Carbon Monoxide as an Attenuator of Vasoconstriction in Piglet Cerebral Arterioles[1]

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Carbon monoxide (CO) is an endogenous dilator in the newborn cerebral circulation. The present study addressed the hypothesis that endogenous CO attenuates pial arteriolar vasoconstriction caused by hypocapnia, platelet activating factor, and elevated blood pressure. Experiments used anesthetized piglets with implanted, closed cranial windows. Topical application of a metal porphyrin inhibitor of heme oxygenase was used to inhibit production of CO. Chromium mesopophyrin increased vasoconstriction in response to hypocapnia. The constrictor response to a topical stimulus, platelet activating factor, was also increased by application of chromium mesoporphyrin. Inhibition of heme oxygenase did not constrict pial arterioles in normotensive newborn pigs (mean arterial pressure of about 70 mmHg), but did constrict pial arterioles of piglets with experimentally induced increases in arterial pressure (mean arterial pressure greater than 90 mmHg). In fact, pial arterioles of normotensive piglets transiently dilated to chromium mesoporphyrin, whereas those of hypertensive piglets progressively constricted during 10 min of chromium mesoporphyrin treatment. Therefore, inhibition of heme oxygenase augments cerebral vasoconstriction in response to several very different constrictor stimuli. These data suggest endogenous CO attenuates vasoconstrictor responses in the newborn cerebral circulation. Exp Biol Med 228:46–50, 2003

Key words: cerebrovascular circulation; heme oxygenase; microvascular; hypocapnia; platelet activating factor; hypertension

Carbon monoxide (CO) is produced physiologically by the heme oxygenase (HO) catalyzed breakdown of heme, which produces biliverdin, free iron, and CO (1). HO is present in vascular endothelium, vascular smooth muscle, and brain parenchyma (1, 2). There are three isoforms of HO that are products of separate genes: HO-1, HO-2, and HO-3 (1–5). HO-3 has less activity than HO-1 and HO-2 and has been studied little. Various stimuli induce HO-1 expression, among them cAMP and hypoxia (1, 2, 6–9). HO-2 is constitutively expressed and so far, its expression has been found to be regulated only by steroids (1, 10). Maximal levels of HO-2 are in the brain (1, 2).

CO is a potent vasodilator. Endogenous CO can be produced in high enough concentrations to cause vasodilation (3, 11–13). CO may increase cGMP inside the cell (3, 13, 14). Also, CO activates calcium-activated potassium channels (K_{Ca}), which hyperpolarizes and consequently relaxes vascular smooth muscle (13, 15, 16). CO may thus join other autocrine and paracrine dilators, such as NO and prostaglandins, in control of cerebral vascular tone. Furthermore, there are clearly interactions among the three (17–19).

CO could be particularly important in the perinatal period. Sex steroids upregulate HO. The HO-1 and HO-2 isoforms are present in pregnant myometrium in 15 times greater concentrations than in the nonpregnant uterus (20). CO appears to be important in control of the fetal vasculature, with a potential contribution of endogenously produced CO to ductus arteriosus patency (21). Finally, levels of HO are far greater in the mature than the immature fetus (22). In short, the developmental level of the animal, the location of HO, and the function of HO and its products point to CO as a potential critical regulator of vascular tone in the brain of mammalian neonates.

As an endogenous dilator influence in the cerebral circulation, CO likely not only mediates dilation in response to appropriate stimuli, but also may attenuate vasoconstriction as in the renal vasculature (23). In the present study, we address the hypothesis that endogenously produced CO reduces cerebral arteriolar constriction to hypocapnia, platelet activating factor (PAF), and elevated blood pressure in newborn pigs. These three constrictor stimuli were selected because all could be involved in cerebral vasoconstriction in the neonatal period. Hypocapnia can be produced intentionally in attempts to dilate constricted pulmonary arteries and unintentionally when resistance and compliance changes occur in ventilated neonates. PAF may be elevated in po-

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tentially injurious situations, particularly intracranial hemorrhage. Finally, the relative instability of blood pressure in neonatal life can result in transient declines and elevations of blood pressure. Thus, these three constrictor stimuli can be part of the etiology of newborn cerebral vasoconstriction and possible ischemic injury.

Material and Methods

All procedures that involve animals were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Closed cranial windows were implanted in anesthetized piglets as described before (19, 24–26). Briefly, newborn piglets (1–3 days old, 1–3.0 kg) were anesthetized with ketamine hydrochloride (33mg/kg i.m.) and acepromazine (3.3 mg/kg i.m.), and anesthesia was maintained with a-chloralose (50 mg/kg i.v., initially, supplemented with 2 mg/kg each hour). The animals were intubated and mechanically ventilated. Catheters were inserted into the femoral vein for maintenance of anesthesia and drug injections and into the femoral artery to record blood pressure and draw samples for blood gases and pH analysis. Blood gases were controlled via mechanical ventilation. P_aCO_2 was held constant around 30 mmHg. Supplemental O_2 was administered in the normotension versus hypertension experiments, but not in the other protocols. Arterial blood pressures, gases, and pH of normally ventilated and hyperventilated piglets before and during chromium mesoporphyrin (CrMP) treatment are shown in Table I. Arterial blood pressures, gases, and pH of piglets in the normotensive and hypertensive groups are shown in Table II. Body temperature was maintained at 37°–38°C using a thermostatically controlled heating pad connected to a rectal probe.

The scalp was retracted, and a 2-cm diameter hole was made in the skull over the parietal cortex. The dura was cut without touching the brain and all cut edges were retracted over the bone so that the periarachnoid space was not exposed to bone or damaged membranes. A stainless steel-and-glass cranial window was placed in the hole and was cemented into place with dental acrylic. The space under the window was filled with artificial cerebrospinal fluid (aCSF; in milligrams per liter: 220 KCl, 1132 $MgCl_2$, 221 $CaCl_2$, 7710 NaCl, 401 urea, 665 dextrose, and 2066 $NaHCO_3$). aCSF was warmed in a water bath to 37°C and was bubbled with a gas mixture of 6% CO_2, 6% O_2, and 88% N_2 to maintain approximate values of pH 7.33, PCO_2 45 mmHg, and PO_2 45 mmHg. aCSF was injected via a syringe port on the side of the window and was allowed to drip freely from the opposite port during the injection. Pial arterioles were observed with a dissecting microscope. Diameters were measured with a video micrometer that was coupled to the microscope, a television camera, and a video monitor. Data from one pial arteriole are reported for each piglet.

Experiments. Three different constrictor stimuli were used: hypocapnia, PAF, and arterial hypertension. Endogenous CO production was inhibited with topical application of CrMP (1.5×10^{-6} *M*) (Porphyrin Products, Logan, UT). Previously, we showed that CrMP blocks cerebral vasodilation to HO substrate (heme-L-lysinate) without affecting dilation to CO, indicating effective blockade of HO (25). Light-degraded CrMP has no effect on any responses, including dilation to the HO substrate, heme-L-lysinate.

Hypocapnia. Piglets were hyperventilated to arterial PCO_2's of about 20 mmHg by increasing the rate of ventilation to approximately 20 breaths per minute and by increasing the peak inspiratory pressure to 20 mmHg from the normal settings of about 14 breaths per minute and 10 mmHg. Identical ventilator settings were used on each piglet before and after HO inhibition and produced virtually identical $PaCO_2$ (Table I). Mean arterial pressure (MAP) and pial arteriole diameter (PAD) were measured at baseline, and at 1, 5, and 10 min. The maximal constriction that is typically attained and sustained from 5 to 10 min was recorded.

PAF. The PAF dose was selected to produce a small, consistent, readily reversible constriction. PAF (10^{-11} *M*) was applied topically, and PADs were recorded at 0, 1, 5, and 10 min. PAF was then removed and the arterioles allowed to return to their baseline levels.

Inhibition of HO. CrMP was used to inhibit HO. CrMP (1.5×10^{-6} *M*) was allowed to sit under the window for 20 min in darkness before resuming experimentation. During use of CrMP, the room lights were out and microscope lights were only turned on for quick (about 5–10 sec) measurements of PAD at 1, 3, 5, and 10 min. The inhibitor was maintained for the duration of experimentation. Constrictions to hypocapnia or PAF were compared in the absence and presence of CrMP. The same arteriole served as control and HO inhibited in each experiment.

The dilation to topical isoproterenol (10^{-6} *M*) was measured at the beginning and end of each experimental preparation to assure the reactivity in general had not changed. For example, in hypocapnia experiments, pial arterioles dilated from 63 ± 3 to 83 ± 5 m prior to CrMP, and the same

Table I. Arterial Blood Pressure, Gases, and pH of Piglets

	ABP (mmHg)	pH	P_{CO2} (mmHg)	P_{O2} (mmHg)
Control	68 ± 5	7.30 ± .03	31 ± 2	89 ± 5
Hyperventilation	62 ± 5	7.53 ± .02	20 ± 1	101 ± 11
CrMP	61 ± 6	7.29 ± .04	35 ± 3	83 ± 10
CrMP-hyperventilation	63 ± 6	7.53 ± .03	21 ± 1	109 ± 12

Table II. Arterial Blood Pressure, Gases, and pH of Piglets

	ABP (mmHg)	pH	P_{CO2} (mmHg)	P_{02} (mmHg)
Normotensive	72 ± 4	7.28 ± .06	31 ± 1	166 ± 527
CrMP-normotensive	69 ± 5	7.30 ± .04	28 ± 2	179 ± 29
Hypertensive	95 ± 1	7.40 ± .04	30 ± 3	169 ± 12
CrMP-hypertensive	92 ± 3	7.41 ± .03	30 ± 3	178 ± 17

arterioles dilated from 64 ± 4 to 83 ± 6 μm in the presence of CrMP.

Cerebral Perfusion Pressure. The arteriolar constriction in response to increased cerebral perfusion pressure was measured by creating hypertension via increased circulatory filling pressure and increased peripheral vascular resistance. First, the vagus nerves were cut bilaterally to prevent baroreceptor-induced bradycardia, and bilateral pneumothorax was produced via 4-cm intercostal incisions to prevent interpleural pressure elevation. A blood pressure cuff was then wrapped around the abdomen and hind legs of the piglet. To increase cerebral perfusion pressure, the cuff was inflated to 300 mmHg. Such inflation effectively increases vascular resistance by eliminating lower body circulation and increases circulatory filling pressure by emptying lower body venous reservoirs into the rostral circulation. MAP, pH, and blood gases were monitored until a maximal pressure was achieved (95 ± 1 mmHg; less than 5 min; Table II). With the MAP elevated and stable, CrMP was infused under the cranial window. PAD and MAP were measured at baseline after obtaining a maximum pressure and then 1, 3, 5, and 10 min after application of the CrMP while maintaining hypertension. The effect of CrMP on PAD was compared between normotensive (sham preparation but no cuff inflation) and hypertensive piglets. When CrMP was present lights were only turned on for the measurements (5–10 sec, each time point).

Data Analysis. Data are presented as means ± SEM. They were analyzed statistically by analysis of variance (ANOVA) with repeated measures followed by Tukey-Kramer Multiple Comparisons Test. $P < 0.05$ was considered significant.

Results

Hypocapnia and PAF experiments were done after a 20-min pre-exposure to the metal porphyrins when diameters were not different from before the inhibitor. CrMP greatly augmented constriction to hypocapnia ($P_aCO_2 \cong 20$ mmHg; Fig. 1). Among five pigs, pial arterioles had a mean constriction of 12% in response to hypocapnia. After application of CrMP, the same degree of hypocapnia caused a mean constriction of 39%.

Similar to the effect on hypocapnia-induced constriction, CrMP augmented pial arteriolar constriction to PAF (Fig. 2). Thus, arterioles constricted to PAF 7.7% in the absence of CrMP and 14.2% in its presence.

Hypertension caused pial arteriolar constriction (same arterioles: 64 ± 3 to 55 ± 3 μm at 72 ± 4 and 95 ± 1 mmHg

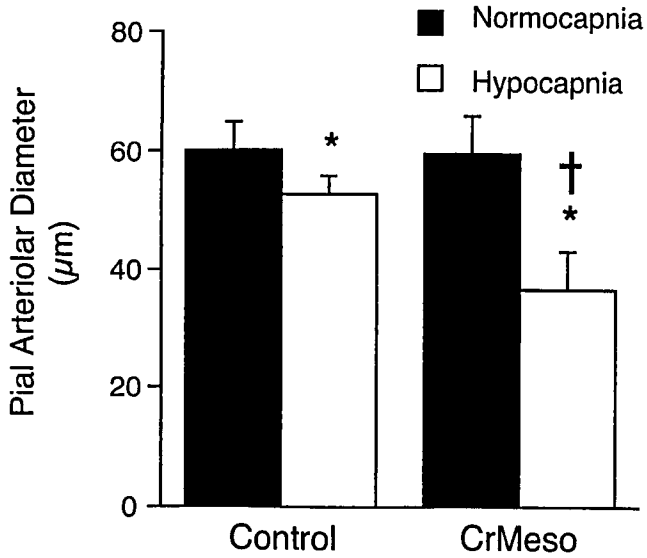

Figure 1. Effects of CrMP on vasoconstriction in response to hypocapnia. Pial arteriole diameters were measured before and during hypocapnia and then with CrMP treatment before and during hypocapnia. ($n = 5$). *$P < 0.05$ compared with normocapnia. +$P < 0.05$ compared with no CrMP.

MAP, respectively). In normotensive piglets, CrMP tended to dilate pial arterioles initially. The magnitude of the PAD increase was highly variable, reaching a peak within 1–5 min, with diameter returning to baseline by 10 min. Constriction was not observed. In hypertensive piglets, CrMP consistently caused further constriction in addition to that which occurred when pressure was increased. The constriction to CrMP was progressive during the period of 5–10 min after treatment. The data shown in Figure 3 are the diameters and pressures 10 min after CrMP, for both the normotensive and hypertensive pigs. These data reinforce the hypothesis of CO as an attenuator of vasoconstriction. Dilations to isoproterenol at the very beginning and at the very end were not different in either sham or hypertensive piglets, suggesting overall stability of vascular reactivity throughout the hypertensive protocol.

Discussion

The new findings of this study are that HO inhibition augments the vasoconstrictor responses of pial arterioles to hypocapnia, PAF, and hypertension, suggesting that endogenously produced CO provides a dilator influence that modulates vasoconstrictor responses in the cerebral circulation of the newborn pig.

These data are consistent with the results of previous

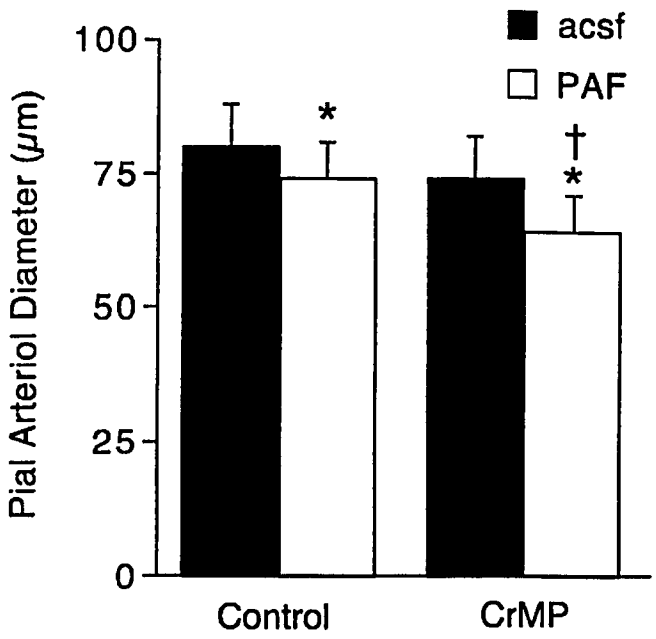

Figure 2. Effects of CrMP on vasoconstriction in response to PAF. Constriction was measured to PAF alone and then to PAF with treatment of the metal porphyrin HO inhibitor ($n = 5$). *$P < 0.05$ compared with no PAF. +$P < 0.05$ compared with no CrMP.

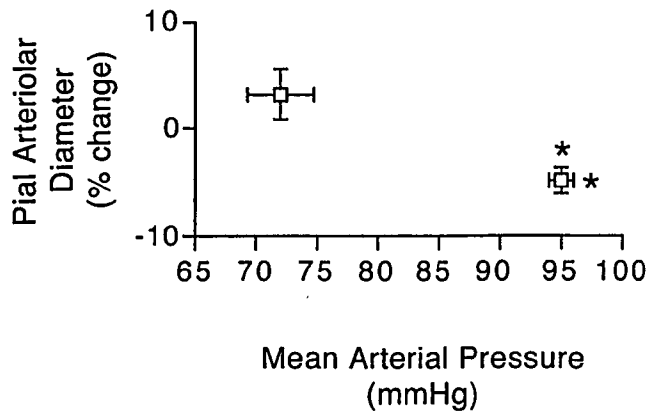

Figure 3. Effect of CrMP on pial arteriolar diameter of normotensive and hypertensive piglets ($n = 4$ for both groups). *$P < 0.05$ when compared with the normotensive group.

work on piglets, which established CO as an endogenous vasodilator in the neonatal cerebral circulation (25). CO produces dose-dependent dilation of piglet pial arterioles. The enzyme that endogenously produces CO is present in high concentrations in cerebral vessels. The substrate of HO also produces dose-dependent dilation of cerebral arterioles. CO appears to be involved in cerebral vasodilation in response to hypoxia (25) and glutamate (27). The present study extends the understanding of the potential physiological significance of CO in newborn cerebral circulation to that of a generalized dilator influence that counteracts vasoconstriction thereby tending to promote cerebral blood flow.

Previous studies have demonstrated K_{Ca} channels modulate the vasoconstriction of cerebral vessels to hypertension (28). Attenuating constrictor responses could play a very important protective role ensuring that vasoconstrictor responses are partially counterbalanced to reduce the likelihood of ischemia. Dilation of piglet pial arterioles to CO involves K_{Ca} channels (25). Inhibitors of K_{Ca} channels block the dose-dependent vasodilation of cerebral vessels to CO. Thus, CO could contribute to K_{Ca} channel modulation of hypertension-induced constriction.

Other potential pathways involved in CO's vascular regulatory action include cAMP and cGMP. CO and heme-L-lysinate have been shown to increase cAMP in piglet brain (25), although the changes were minute when compared with the dose-dependent increases produced by iloprost or isoproterenol (26). Both nitric oxide (NO) and CO can activate guanylyl cyclase; however, in contrast to NO, CO did not elicit detectable increases of cerebrospinal fluid concentration of cGMP in piglets even at doses that produce strong dilation (25).

In normotensive (72 ± 4 mmHg) piglets, CrMP did not constrict pial arterioles. Conversely, hypertensive (95 ± 1 mmHg) piglets showed cerebral vasoconstriction when CrMP was applied that progressed from 5 to 10 min of treatment. These data suggest basal CO production makes little contribution to maintenance of lower cerebrovascular tone in baseline newborn conditions. However, vasoconstrictor influences whether hypocapnia, PAF, or increased perfusion pressure appear to increase the vasodilatory influence of CO, possibly by increasing CO production. At 10 min of treatment with CrMP of hypertensive piglets, PAD had decreased significantly (–4.9% ± 1.8%; $P < 0.01$) in contrast to normotensive piglets where the change of PAD (+3.2% ± 2.4%) was not significantly different from zero. Clearly, inhibition of HO constricted pial arterioles of piglets with blood pressures over 90mmHg, but not those of piglets with blood pressures around the normal of 70 mmHg.

In short, it appears that CO is among the mediators in an increasingly complex collection that regulates cerebral blood flow. Our data support the hypothesis that CO is an endogenous attenuator of vasoconstriction whether that constriction is induced by hypocapnia, hypertension, or PAF. This places CO among the many mediators involved in maintaining homeostasis of the cerebral vasculature in the face of acute physiologic challenges. Specifically, CO appears to have a role in preventing an overzealous vasoconstriction in response to hypocapnia, hypertension, or paracrine mediators.

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