Cerebroprotective effects of the CO-releasing molecule CORM-A1 against seizure-induced neonatal vascular injury

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Submitted 20 March 2007; accepted in final form 11 July 2007

Zimmermann A, Leffler CW, Tcheranova D, Fedinec AL, Parfenova H. Cerebroprotective effects of the CO-releasing molecule CORM-A1 against seizure-induced neonatal vascular injury. Am J Physiol Heart Circ Physiol 293: H2501–H2507, 2007. First published July 13, 2007; doi:10.1152/ajpheart.00354.2007.—Endogenous CO, a product of heme oxygenase activity, has vasodilator and cytoprotective effects in the cerebral circulation of newborn pigs. CO-releasing molecule (CORM)-A1 (sodium boranocarbonate) is a novel, water-soluble, CO-releasing compound. We addressed the hypotheses that CORM-A1 1) can deliver CO to the brain and exert effects of CO on the cerebral microvasculature and 2) is cerebroprotective. Acute and delayed effects of topically and systemically administered CORM-A1 on cerebrovascular and systemic circulatory parameters were determined in anesthetized newborn pigs with implanted closed cranial windows. Topical application of CORM-A1 (10−7–10−5 M) to the brain produced concentration-dependent CO release and pial arteriolar dilation. Systemically administered CORM-A1 (2 mg/kg ip or iv) caused pial arteriolar dilation and increased cortical cerebral fluid CO concentration. Systemic CORM-A1 did not have acute or delayed effects on blood pressure, heart rate, or blood gases. Potential cerebroprotective vascular effects of CORM-A1 (2 mg/kg ip, 30 min before seizures) were tested 2 days after bicuculline-induced epileptic seizures (late postictal period). In control piglets, seizures reduced postictal cerebrovascular responsiveness to selective physiologically relevant vasodilators (bradykinin, hemin, and isoproterenol) indicative of cerebrovascular injury. In contrast, in CORM-A1-pretreated animals, no loss of postictal cerebrovascular reactivity was observed. We conclude that systemically administered CORM-A1 delivers CO to the brain, elicits the vasodilator and cytoprotective effects of CO in the cerebral circulation, and protects the neonatal brain from cerebrovascular injury caused by epileptic seizures.

Epileptic seizures cause long-term cerebrovascular dysfunction in human patients and animal models, as indicated by symptoms of the postictal state and reduced vasoreactivity to physiologically relevant vasodilators, respectively (6, 9, 26). Seizures occur when there is excessive synchronized depolarization of neurons within the central nervous system due to unrestrained glutamate release. The GABA_A receptor blocker bicuculline causes convulsions in newborn piglets, a well-defined model of neonatal epileptic seizures, documented by increased electroencephalographic amplitude and spectral power within the 1- to 15-Hz frequency range sustained for >2 h (27).

We previously reported that pharmacological induction of HO-1, the inducible HO isomerase, was protective against long-term cerebrovascular dysfunction caused by seizures in vivo (26) and glutamate-triggered apoptosis in cultured piglet microvascular endothelial cells (25). The favorable impact of enhanced HO activity could be due to the degradation of prooxidant heme and the production of CO and/or biliverdin/bilirubin, since CO has antiapoptotic and antioxidant properties, whereas bilirubin is a powerful antioxidant naturally occurring abundantly in the body (15).

The effects of gaseous CO can be mimicked by a group of pharmacological compounds collectively termed CO-releasing molecules (CORMs) (1, 11, 12, 22, 23). For our in vivo experiments, we selected CORM-A1 [Na_2(H_3BCO_2)], a novel water-soluble compound that spontaneously releases gaseous CO in physiological solutions (1, 23). In contrast to other CORMs, CORM-A1 does not contain heavy metals that may affect ion channel function or induce HO-1 and, thus, stimulate endogenous CO production (23). The effects of CORM-A1 in the cerebral circulation in vivo have not been investigated.

We hypothesize that peripherally administered CORM-A1 1) delivers CO to the brain, 2) exerts the effects of gaseous CO on the cerebral circulation, and 3) is vasoprotective against seizure-induced sustained cerebrovascular dysfunction in newborn pigs in vivo.

METHODS

Protocols and procedures using animals were approved by the Animal Care and Use Committee at the University of Tennessee Health Science Center.

Cranial windows and cerebrovascular reactivity. Newborn pigs (1–5 days old, 1.5–2.5 kg body wt, either sex) were anesthetized

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initially with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im) and maintained on α-chloralose (30 mg/kg iv). Catheters inserted into the femoral artery were used to monitor arterial blood gases, pH, and blood pressure, and those inserted into the femoral vein were used to inject drugs and fluids. The animals were intubated and artificially ventilated with room air to maintain physiological levels of arterial blood gases and pH. Body temperature was maintained at 37–38°C with a servo-controlled heating pad. A closed cranial window and intravital microscopy were used to determine pial arteriolar diameter (PAD), as described previously (6). The space under the window was filled with artificial cerebrospinal fluid (aCSF) that consisted of (in mM) 3.0 KCl, 1.5 MgCl₂, 1.5 CaCl₂, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO₃ and was equilibrated with 6% CO₂-6% O₂-88% N₂ to pH 7.3–7.35 at 37°C. Two or three pial arterioles (60–80 μm) from each animal were used to test vascular reactivity. Control PAD values were measured over a 10-min period under basal conditions. Bradykinin (10⁻⁵ M), isoproterenol (10⁻⁶ M), sodium nitroprusside (10⁻⁴ M), or hemin (10⁻⁶ M) was applied to the cerebral surface, and the maximal PAD achieved between 3 and 10 min was taken as the response.

Seizure induction. Epileptic seizures were induced by bicuculline in ketamine-acepromazine-anesthetized and ventilated newborn piglets, as we described previously (26, 27). Piglets were intubated through the mouth and ventilated with supraphysiological minute volume and a gas mixture of 4% CO₂-21% O₂-75% N₂ to maintain physiological levels of blood gases. Body temperature was maintained at 37–38°C with a servo-controlled heating pad. A butterfly needle was inserted into the ear vein for administration of pancuronium (0.2 mg/kg iv) before seizure induction. Seizures were induced by bicuculline (3 mg/kg ip), a GABA receptor blocker that induces spontaneous seizures in piglets by disrupting the normal balance between excitatory and inhibitory neurotransmitters. Bicuculline was dissolved in 0.1 N HCl (3 mg/ml), titrated with 1 N NaOH to pH 4–5, and diluted with 5 ml of saline. Bicuculline and other drugs were administered aseptically through a 0.22-μm Millipore syringe filter. The animals were kept on the ventilator for 2 or 3 h until the seizure activity subsided. When fully conscious, piglets were transferred to the animal care facility and kept in warmed cages with food and drink ad libitum for 2 days.

CO detection by gas chromatography–mass spectrometry. For detection of CO release in vitro, CORM-A1 (10⁻µM) was dissolved in Krebs buffer, protected from light, and exposed to open air. The medium was sampled at 1, 2, 5, and 24 h, transferred to the sealed vials containing the internal standard of a saturated solution of 13C18O (1 mM), and incubated at 37°C for 10 min; the head-space gas was collected for CO detection. For detection of CO in cerebral circulation in vivo, the samples of cortical periarachnoid CSF were collected from the brain surface under the cranial window during 1) basal conditions, 2) after topical application of active or inactivated CORM-A1 (10⁻⁷–10⁻⁴ M), or 3) after systemic injection of CORM-A1 (2 mg/kg ip or iv). The volume under the window was 0.4 ml. CSF (0.4 ml) under the cranial window was collected in 10-min intervals through a sputum directly into the sealed vials containing 1.3 ml of Krebs buffer and 13C18O (1 mM). The vials were kept at 4°C for 1–4 days. For CO detection, the vials were heated at 70°C for 10 min. The head-space gas CO concentration was determined by gas chromatography–mass spectrometry (GC-MS) to ensure complete separation and identification of gaseous molecules with identical molecular masses. 1) The components of the head-space gas are separated on a molecular sieve-coated capillary column (CP-Molsieve 5Å column (30 m, 0.32 mm ID), Varian, Palo Alto, CA) to effectively separate CO from other gases (retention times of 1.5 min for O₂ peak, 2.3 min for N₂ peak, and 6.3 min for CO peak). 2) The CO peak is quantitatively detected on the basis of the peak areas with the mass-to-charge ratios corresponding to 12C16O and 13C18O (6).

Experimental design. First, we investigated acute cerebrovascular effects of topical and systemic CORM-A1 in newborn pigs. CORM-A1 was 1) applied to the area under the closed cranial window in consecutively increasing concentrations (10⁻⁷–10⁻³ M, 10 min each) or 2) administered systemically (2 mg/kg ip or iv, 60–90 min). PAD and systemic parameters [mean arterial blood pressure (MABP), heart rate (HR), blood gases, and hematocrit] were measured, and cortical periarachnoid CSF was collected for CO detection (see above).

Second, we compared delayed postictal cerebrovascular reactivity in control and CORM-A1-treated animals. Epileptic seizures were induced by bicuculline, and animals were allowed to recover for 2 days (see above). Cerebrovascular reactivity was tested in 1) control intact piglets (group I, n = 9), 2) postictal (2 days after seizure induction) animals (group II, n = 7), 3) CORM-A1-pretreated postictal (2 days after seizures, 2 mg/kg CORM-A1 ip 30 min before seizure induction) animals (group III, n = 5), and 4) CORM-A1-treated (2 days after CORM-A1, 2 mg/kg ip) animals (group IV, n = 6).

Active CORM-A1 (10⁻³ M stock solution) was prepared immediately before use to avoid loss of released CO. Inactivated CORM-A1 was prepared by exposure of the CORM-A1 stock solution to open air for 20 h at room temperature to fully decompose the parent compound (23). For topical administration, stock solutions of active or inactivated CORM-A1 were diluted with aCSF. For systemic administration, CORM-A1 was dissolved in saline (1 mg/ml) and immediately applied (intravenously or intraperitoneally) aseptically through a 0.22-μm Millipore syringe filter.

Statistical analysis. Values are means ± SE of absolute values or percentage of control. ANOVA with repeated measures and Tukey-Kramer multiple comparisons test were used to confirm differences among and then between groups, respectively. P < 0.05 was considered significant in all statistical tests.

Materials. CORM-A1 was a generous gift from Hector Knight (Tyco-Mallincrodt Medical, Petten, Holland). Pancuronium bromide was obtained from Astra Pharmaceutical Products (Westborough, MA). Bicuculline, bradykinin, isoproterenol, sodium nitroprusside, and hemin were purchased from Sigma (St. Louis, MO).

RESULTS

CO release from CORM-A1 in vitro. CORM-A1 dissolved in the physiological Krebs solution (pH 7.4) releases CO in a concentration- and time-dependent manner, as detected by GC-MS (Fig. 1). Stable CO release from CORM-A1 was observed during the first 1–2 h in solution and then declined progressively. The half-life (t1/2) of CORM-A1 at room temperature (pH 7.4) is ~3 h (Fig. 1B). After 20 h of exposure to...
open air at room temperature, CORM-A1 was completely decomposed and incapable of releasing CO (inactivated CORM-A1, CO was below the detection limits). Therefore, CORM-A1 provides a dose- and time-dependent CO release at physiological pH within 5–6 h.

Cerebrovascular effects of topically administered CORM-A1. We compared cerebrovascular effects of topically administered CORM-A1 (dissolved in aCSF) and gaseous CO (1 mM solution in aCSF). CO or CORM-A1 solutions were applied to the brain surface under the cranial window in consecutively increasing concentrations (10^{-6}, 10^{-5}, and 10^{-4} M, 10 min each). First, we detected CO concentrations under the cranial window (Fig. 4) and caused vasodilation of pial arterioles. CORM-A1 increased CO concentration in cortical periarachnoid CSF (Fig. 4) and caused vasodilation of pial arterioles. Systemic (intravenously or intraperitoneally administered) CORM-A1 had a dose-dependent vasodilator effect on pial arterioles that was comparable to topically applied gaseous CO (Fig. 3). Maximal pial arteriolar dilation (15 ± 2%) was achieved at 10^{-6} M topical CORM-A1 or gaseous CO (n = 6 piglets). In contrast, inactivated CORM-A1 was not capable of releasing CO (100 ± 21, 90 ± 21, 90 ± 23, and 94 ± 22 nM CO under the cranial window at 0, 10^{-6}, 10^{-5}, and 10^{-4} M CORM-A1, respectively, n = 5 piglets) and failed to cause cerebral vasodilation (2 ± 1, 1 ± 1, and 2 ± 2% at 10^{-6}, 10^{-5}, and 10^{-4} M CORM-A1, respectively, n = 5 piglets).

Acute systemic and cerebrovascular effects of systemically administered CORM-A1. For systemic administration, CORM-A1 (2 mg/kg) was dissolved in saline immediately before the experiment and injected intravenously or intraperitoneally. Systemic CORM-A1 did not affect systemic circulatory parameters, including MABP, HR, and blood gases/oxygenation (Table 1). Systemic (intravenously or intraperitoneally administered) CORM-A1 increased CO concentration in cortical periarachnoid CSF (Fig. 4) and caused vasodilation of pial arterioles (Fig. 5). Maximal cerebrovascular responses (10–15% dilation, 1.5- to 2-fold cortical CO increase) were observed within 20–40 min of systemic CORM-A1 administration (Figs. 4 and 5). Intravenous and intraperitoneal deliveries of CORM-A1 affected cerebrovascular parameters equally. Brain CO and PAD returned to baseline values within 1–1.5 h of CORM-A1 administration. These data indicate that systematically injected CORM-A1 delivers CO to the brain without altering physiologic variables.

Long-term effects of CORM-A1 on postictal cerebrovascular function. Postictal cerebrovascular response to endothelium-dependent (bradykinin and hemin) and endothelium-independent (isoproterenol and sodium nitroprusside) stimuli was tested 2 days after epileptic seizures. We compared systemic and cerebrovascular parameters in four experimental groups of control and postictal piglets that were untreated or pretreated (30 min before seizures) with systemic CORM-A1 (2 mg/kg ip). MABP, HR, blood gases, and pH were within the physiologic range for newborn piglets, and no differences were observed among the experimental groups (Table 2). Therefore, seizures and/or CORM-A1 treatment had no long-term adverse effects on the systemic parameters in newborn pigs. We tested cerebrovascular function by responsiveness of pial arterioles to endothelium-dependent (bradykinin and hemin) and endothelium-independent (isoproterenol and sodium nitroprusside) vasodilators in control intact animals (group I), 2 days after seizure induction (group II), seizure induction combined with CORM-A1 treatment (group III), or CORM-A1 treatment alone (group IV). At 2 days after epileptic seizures, cerebrovascular responses to bradykinin (10^{-5} M), hemin (10^{-5} M), and isoproterenol (10^{-6} M) were significantly decreased (group I; Figs. 6 and 7). Postictal cerebral vasodilation to sodium nitroprusside was not reduced, in contrast to our previous results (26). Because we used a lower concentration (10^{-6} vs. 10^{-5} M) of nitroprusside, it is possible that loss of smooth muscle function might not be manifested at submaximal stimulation. Overall, epileptic seizures caused sustained loss of responsiveness toward a variety of endothelium-dependent and -independent vasodilator stimuli, confirming our pre-
CORMs are designed to liberate CO and elicit its biological effects in vivo. However, when animals were pretreated with systemic CORM-A1 (2 mg/kg ip), the loss of cerebrovascular reactivity was partially reduced or completely prevented (group III; Figs. 6 and 7). Postictal cerebrovascular reactivity to sodium nitroprusside remained unchanged in untreated and CORM-pretreated animals, suggesting that vascular smooth muscle may be less affected by seizures. CORM-A1 alone had no effects on cerebrovascular responsiveness to all tested vasodilators (group IV; Figs. 6 and 7). These results provide evidence that systemically administered CORM-A1 is protective against sustained cerebrovascular dysfunction caused by seizures.

**DISCUSSION**

The major novel findings of our study are that systemic administration of CORM-A1 to newborn piglets in vivo 1) liberates CO and delivers functionally effective levels of the CO gas to the brain and cerebral microvasculature, 2) prevents sustained postictal cerebrovascular injury caused by epileptic seizures, and 3) has no effects on arterial blood pressure or HR. These data indicate that systemically administered CORM-A1 can result in vasoactive and cytoprotective effects of CO on the neonatal cerebral circulation.

CORMs are designed to liberate CO and elicit its biological activities, including vasodilator effects (7, 11, 12, 22–24, 31, 32). First-generation CORMs, CORM-1 (dimanganese decacarbonyl) and CORM-2 (tricarbonyldichlororuthenium II dimer), contain heavy metals, are soluble in organic solvents, and may require light irradiation for liberation of CO (CORM-1). Water-soluble CORM-3, tricarbonylchloroglycinato(ruthenium(II), also contains a heavy metal and liberates CO rapidly, providing a relatively short-term experimental window (11, 24). Recently introduced water-soluble CORM-A1 contains no heavy metal and releases CO spontaneously and slowly in physiologically buffered solutions (1, 23). Therefore, CORM-A1 is highly suitable for in vitro and in vivo experiments. In vivo, CORM-A1 exhibits biological effects of CO on the heart and kidneys (23, 24, 28, 30). Vasodilator effects of CORMs have been demonstrated in several vascular beds, including coronary, renal, and cerebral arteries (2, 10, 11, 14, 24, 28). In newborn pigs, gaseous CO is a cerebral vasodilator that dilates cerebral vessels by directly activating large-conductance Ca^2+^-activated K^-channels on smooth muscle cells (17). In isolated pressurized cerebral arterioles from newborn pigs, CORM-1 stimulated by light to liberate CO caused endothelium-dependent dilation (4, 10). However, biological effects of CORMs on the cerebral circulation in vivo have not been investigated.

We demonstrate that topically or systemically administered CORM-A1 delivers CO to the brain and has cerebrovascular effects in vivo. In our experiments, CORM-A1 dissolved in physiological solutions (pH 7.4) dose dependently released CO for 7–5 h (t_{1/2} at room temperature ~3 h). CO liberation from CORM-A1 is temperature dependent, and t_{1/2} of 20 min has been reported at the incubation temperature of 37°C (23). Topical application of CORM-A1 directly to the brain surface prevents or completely prevents (group III; Figs. 6 and 7). Postictal cerebrovascular reactivity to sodium nitroprusside remained unchanged in untreated and CORM-pretreated animals, suggesting that vascular smooth muscle may be less affected by seizures. CORM-A1 alone had no effects on cerebrovascular responsiveness to all tested vasodilators (group IV; Figs. 6 and 7). These results provide evidence that systemically administered CORM-A1 is protective against sustained cerebrovascular dysfunction caused by seizures.

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**Table 1. Systemic circulatory parameters before and after systemic administration of CORM-A1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
<th>Arterial PCO2, mmHg</th>
<th>Arterial PO2, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>67±2</td>
<td>154±14</td>
<td>32±2</td>
<td>113±6</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>CORM-A1</td>
<td>68±2</td>
<td>150±13</td>
<td>33±1</td>
<td>115±6</td>
<td>7.46±0.02</td>
</tr>
<tr>
<td>20 min</td>
<td>66±2</td>
<td>149±15</td>
<td>34±1</td>
<td>111±6</td>
<td>7.45±0.02</td>
</tr>
<tr>
<td>40 min</td>
<td>64±2</td>
<td>153±16</td>
<td>34±1</td>
<td>110±6</td>
<td>7.44±0.02</td>
</tr>
<tr>
<td>Control 2</td>
<td>65±2</td>
<td>152±16</td>
<td>33±1</td>
<td>119±6</td>
<td>7.46±0.03</td>
</tr>
<tr>
<td>CORM-A1</td>
<td>67±1</td>
<td>150±14</td>
<td>32±1</td>
<td>120±6</td>
<td>7.46±0.03</td>
</tr>
<tr>
<td>20 min</td>
<td>66±2</td>
<td>150±14</td>
<td>32±1</td>
<td>121±6</td>
<td>7.46±0.03</td>
</tr>
<tr>
<td>40 min</td>
<td>67±2</td>
<td>149±15</td>
<td>33±1</td>
<td>119±6</td>
<td>7.53±0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 5 piglets). MABP, mean arterial blood pressure; HR, heart rate; CORM, CO-releasing molecule (2 mg/kg iv).

**Fig. 4.** Effects of systemically administered CORM-A1 on CO level in cortical periarachnoid CSF. CORM-A1 (2 mg/kg) was administered systemically (iv or ip), and CSF under the cranial window was collected at 0–90 min. CO concentration in CSF was determined by gas chromatography-mass spectrometry. Values are means ± SE (n = 5 animals). *P < 0.05 vs. baseline.

**Fig. 5.** Effects of systemically administered CORM-A1 on pial arteriolar diameter. CORM-A1 (2 mg/kg) was administered systemically (iv or ip), and pial arteriolar diameter was measured from 0 to 90 (iv) or from 0 to 60 (ip) min. Values are means ± SE (n = 5 animals). *P < 0.05 vs. baseline.
moderate hypotensive effects in response to CORMs were not change HR or myocardial contractility (24). Conversely, cardiovascular function. In isolated rat hearts, CORM-A1 did are few controversial reports on the effects of CORM-A1 on tion of CORM-A1 (2 mg/kg) had no acute or delayed effects on dependent vasodilators was not changed.

cerebrovascular reactivity to endothelium-dependent and -in-
dependent vasodilators was not changed. We found no adverse or long-lasting effects of systemic CORM-A1 on cerebrovascular function. Cerebrovascular effects of CORM-A1 are sustained for no longer than 1–1.5 h after a single systemic administration. This correlates with temperature-dependent $t_{1/2}$ of the compound (0.5–3 h). Intra-peritoneal administration of CORM-A1 appears to produce longer-lasting changes in the cerebral circulation and, therefore, could be advantageous when more prolonged treatment is desired. At 2 days after systemic administration of CORM-A1, cerebrovascular reactivity to endothelium-dependent and -independent vasodilators was not changed.

In newborn pigs, intravenous or intraperitoneal administration of CORM-A1 (2 mg/kg) had no acute or delayed effects on blood pressure, HR, blood gases, pH, or oxygenation. There are few controversial reports on the effects of CORM-A1 on cardiovascular function. In isolated rat hearts, CORM-A1 did not change HR or myocardial contractility (24). Conversely, moderate hypotensive effects in response to CORMs were reported in normotensive and hypertensive rats in vivo (12, 22, 23).

We found that CORM-A1 exhibits potent cerebroprotective effects in the newborn cerebral circulation in vivo. Previously, we demonstrated that epileptic seizures cause substantial loss of cerebrovascular function in newborn piglets indicative of sustained cerebrovascular injury (26). Reduced responsiveness to major functionally relevant vasodilators was observed 2 days after seizures (delayed postictal period). In our present study, we observed reduction of cerebrovascular responsiveness to endothelium-dependent (bradykinin and heme) and endothelium-independent (isoproterenol, but not nitroprusside) vasodilators. Overall, our previous and present data indicate that endothelium-dependent responses are more susceptible to seizure-induced damage, although some cerebrovascular smooth muscle damage may also occur. However, when CORM-A1 was administered systemically (2 mg/kg iv or ip) shortly before seizures, the loss of cerebrovascular reactivity during the postictal period was partially or completely pre-vented. Previously, we demonstrated that HO [constitutive and inducible (HO-2 and HO-1)] is essential in protecting the

tic seizures (6, 26). Taken together, these data show that CO, formed from an exogenous source (CORM-A1) or endog-

enously via HO-catalyzed heme degradation, is protective against seizure-induced cerebrovascular injury.

![Fig. 6. Postictal cerebrovascular reactivity to endothelium-dependent vasodilators. Cerebrovascular reactivity to bradykinin (10^{-5} M) and hemin (10^{-6} M) was tested 2 days after bicuculline-induced epileptic seizures (delayed postictal state). Control animals were untreated (group I, n = 9) or pretreated with CORM-A1 (group IV, 2 mg/kg ip) 2 days before testing (n = 7). Seizures were induced in untreated (postictal, group II, n = 7) or CORM-A1-pretreated (2 mg/kg ip, group III, n = 5) piglets 30 min before seizures. Values are means ± SE. *P < 0.05 vs. baseline. †P < 0.05 vs. corresponding postictal.]

![Fig. 7. Postictal cerebrovascular reactivity to endothelium-independent vasodilators. Cerebrovascular reactivity to isoproterenol (10^{-6} M) and sodium nitroprusside (10^{-6} M) was tested 2 days after bicuculline-induced epileptic seizures (delayed postictal state). Control animals were untreated (group I, n = 9) or pretreated with CORM-A1 (2 mg/kg ip) 2 days before testing (group IV, n = 7). Seizures were induced in untreated (group II, n = 7) or CORM-A1-pretreated (2 mg/kg ip) piglets 30 min before seizures (group III, n = 5). Values are means ± SE. *P < 0.05 vs. baseline. †P < 0.05 vs. corresponding group II.]

**Table 2. Systemic circulatory parameters in intact control and postictal newborn piglets**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
<th>Arterial Po$_2$, mmHg</th>
<th>Arterial Po$_4$, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>9</td>
<td>77±3</td>
<td>145±2</td>
<td>31±2</td>
<td>95±8</td>
<td>7.42±0.03</td>
</tr>
<tr>
<td>Group II</td>
<td>7</td>
<td>75±2</td>
<td>107±8</td>
<td>32±2</td>
<td>116±9</td>
<td>7.45±0.01</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>78±2</td>
<td>136±7</td>
<td>38±2</td>
<td>85±9</td>
<td>7.44±0.02</td>
</tr>
<tr>
<td>Group IV</td>
<td>6</td>
<td>79±1</td>
<td>144±7</td>
<td>30±2</td>
<td>97±6</td>
<td>7.44±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of piglets. Group I, control; group II, 2 days after seizure induction; group III, 2 days after seizure induction + CORM-A1; group IV, 2 days after CORM-A1.
CORM-A1-delivered CO may also affect brain neurons. We previously reported that endogenous CO produced via brain HO activity has a moderate proconvulsant effect in the newborn pig seizure model (72). Therefore, we anticipate that systemic CORM-A1 may aggravate epileptic neuronal activation. If cerebroprotection were secondary to the effects of CO on neurons, we would expect aggravation of cerebrovascular dysfunction. However, our present results demonstrate that CORM-treated piglets were protected from postictal cerebrovascular dysfunction and, therefore, that the cerebroprotective vascular effect is not neuron mediated. This finding suggests that the dynamics of brain neuronal activity and postictal cerebrovascular dysfunction involve independent diverse CO-mediated responses of neurons and cerebral microvessels that mediate cell activation and cell survival.

Protective effects of CORMs have been reported in several tissue injury models. In kidneys, CORM-A1 and CORM-3 improved renal vascular function after cold storage (30) and prevented ischemia-induced acute renal failure (37). CORMs provided cardioprotection against ischemia-reperfusion injury (8, 13, 34, 36). CO has anti-inflammatory properties as well. CORM-3 inhibited TNF-α production by microglial cells and macrophages (3, 31). CORM-2 reduced systemic inflammatory response syndrome after skin burn injury in mice (35) and inhibited the inflammatory response to cytokines in epithelial cells (20). Epileptic seizures induce inflammation and TNF-α formation in the central nervous system, which might contribute to cerebrovascular damage (38). Thus, immunomodulation by CORM-A1 might also contribute to its protective action against seizure-induced cerebrovascular damage.

Apoptosis of cerebrovascular endothelial cells is a potential factor in cerebrovascular dysfunction during the late postictal period. Seizures are related to excessive production of excitotoxic and inflammatory mediators. Glutamate, the major excitatory neuromediator, and TNF-α, the proinflammatory cytokine, are excessively produced in the brain during seizures and may lead to cerebrovascular injury. Glutamate and TNF-α caused apoptosis of cerebrovascular endothelial cells that was completely prevented by CORM-A1 (5, 25). Endogenously produced CO is also cerebroprotective. HO-1 overexpression prevented, whereas HO-2 downregulation or gene deletion greatly exacerbated, seizure-induced cerebrovascular injury and dysfunction in vivo and in vitro (5, 25, 26). In rat hepatocytes, prolonged treatments (6–12 h) with CO donors and authentic CO gas induced HO-1 expression (16). In our experiments, CORM-A1 was administered 30 min before seizures and, therefore, exhibited immediate (early) cerebroprotective effects, whereas de novo enzyme synthesis requires ≥6 h (16). Therefore, it is unlikely that the cerebroprotective effects of CORM-A1 following epileptic seizures in newborn pigs were mediated via the induction of HO-1.

Oxidative stress related to excessive release of the excitatory neuromediator glutamate and the proinflammatory cytokine TNF-α during seizures is a potential contributor to extended cerebrovascular damage and apoptosis. Increased reactive oxygen species (ROS) formation was observed in endothelial cells from cerebral microvessels stimulated by glutamate and TNF-α (5, 25). We found that CORM-A1 greatly reduced oxidative stress stimulated by these proapoptotic agents in cerebrovascular endothelial cells (5, 25). In other vascular beds, antiapoptotic effects of CO also have been related to inhibition of ROS generation (18, 19, 33, 39–41).

Mechanisms by which CO prevents oxidative stress and apoptosis in response to excitoactivity and inflammation in the cerebral circulation and other vascular beds are not completely understood. Overall, the products of HO activity have antioxidant potential. Biliverdin/bilirubin, along with biliverdin reductase, act as ROS scavengers (15, 17). In contrast, the ability of CO to reduce oxidative stress appears to be related to its ability to block formation of ROS by interacting with the ROS-producing enzymes. CO has a high affinity to heme and, therefore, may block the action of numerous heme-containing enzymes, including the mitochondria respiratory chain components cytochrome c, MAPK, and p53 kinase (18, 19, 21, 29, 33, 40, 41). CO uncouples mitochondrial respiration from ATP production by enhancing the proton leakage across the inner mitochondrial membrane and, thus, may act to preserve energy and maintain the cellular antioxidant defense mechanisms (29).

Overall, we conclude that CORM-A1 provides a pharmacological tool for delivery of CO to the brain and protection against sustained cerebrovascular injury caused by epileptic seizures. CORM-A1 might be the basis of novel pharmacological tools for cerebroprotection.

ACKNOWLEDGMENTS

The authors thank Danny Morse for helping with preparation of the figures.

GRANTS

This research was supported by the National Institute of Neurological Disorders and Stroke and the National Heart, Lung, and Blood Institute. A. Zimmermann was supported by the National Scientific Research Fund of Hungary.

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