumbar puncture. Ewes were kept sedated but breathed spontaneously throughout surgery. Under sterile conditions, the left forelimb of the fetal lamb was delivered through a uterine incision. A skin incision was made under the left forelimb after local infiltration with 1% lidocaine. Polyvinyl catheters were inserted into the axillary artery and advanced into the ascending aorta (Ao). A left axillary to sternal thoracotomy exposed the heart and great arteries. Polyvinyl catheters were inserted into the left pulmonary artery (LPA), main pulmonary artery (MPA), and left atrium (LA) by direct puncture and secured into position with purse-string sutures. The LPA catheter was placed immediately proximal to the DA. Because most of the right ventricular output crosses the DA from the MPA, the MPA catheter delivers the drug directly to the DA. A 6-mm ultrasonic flow transducer (Transonic Systems, Ithaca, NY) was placed around the LPA to measure blood flow to the left lung (Qp).

Physiological Measurements

The Ao, MPA, and LA catheters were connected to a computer-driven pressure transducer and recorder (Biopac Systems, Santa Barbara, CA). Pressures were referenced to LA pressures. The pressure transducer was calibrated with a mercury manometer. The flow transducer cable was attached to an internally calibrated flowmeter (Transonic Systems) for continuous measurements of LPA flow. The absolute values of flow were determined from phasic blood flow signals as previously described (16, 22). Pulmonary vascular resistance (PVR) in the left lung was calculated with the following equation: $PVR = \frac{mean \text{ MPA pressure} - mean \text{ LA pressure}}{LPA \text{ flow}}$. Arterial blood gas tensions, pH, hemoglobin, oxygen saturation, carboxyhemoglobin, methemoglobin, and oxygen content were measured from blood samples that were drawn from the Ao catheter and measured at 39.5°C with a blood gas analyzer and hemoximeter (model OSM-3, Radiometer, Copenhagen, Denmark).

Study Drugs and Gases

The NO gas (BOC Group, Murray Hill, NJ) used in these experiments was certified at a concentration of 409 parts/ million (ppm) NO (chemiluminescence method), with <1% contamination by other oxides of nitrogen. The ICO and N02 were measured with an electrochemical sensor calibrated against a reference NO tank. The CO gas (Spectra Gases, Alpha, NJ) was certified at a concentration of 0.5% (5,000 ppm), with the balance nitrogen. Concentrations of ICO were measured with an electrochemical sensor (Biosystems, Middletown, CT) calibrated against the CO tank.

Zinc protoporphyrin IX (Sigma, St. Louis, MO) solutions were prepared in subdued light by dissolving a known amount (either 2.5 or 15 mg) of metalloporphyrin in 1 ml of 0.2 M NaOH until completely dissolved (2, 18). Four milliliters of sterile normal saline were added for a final volume of 5 ml, and the solution was adjusted to a final pH of 9.0 with the dropwise addition of 0.1 N HCl. The required amount of solution was drawn up into a syringe and stored at room temperature in subdued light until the time of study. All solutions were made immediately before the start of the study, and catheters and syringes were light protected during the course of the study. The drug was infused intravenously with an infusion pump set to deliver 5 ml over a 30-min period.

Experimental Design

Protocol 1: Effects of ICO on the transitional ovine pulmonary circulation. After stabilization of physiological parameters, pancuronium (0.1 mg/kg) was administered to the fetus, the fetal head was exteriorized, and a tracheotomy was performed for placement of an endotracheal tube (4.5-mm internal diameter). Mechanical ventilation was initiated with a continuous-flow, time-cycled, pressure-limited neonatal ventilator at the following settings: positive inspiratory pressure of 28 cmH2O; positive end-expiratory pressure of 4 cmH2O; respiratory rate of 20 breaths/min; inspiratory time of 1.0 s; and inspired O2 fraction < 0.10. The mechanical ventilator settings were modified during the course of the studies based on the results of aortic blood gas samples. The management strategy was guided by target arterial PCO2 and PO2 values.

After baseline measurements were obtained, animals received ICO and INO via an endotracheal tube. Fetal lambs were exposed to ICO at 5, 100, 500, and 2,500 ppm, to INO at 5 and 20 ppm, and to combined INO at 5 ppm and ICO at 100 and 2,500 ppm. Each treatment period was 10 min. The order of gas administration was randomized. Two animals were first exposed to ICO and then combined gases; and the fifth animal received combined ICO and INO separately. After exposure to study gases, the animals were exposed to 100% O2 for a 10-min period. Hemodynamic measurements and arterial blood gas samples were recorded every 10 min throughout the entire study.

At the conclusion of the study, the animals were killed by administration of a lethal dose of a euthanasia solution (pentobarbital sodium; Fort Dodge Laboratories, Fort Dodge, IA).

Protocol 2: Hemodynamic effects of zinc protoporphyrin IX treatment in the late-gestation ovine fetus. Animals were studied after a 48-h recovery period after surgery. Baseline hemodynamic measurements were recorded for Qp, MPA pressure, aortic pressure (AoP), LA pressure, and heart rate. After baseline measurements were stable for a 30-min period, normal saline (5 ml) was infused over 30 min to study the effect of vehicle alone on hemodynamics. After the control infusion, zinc protoporphyrin IX (2.5 mg, 13 µmol/kg) was infused into the LPA over 30 min to study the effects on pulmonary hemodynamics. Hemodynamic measurements were recorded for an additional 30 min after the infusion period ended. Zinc protoporphyrin IX (15 mg) was infused over 1 min directly into the MPA proximal to the DA to better study the effects on DA tone. Hemodynamic measurements were recorded for an additional 60 min.
Data Analysis

Statistical analysis of the hemodynamic data was performed by one-way analysis of variance. Where significant differences were identified, post hoc analysis was performed with Student-Newman-Keuls test. All statistical measurements were performed with a commercially available statistics package (StatMost, DataMost, Salt Lake City, UT). The level of significance was set at $P < 0.05$. The results are reported as means $\pm$ SE.

RESULTS

Protocol 1: Effects of Exogenous CO on Pulmonary Hemodynamics

$ICO$ did not affect pulmonary vascular tone at any of the study doses (5, 100, 500, and 2,500 ppm). In particular, PVR and $Q_p$ did not change during exposure to $ICO$ (Figs. 1 and 2). Although MPA pressure and AoP decreased during exposure to $ICO$ at 2,500 ppm, there was no effect on these parameters at lower doses (Fig. 2; Table 1). Arterial blood gas tensions revealed no change in pH or arterial PO$_2$ values after treatment with $INO$, $ICO$, or the combined gases. Adequate exposure to $ICO$ was demonstrated by an increase in carboxyhemoglobin levels after each treatment dose of $ICO$ (Table 1).

In contrast, exposure to $INO$ at 5 and 20 ppm caused a marked fall in PVR and MPA pressure (Figs. 1 and 2). The combination of $INO$ and $ICO$ also lowered PVR, but there was no difference in the response to combined treatment compared with $INO$ alone (Fig. 1). Treatment with 100% O$_2$ at the conclusion of the study showed no further increase in $Q_p$.

Fig. 1. Effect of exposure to inhaled carbon monoxide ($ICO$), inhaled nitric oxide ($INO$), and $INO + ICO$ on pulmonary vascular resistance (PVR) in ovine fetal circulation. Nos. on x-axis, concentration in parts/million. BL, baseline; N, nitric oxide; C, carbon monoxide. Although $INO$ caused a marked decrease in PVR, $ICO$ treatment had no effect. $^*P < 0.05$ vs. BL.

Fig. 2. Effect of treatment with $ICO$, $INO$, and $INO + ICO$ on main pulmonary arterial pressure (PAP; A) and left pulmonary artery (LPA) blood flow (B) in ovine fetal circulation. Nos. on x-axes, concentration in parts/million. Values are means $\pm$ SE. $^*P < 0.05$ vs. BL.
Protocol 2: Effects of Zinc Protoporphyrin IX on Pulmonary Hemodynamics

Infusion of zinc protoporphyrin IX into the LPA or MPA did not alter MPA pressure, Qp, or PVR from baseline measurements (Fig. 3, Table 2). Systemic blood pressure (AoP) was also unchanged after metalloporphyrin administration.

Despite infusion of relatively high doses of zinc protoporphyrin IX (15 mg) directly into the MPA, there was no change in the pressure gradient between the MPA and Ao. These findings suggest that zinc protoporphyrin IX had no apparent effect on tone of the DA in study animals.

DISCUSSION

We found that administration of exogenous CO (Ico) did not cause pulmonary vasodilation in the transitional ovine circulation. Ico did not alter PVR at any of the study doses ranging from 5 to 2,500 ppm. These findings are in contrast to the marked fall in PVR after exposure to low-dose INO, which has been reported in previous studies of this animal model (1, 19). Combined treatment with Ico did not enhance the vasodilator response to INO. In addition, we also report that treatment with a HO inhibitor, zinc protoporphyrin IX, did not affect pulmonary or systemic hemodynamics in the fetal lamb. There was no apparent effect on tone of the DA even after infusion of high doses of zinc protoporphyrin IX directly into the MPA. These findings suggest that endogenous CO production plays little role in the control of basal tone of the pulmonary circulation or DA.

Based on extensive in vitro studies (4, 11, 17, 26), there is increasing interest in the potential role of CO as a regulator of vascular tone. However, this is the first in vivo study to directly examine the effects of Ico on the vascular tone of the intact perinatal lung. We found no effect of Ico on pulmonary vascular tone. Endogenous production of CO has also been suggested as a potential modulator of tone in the DA (8), but we were unable to find a significant response during treatment with zinc protoporphyrin IX.

Previous studies (3, 7, 27, 28, 30, 33) have demonstrated that CO can stimulate guanylate cyclase and increase cGMP under a variety of conditions. In vitro studies have demonstrated that endogenous CO causes a time-dependent increase in cGMP levels in cultured vascular smooth muscle cells (28) and that this increase in cGMP is blocked in the presence of the HO inhibitor tin protoporphyrin IX (27). In addition, CO has been shown to cause vasodilation of isolated vessel preparations of femoral arteries, carotid arteries, coronary arteries, and cerebral arteries (13, 35), although this vasorelaxation is less potent than that seen with NO. These findings are consistent with a study (31).

Table 2. Hemodynamic and arterial blood gas values for lambs treated with zinc protoporphyrin IX

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Zinc Protoporphyrin IX</th>
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<tbody>
<tr>
<td></td>
<td>2.5 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>Qp, ml/min</td>
<td>57 ± 6.1</td>
<td>59 ± 7.7</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>45 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>43 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>PAP-AoP gradient</td>
<td>2.3 ± 1.0</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>161 ± 5.8</td>
<td>160 ± 2.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.33 ± 0.04</td>
</tr>
<tr>
<td>PaO2, Torr</td>
<td>22.0 ± 1.0</td>
<td>34.3 ± 14</td>
</tr>
<tr>
<td>PaCO2, Torr</td>
<td>43.5 ± 0.5</td>
<td>430 ± 5.3</td>
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Values are means ± SE. *P < 0.05 vs. baseline.
showing that a high concentration of CO (11.5%) in the isolated lung model causes pulmonary vasodilation and reduces hypoxic vasoconstriction (31). Other authors (4), however, found that although CO did bind to the heme moiety of sGC, it failed to activate sGC in vitro. In addition, a recent study (38) showed that HO-1-null mice failed to show evidence of more severe pulmonary vascular remodeling or pulmonary hypertension after exposure to chronic hypoxia. Our present study failed to demonstrate that exogenous CO causes pulmonary vasodilation in the perinatal lung despite administration of high doses directly into the lung, which is consistent with these in vitro and in vivo observations (4, 38).

CO has a lower affinity for heme than for NO and causes less potent activation of sGC (24, 33). CO can also bind to NO synthase, which may directly inactivate and inhibit transcription of NO synthase (24, 37). There are complex interactions between HO, CO, and other known mediators of vascular tone such as ET-1 and PGE$_2$, which may also alter the expression and function of CO.

One of the potential limitations of this study is that we examined the effect of CO only under normal conditions in the perinatal lung. It is unknown what effect CO may have on vascular tone under stress conditions such as hypoxia or in conditions where NO is known to be downregulated as in some models of pulmonary hypertension. Another possible limitation is that the highest dose of CO studied (2,500 ppm) may have produced toxic effects, causing alteration in cardiac performance. However, the order of gas administration was randomly alternated so that those animals exposed first to ICO still showed an intact vasodilatory response to I$_{NO}$.

We also report that zinc protoporphyrin IX had no effect on basal pulmonary vascular or DA tone, suggesting that endogenous CO production does not modulate basal pulmonary hemodynamics in the fetal lung. However, there are some limitations in this study. Because we cannot directly measure CO production in vivo, we are uncertain whether we adequately achieved CO blockade with the doses of metalloporphyrin studied. However, in comparison with other published studies (2, 18, 34), the doses in our study were relatively high and the concentrations of drug achieved in the LPA were particularly higher because the drug was infused directly into the left lung, which has a low basal flow rate.

A previous study (9) has also examined the role of endogenous CO in maintaining the patency of the DA in the fetus. There is evidence to support the role of several mediators of vascular tone in the DA, including NO, PGE$_2$, and ET-1 (8). In some animal models, infusion of indomethacin, a cyclooxygenase inhibitor, into the DA caused a rapid and dramatic increase in the MPA-Ao pressure gradient, consistent with DA closure (14). Because CO acts in a similar fashion to NO, it has been suggested that CO helps to maintain ductal tone in the fetus through vasodilatory properties (8, 26). An in vitro study (11) has demonstrated that the HO inhibitor zinc protoporphyrin IX had no effect on the tone of the DA under basal conditions but did cause ductal constriction after pretreatment with endotoxin. In addition, studies have shown that HO and CO are present in the DA (11) and that exogenous CO can cause potent relaxation of the isolated DA in the lamb (9). However, this relaxation of the DA is maintained in the presence of guanylate cyclase inhibitors, and the degree of relaxation does not correlate with tissue levels of cGMP (10). Past work has also demonstrated that although a nonselective inhibitor of NO synthesis caused minimal increases in DA tone, inhibition of sGC did increase tone (14). It is likely that non-NO-dependent stimulation of sGC contributes to DA patency, possibly through the production of CO (14), but we saw no effect with zinc protoporphyrin IX in the normal fetus.

We conclude that administration of exogenous CO does not cause pulmonary vasodilation in the near-term ovine circulation and does not alter the pulmonary vasodilatory response to I$_{NO}$. In addition, endogenous CO does not appear to contribute to baseline pulmonary hemodynamics or to the maintenance of patency of the DA in the late-gestation fetus.

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