Epileptic seizures cause extended postictal cerebral vascular dysfunction that is prevented by HO-1 overexpression

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blood flow during the seizures resulted in severe impairment of cerebral vascular function. During the immediate recovery period (2 h after the seizure onset), cerebral vascular reactivity to physiologically relevant vasodilators was greatly reduced in animals with inhibited HO activity (12). In contrast, no decrease in cerebral vascular reactivity was observed during the immediate postictal recovery in control animals with normal HO activity (12).

Although it is recognized that cerebral vascular dysfunction may substantially contribute to neurological problems in patients with epilepsy, no experimental data are available on cerebral vascular reactivity to physiologically and pharmacologically relevant stimuli during the extended postictal state. We hypothesized that 1) seizures cause prolonged postictal cerebral vascular dysfunction and 2) postictal cerebral vascular dysfunction can be accentuated by HO inhibition and rescued by HO overexpression. Therefore, we assessed the cerebral vascular reactivity during the extended postictal period, 2 days after sustained seizures, because neurological complications of the postictal state in patients are typically extended over a several-day period after the ictal episode (18). Cerebral vascular reactivity to endothelium-dependent and -independent vasodilators was detected in animals with 1) intact, 2) inhibited, and 3) upregulated HO activity. To inhibit HO activity in the brain in vivo, we used systemically administered SnPP because it selectively and effectively blocks HO activity and HO-mediated cerebral vascular responses and has little nonspecific effects in cerebral circulation (12). Systemically administered SnPP inhibited cerebral vasodilation to seizures and was better tolerated by animals than other HO inhibitors (12, 33). To upregulate HO activity in the brain before seizures, we used cobalt-containing metallocorphyrin (CoPP), a potent inducer of HO-1 expression by transcriptional upregulation (1, 24).

**METHODS**

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

**Induction of sustained seizures.** Newborn pigs (1–5 days old, 1.5–2.5 kg) were anesthetized with ketamine hydrochloride (15 mg/kg im) and acepromazine (1.6 mg/kg im) and then intubated through the mouth. To maintain physiological levels of blood gases and to keep arterial PO2 (PaO2) fixed, the animals were ventilated with a physiological minute volume with a gas mixture of 4% CO2-21% O2 and 5% dextrose (4 ml/min) and ventilated with room air, and equipped with catheters for infusion of artificial cerebrospinal fluid (aCSF) (in mM): 3.0 KCl, 1.5 MgCl2, 1.5 CaCl2, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO3 equilibrated with 6% CO2-6% O2-88% N2 to pH 7.3–7.35 at 37°C.

**Vascular reactivity.** To test vascular reactivity, three pial arterioles (60–80 μm) in each animal were selected for observation. For the control diameter values, pial arteriolar diameter (PAD) was measured over a 10-min period during baseline conditions. Hypercapnia (PaCO2, 84 ± 5 mmHg, PaO2, 100 ± 8 mmHg, pH, 7.00 ± 0.05) was induced for 10 min by ventilating piglets with a mixture of 10% CO2 and 21% O2 in 69% N2. At the end of the hypercapnia, the ventilation gas was returned to air, and the brain surface was flushed with aCSF for 20 min to allow all parameters (arterial blood gases and PAD) to return to baseline values. Bradykinin (10–8 M), isoproterenol (10–6 M), sodium nitroprusside (10–5 M), or hemin (10–5, 10–7, and 10–6 M) were applied to the cerebral surface, and changes in PAD were recorded. Between the applications, the brain surface was flushed with aCSF for 20 min. PAD was repeatedly measured three times over a 10-min period; the stable diameter achieved between 5 and 10 min was taken as the response.

**Experimental groups.** To test the postictal cerebral vascular reactivity, we selected 2 days after sustained seizures, because clinical complications of the postictal state are extended to several days after the ictal episode (18). Postictal cerebral vascular reactivity was tested in 1) intact animals (group I, n = 9); 2) saline-sham control animals (2 days after sham operation/anaesthesia, group II, n = 5); 3) saline-postictal animals (2 days after seizures, group III, n = 6); 4) SnPP-sham control animals (2 days after administration of SnPP, 3 mg/kg iv, and sham operation/anaesthesia, group IV, n = 4); as we have previously demonstrated, this dose of systemic SnPP inhibits HO in cerebral circulation as detected by CO production by the brain surface and by decreased cerebral vascular reactivity to a heme derivative (12); 5) SnPP-postictal animals (2 days after seizures and SnPP, 3 mg/kg, administered 30 min before bicuculline, group V, n = 5); 6) CoPP-sham control animals (2 days after sham operation/anaesthesia, CoPP, 50 mg/kg iv, was administered 24 h before the sham operation, group VI, n = 4); as our present results demonstrate, 50 mg/kg CoPP increases HO activity and enhances HO-mediated responses in cerebral circulation (see RESULTS); and 7) CoPP-postictal animals (2 days after seizures and CoPP, 50 mg/kg iv, administered 24 h before bicuculline, group VII, n = 5). SnPP or CoPP were dissolved in 0.4 ml of 10% ethanolamine, diluted with 6 ml saline, and administered intraperitoneally by a 0.45-μm Millipore filter-equipped syringe; both light-sensitive compounds were protected from light at all times. Ethanolamine alone (final concentration, 0.6%) had no effects on diameter of cerebral vessels or cerebral vascular reactivity.

**Isolation of cerebral microvessels and the brain parenchyma.** The brain was obtained from ketamine-acepromazine-anesthetized newborn pigs (31). The cerebral cortex was homogenized by using a glass homogenizer with a loosely fitted pestle in Krebs buffer (in mM: 120 NaCl, 5 KCl, 0.6 MgSO4, 1.8 CaCl2, 10 HEPES, and 6 glucose; pH 7.4). The homogenate was filtered through a 300-μm mesh filter and subsequently refiltered through a 60-μm filter. Cerebral microvessels (60–300 μm) collected on the last filter were resuspended in the Krebs buffer. The filtrate passed through a 60-μm filter was used as the vasculature-free brain parenchyma. The amounts of protein in preparations of cerebral microvessels and the brain parenchyma were quantified by the Lowry method (31).

**Cerebral microvascular endothelial cells in primary culture.** Cerebral microvascular endothelial cells were isolated from cerebral microvessels using collagenase-dispase digestion followed by Percoll density gradient centrifugation (31). Cerebral microvascular endothelial cells were plated on Matrigel-coated plates and grown in Dulbecco’s modified Eagle’s medium with 20% FBS, 30 μg/ml endothelial cell growth supplement, 1 μM hepaticin, and antibiotic-antimycotic mixture. Experiments were performed on confluent quiescent...
cells. Cells were incubated for 24 h in serum-depleted Dulbecco's modified Eagle's medium (0.1% FBS) without or with CoPP (10–100 μM). After the incubation, cells were rinsed with cold PBS, harvested by scraping, and lysed in the sample buffer (100°C for 10 min) for Western immunoblotting.

**HO activity in cerebral microvessels and in the brain parenchyma.** Cerebral microvessels and the brain parenchyma were isolated from the cerebral cortex of saline-control (n = 9 animals) or CoPP-treated piglets (n = 9 animals) as described above. HO activity was measured as CO production from exogenous HO substrate, a stable heme derivative heme-1-lysinate (HLL) (21). For HO activity detection, aliquots of cerebral microvessels or brain parenchyma (20–40 μg protein/tube) were incubated with 2 × 10^{-5} M HLL in the Krebs buffer for 40 min at 37°C (total volume, 1.7 ml). For quantification purposes, a saturated solution of heavy^{13}CO (1 mM, Isotech, Miamisburg, OH) was injected as the internal standard, and the vials were tightly sealed with Teflon-lined caps. During the incubation, CO formation in a HO-catalyzed reaction is released into the headspace. The reaction was stopped by heating the vials at 75°C for 10 min. CO detection in the headspace gas was performed using gas chromatography/mass spectrometry (Hewlett-Packard 5970 mass-selective ion detector interfaced to a Hewlett-Packard 5890A gas chromatograph) and quantified using the values for standard^{13}CO (21). The results were normalized to the amount of protein in the tissue samples. Each assay was performed in quadruplicates.

**Western immunoblotting.** Cell lysates (20 μg protein/lane) were resolved by 9% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Nonspecific binding was blocked with 5% bovine serum albumin-0.1% Tween 20. The membranes were probed with polyclonal anti-human HO-1 (SPA 895 from StressGen, at 1:5,000 dilution) or with polyclonal anti-human HO-2 (SPA 897 from Stress-Gen Biotechnologies, Victoria, Canada, at 1:5,000 dilution), followed by Peroxidase-conjugated donkey anti-rabbit IgG (dilution 1:10,000) (Jackson ImmunoResearch, West Grove, PA). As positive controls, we used recombinant rat HO-1 and human HO-2 proteins (StressGen). To detect other inducible endothelial vasorelaxant systems, membranes were probed with anti-inducible nitric oxide synthase (iNOS) monoclonal antibodies (Transduction Labs, at 1:2,000 dilution) followed by Peroxidase-conjugated donkey anti-mouse IgG (dilution 1:10,000) (Jackson Immunoresearch) or with COX-2 (human) antiserum (Cayman, at 1:10,000 dilution) followed by Peroxidase-conjugated donkey anti-rabbit IgG (dilution 1:10,000) (Jackson ImmunoResearch). For quantification purposes, the membranes were reprobed with monoclonal antibodies against actin (Chemicon International, Temecula, CA) (dilution 1:10,000) followed by Peroxidase-conjugated donkey anti-mouse IgG (Jackson Immunoresearch). The bands were visualized with the Western Lightning chemiluminescence kit (Perkin-Elmer Life Science Products, Boston, MA) and quantified using NIH image 1.63. The levels of optical saturation measured were verified to be submaximal and within the linear range of detection on all membranes.

**Statistical analysis.** Values are presented as means ± SE of absolute values or percentage of control. Analysis of variance with repeated measures and Fisher’s protected least significant difference test were used to confirm differences among and then between groups, respectively. A level of P < 0.05 was considered significant in all statistical tests.

**Materials.** HLL was prepared by using methods described previously (22), protected from light, and stored at −20°C. SnPP and CoPP were purchased from Frontier Scientific (Logan, UT). Pancuronium bromide was from Astra Pharmaceutical Products (Westborough, MA). Bicuculline, bradykinin, isoproterenol, sodium nitroprusside, and hemin were from Sigma (St. Louis, MO).

### RESULTS

**Systemic parameters in control and postictal experimental groups of animals.** We compared major systemic parameters in all intact control, sham control, and postictal groups of newborn piglets. No differences in mean arterial blood pressure, heart rate, blood gases, pH, and body temperature were observed among the experimental groups (Table 1), with all the parameters being within the physiological range for newborn piglets. These data indicate that sustained seizures have no prolonged postictal effects on the systemic parameters measured in newborn pigs.

**Postictal cerebral vascular responsiveness in animals with intact HO activity.** Two days after the seizures, we investigated cerebral vascular responses to endothelin-dependent (hypercarnia, bradykinin) and endothelium-independent (isoproterenol, sodium nitroprusside) vasodilators in animals with intact HO activity (saline-postictal group III). Cerebral vascular reactivity to both endothelium-dependent and -independent dilator stimuli was reduced by 30–50% compared with the reactivity in intact animals (group I) or in saline-sham control animals (group II) (Fig. 1). These data indicate that during the extended postictal period, a general impairment of cerebral vascular function is observed in animals with intact HO activity.

**Postictal cerebral vascular responsiveness in animals with inhibited HO activity.** Systemic SnPP (3 mg/kg) rapidly inhibits HO activity in the cerebral microcirculation in vivo as indicated by decreased CO production by the brain and by reduced cerebral vasodilation to HLL, a HO substrate (12). Cerebral vascular reactivity to heme, another HO substrate, was also greatly reduced 30 min after the administration of SnPP (see Fig. 5), consistent with the inhibition of HO activity in cerebral microvasculature. We investigated delayed effects of HO inhibition on cerebral vascular reactivity 2 days following the seizures and the inhibitor administration. SnPP (3

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**Table 1. Postictal systemic parameters in experimental groups of newborn piglets**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MABP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>P_{CO_2}, mmHg</th>
<th>P_{O_2}, mmHg</th>
<th>pH</th>
<th>T, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact animals (Group I)</td>
<td>9</td>
<td>64±4 (13)</td>
<td>136±9 (24)</td>
<td>39±2 (4)</td>
<td>104±6 (11)</td>
<td>7.38±0.02 (0.06)</td>
<td>37</td>
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<td>Salini</td>
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<tr>
<td>Sham control animals (Group II)</td>
<td>5</td>
<td>61±5 (9)</td>
<td>129±19 (33)</td>
<td>27±2 (4)</td>
<td>97±3 (5)</td>
<td>7.41±0.04 (0.09)</td>
<td>37</td>
</tr>
<tr>
<td>Postictal animals (Group III)</td>
<td>5</td>
<td>64±5 (12)</td>
<td>110±5 (13)</td>
<td>26±1 (2)</td>
<td>106±7 (17)</td>
<td>7.44±0.03 (0.09)</td>
<td>37</td>
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<tr>
<td>SnPP</td>
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<tr>
<td>Sham control animals (Group IV)</td>
<td>4</td>
<td>61±7 (14)</td>
<td>149±13 (31)</td>
<td>32±5 (9)</td>
<td>98±8 (15)</td>
<td>7.34±0.07 (0.13)</td>
<td>37</td>
</tr>
<tr>
<td>Postictal animals (Group V)</td>
<td>5</td>
<td>72±4 (14)</td>
<td>132±13 (28)</td>
<td>27±1 (2)</td>
<td>110±6 (13)</td>
<td>7.45±0.02 (0.07)</td>
<td>37</td>
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<tr>
<td>CoPP</td>
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<tr>
<td>Sham control animals (Group VI)</td>
<td>4</td>
<td>70±6 (10)</td>
<td>140±15 (29)</td>
<td>32±4 (7)</td>
<td>98±5 (11)</td>
<td>7.32±0.07 (0.12)</td>
<td>37</td>
</tr>
<tr>
<td>Postictal animals (Group VII)</td>
<td>5</td>
<td>69±4 (13)</td>
<td>139±11 (27)</td>
<td>36±3 (3)</td>
<td>100±5 (12)</td>
<td>7.39±0.02 (0.06)</td>
<td>37</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of piglets. MABP, mean arterial blood pressure; P_{CO_2}, arterial P{CO_2}; P_{O_2}, arterial P{O_2}; T, temperature; SnPP, tinprotoporphrin; CoPP, cobalt protoporphyrin. Values in parentheses represent significant difference.
mg/kg ip, SnPP-sham control group IV itself did not cause changes in cerebral vascular reactivity to hypercapnia, bradykinin, isoproterenol, or nitroprusside 2 days after the inhibitor administration and sham operation/anesthesia (Fig. 2). However, when seizures were induced in SnPP-pretreated animals (SnPP-postictal group V), cerebral vascular responses to endothelium-dependent and -independent dilators 2 days after the seizures were severely impaired (40–50% of inhibition, Fig. 2). Moreover, during the extended postictal period, two animals in SnPP-postictal group V showed behavioral abnormalities, including improper body coordination during standing and walking. In these animals, vasodilator responses of pial arterioles were inhibited to even a greater extent (70–90% inhibition).

Effects of CoPP on HO expression and activity in cerebral microcirculation in vitro and in vivo. In primary cultures of cerebral microvascular endothelial cells, CoPP (10–50 μM, 17–24 h) dose-dependently upregulated the inducible HO-1 isoform (Fig. 3) without altering HO-2 expression (data not shown). CoPP did not upregulate other immediate response genes iNOS or COX-2 and did not alter the expression of actin, a housekeeping gene product (Fig. 3), indicating selective targeting of HO-1 expression. We investigated whether CoPP can upregulate HO-1 expression and activity in cerebral circulation in vivo. Systemic administration of CoPP to newborn piglets (50 mg/kg ip) resulted in HO-1 (but not HO-2) upregulation in the brain tissue and in cerebral microvessels within 24 h as detected by Western immunoblotting (Fig. 4, inset). HO activity was detected as CO production from exogenous substrate, a stable heme derivative HLL, by cerebral microvessels and the brain parenchyma isolated 24 h after the treatment with CoPP or saline. In CoPP-treated animals, a greater HO activity was detected in cerebral microvessels (2.5 ± 0.3-fold over the control level) and, to a significantly smaller extent (1.4 ± 0.2-fold over the control), in the brain parenchyma (Fig. 4). CO-dependent vasodilator responses to hemin (a HO substrate) were greatly increased in CoPP-treated animals 24 h after the treatment (Fig. 5). Overall, these data demonstrate that systemic administration of CoPP results in functional upregulation of HO activity in the cerebral microcirculation.

Postictal cerebral vascular responsiveness in animals with upregulated HO activity. We investigated delayed effects of HO-1 upregulation on postictal cerebral vascular reactivity. CoPP was systemically administered (50 mg/kg ip) to animals 24 h before the sham operation (CoPP-sham control group VI) or before seizures (CoPP-postictal group VII), and cerebral vascular responses to hypercapnia, bradykinin, isoproterenol, and nitroprusside were assessed 2 days after the sham operation or the seizure episode. Systemic administration of CoPP itself (group IV) did not cause any long-term changes in cerebral vascular reactivity compared with saline-sham control group VI.
The sustained postictal state is associated with a variety of neurological problems in patients. Inadequate cerebral vascular functioning and loss of cerebral autoregulation during the postictal period may greatly contribute to neuronal damage. However, cerebral vascular function during the extended postictal period has been completely ignored. Using a bicuculline-induced model of seizures in newborn piglets, we questioned whether epileptic seizures cause extended postictal cerebral vascular dysfunction as a manifestation of vascular injury, and whether enhancement of a potential cellular defense system can protect against vascular damage. Our novel findings are as follows: 1) sustained seizures cause severe postictal cerebral vascular dysfunction that is evident at least 2 days after the ictal episode; 2) during the extended postictal period, cerebral vascular responses to both endothelium-dependent and -independent, physiologically relevant vasodilators (hypercapnia, bradykinin, isoproterenol, and sodium nitroprusside) are greatly reduced; 3) inhibition of HO-2 activity in cerebral circulatory system by systemic SnPP during the ictal state exacerbates postictal cerebral vascular dysfunction, including endothelium-dependent vascular dilator responses; and 4) upregulation of HO-1 expression and activity in the cerebral microcirculation by systemic CoPP before and during the ictal state completely prevents cerebral vascular dysfunction during the postictal period.

Seizures consist of massive depolarization of network neurons that result in excessive release of excitatory neurotransmitters, bursts of action potentials, and massive entry of calcium (25). Animal and human studies suggest that seizures can damage the brain cells, modify brain functions, and increase the risk for persistent epilepsy (7, 25, 32, 39). Neuronal death has been described following status epilepticus in hypermetabolic brain areas, mostly neocortex and hippocampus (25, 37, 39, 41). In young experimental animals, repeated seizures had long-lasting debilitating effects on brain development, learning abilities, and behavioral milestones (3, 35, 37, 41).

Very little is known about long-term consequences of seizures on the cerebral microcirculation. Clinical evidence strongly suggests that epileptic seizures can be associated with cerebral vascular injury, including acute vascular lesions in the brain (25) and vascular malformations in the cerebral cortex (40). Seizure-traumatized human brain regions in human patients and in animal models are characterized by ultrastructural changes in brain capillaries and severe disruption of the blood brain barrier (8, 14, 29). Likely consequences of the seizure-induced cerebral vascular damage could be disturbances in cerebral blood flow regulation, inability to adequately adjust cerebral vascular reactivity to vasodilators (Fig. 6). When seizures were induced in CoPP-pretreated animals, postictal cerebral vascular reactivity to endothelium-dependent and -independent stimuli was not decreased (Fig. 6).

Overall data summarized in Fig. 7 show a dramatic decrease in overall cerebral vascular reactivity to physiologically relevant vasodilators during the extended postictal period (2 days after the seizure episode) in control animals and, especially, in animals with inhibited HO activity. In contrast, no sustained postictal cerebral vascular dysfunction was observed in animals with upregulated HO expression/activity. These data indicate that in contrast to the animals with normal HO activity, the animals with upregulated HO activity were completely protected against postictal cerebral vascular dysfunction.

**DISCUSSION**

The sustained postictal state is associated with a variety of neurological problems in patients. Inadequate cerebral vascular functioning and loss of cerebral autoregulation during the postictal period may greatly contribute to neuronal damage. However, cerebral vascular function during the extended postictal period has been completely ignored. Using a bicuculline-induced model of seizures in newborn piglets, we questioned whether epileptic seizures cause extended postictal cerebral vascular dysfunction as a manifestation of vascular injury, and whether enhancement of a potential cellular defense...
blood supply to the brain in a constantly changing environment, and, possibly, subsequent neuronal damage. Remarkably, only a few studies have investigated the postictal cerebral vascular reactivity. In newborn piglets, immediately following the seizures, a loss of autoregulation of cerebral blood flow was observed (27), whereas cerebral vascular reactivity to adenosine, hypercapnia, and isoproterenol remained unaltered (16). To the best of our knowledge, no data are available on cerebral vascular dysfunction and, possibly, vascular injury. Remarkable, only a few studies have investigated the postictal cerebral vascular reactivity. In newborn piglets, immediately following the seizures, a loss of autoregulation of cerebral blood flow was observed (27), whereas cerebral vascular reactivity to adenosine, hypercapnia, and isoproterenol remained unaltered (16). To the best of our knowledge, no data are available on cerebral vascular function during the extended postictal period.

To assess cerebral vascular function during the extended postictal period, we measured cerebral vascular reactivity to endothelium-dependent (hypercapnia, bradykinin) and endothelium-independent dilators (isoproterenol, a β-adrenoreceptor agonist, and sodium nitroprusside, a nitric oxide donor) that dilate smooth muscle via cAMP- and cGMP-dependent mechanisms, respectively. Our previous data demonstrate that during the early recovery period after sustained seizures, cerebral vascular responses to these vasodilators were within normal range (12). In contrast, our present data show that during extended postictal period (2 days after the ictal episode), cerebral vascular responses to hypercapnia and bradykinin were greatly reduced, reflecting possible endothelial dysfunction. Moreover, postictal cerebral vascular responsiveness to smooth muscle-directed dilators was also substantially decreased. Although a few morphological studies present evidence of endothelial damage in cerebral microvessels following sustained seizures in human patients and in animal models (8, 14, 29), no data on cerebral smooth muscle injury are available. Our data clearly indicate sustained postictal general cerebral vascular dysfunction and, possibly, vascular injury. Cerebral vascular dysfunction may result in loss of autoregulation and inadequate blood supply to the brain, potentially exacerbating the long-term damaging effects of sustained seizures in the brain function.

Our previous data demonstrate that when brain HO activity (HO-2 isoform) was inhibited during the ictal episode, the amplitude and duration of the cerebral vasodilator response to seizures was greatly reduced despite continuous neuronal discharges (12, 30). As a result of mismatch between sustained epileptic neuronal discharges and cerebral blood flow, in animals with inhibited HO activity cerebral vascular responses were substantially impaired during early recovery period (12). Our present data demonstrate that in SnPP-treated animals cerebral vascular dysfunction was extended for at least up to 2 days of the postictal period; endothelium-dependent responses in these animals were impaired even to a greater extent than endothelium-independent reactions. Inhibition of HO-2 activity at the time of the seizure episode is critical in cerebral vascular damage observed during early (2 h) and extended (2 days) postictal periods.

HO-2 is the only constitutively expressed isoform in the brain and in cerebral vasculature (12, 31). Cobalt-containing metalloporphyrins induce HO-1 expression in vitro and in vivo by transcriptional upregulation (1, 24). CoPP upregulated HO-1 in cerebral vascular endothelium in vitro without altering the expression of other immediate early genes iNOS and COX-2. Treatment of newborn piglets with CoPP in vivo (50 mg/kg ip for 24 h) resulted in HO-1 induction in the cerebral microcirculation as characterized by HO-1 expression and increased HO activity in the brain and in the cerebral microvessels. HO-mediated vasodilation to heme was greatly amplified in CoPP-treated piglets, indicating that HO-1-derived CO acts as a vasodilator in cerebral microcirculation. Overall, these data strongly suggest that systemically administered CoPP does induce functionally active HO-1 in the cerebral microcirculation in vivo.

We investigated whether HO-1 overexpression in the brain during the ictal episode can reduce postictal cerebral vascular dysfunction. When seizures were induced in animals with overexpressed HO (CoPP-treated piglets), no cerebral vascular dysfunction was observed during early (2 h) and extended (2 days) postictal periods.
dysfunction was observed during extended postictal period. In clear contrast to animals with intact HO activity, in HO-
overexpressing piglets, postictal cerebral vascular reactivity to endothelium-dependent and -independent vasodilators as-
essessed 2 days after the ictal episode was within the control range, or even slightly exaggerated. This finding indicates that overexpression of HO-1 in the brain can prevent long-term debilitating effects of seizures in the cerebral circulation.

What are the mechanisms by which HO-1 overexpression in the brain can prevent postictal cerebral vascular injury? The vasodilator CO, formed via the activity of constitutively expressed HO-2, is important in the regulation of blood flow to the brain during seizures (12, 28, 33). Seizures are associated with a rapid increase in CO formation in the cerebral circulation that correlates with pial arteriolar dilation (12). However, the CO level returned to baseline by 1 h after the seizure induction, while continuous neuronal discharges were extended for at least 2 h (12, 30). Mismatch between the brain perfusion and neuronal activity during sustained seizures could account for the extended postictal cerebral vascular dysfunction. Moreover, when brain HO activity was inhibited during the ictal episode, a severe reduction of cerebral vascular responsiveness indicative of vascular injury was observed in early postictal period (12). Therefore, it is possible that when HO activity is enhanced by HO-1 induction, a CO-dependent component of the blood flow to the brain is better correlated with the neuronal activation, thus protecting neurons and preventing cerebral vascular injury.

Another aspect of the protective role of HO activity in cerebral circulation could be related to antioxidant properties of the HO pathway. Excessive formation of reactive oxygen species in the brain that occurs during the ictal state is recognized as a leading factor in seizure-induced brain injury (2, 25, 37, 39). In addition, seizures are often associated with the brain hemorrhage due to blood flow, perfusion pressure, and intracranial pressure fluctuations. HO activity results in a decrease of the amount of prooxidant heme and in formation of a potent antioxidant bilirubin. Antioxidative capacity of bilirubin is attributed to the ability to undergo redox cycling (4, 26, 38). Bilirubin may be particularly important as a cytoprotector for tissues with weak antioxidant defenses, such as the nervous system (17, 24). Bilirubin, associated with the physiological jaundice in newborn babies, is neuroprotective against retinop-
athy in premature babies (5, 19). In addition, recent data in vascular endothelial and smooth muscle cells indicate that CO itself may have anti-apoptotic effects (10, 23). Of course, CO binds strongly to heme and can be lethal to animals at high doses by blocking oxygen transport via hemoglobin. However, CO at low concentrations prevented apoptosis induced by cytokines in vascular smooth muscle cells (23). It has been suggested that the mechanism of CO protection involves interaction with the key components of a protein phosphorylation cascade (10) and inhibition of the cytochrome C release from mitochondria (23).

Overall, our experimental data in newborn piglets clearly indicate that cerebral vascular dysfunction indicative of cere-
bral vascular injury is a long-term consequence of sustained seizures. Upregulation of HO-1, a potent vasodilator and anti-
oxidant cell defense system in the brain, can prevent postictal cerebral vascular injury. These findings are of potential clinical importance because inappropriate cerebral vascular respon-
siveness to a constantly changing environment may contribute to long-term neurological complications that occur in newborn babies following sustained seizures. Neonatal early childhood convulsions have been correlated with neuronal loss, atrophy, and memory impairment in patients (13, 20), and with increased chances of subsequent development of chronic epi-
lepsy (15, 20, 35, 41). In the United States, there are approxi-
mately 125,000 new cases of epilepsy each year; the largest number of newly diagnosed cases occurs among children under the age of 2 years (36). It has been repeatedly emphasized that seizure-induced brain damage cannot be ignored and that damage to the brain as a consequence of seizures should be broadened to include potentially irreversible or permanent dysfunction to all brain components.

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GRANTS

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