Postischemic Cerebral Microvascular Responses to Norepinephrine and Hypotension in Newborn Pigs

Charles W. Leffler, PhD, David W. Busija, PhD, Donathan G. Beasley, MS, William M. Armstead, PhD, and Robert Mirro, MD

We examined the effects of 20 minutes' cerebral ischemia on cerebral microcirculatory responses to topical norepinephrine and systemic hypotension in three groups (sham-operated control, 2–3 hours postischemia, and 24 hours postischemia) of anesthetized newborn pigs equipped with closed cranial windows. Cerebral ischemia may eliminate the prostanoid vasodilator system from the cerebral circulation. Norepinephrine (10⁻⁴ M) decreased pial arteriolar diameters similarly in all three groups (27%, 28%, and 21%, respectively), but only the sham-operated group exhibited pial arteriolar dilation in response to hypotension (28% at 33 mm Hg). Two–three and 24 hours after cerebral ischemia, hypotension decreased pial arteriolar diameters (21% and 17%, respectively). In sham-operated piglets, norepinephrine and hypotension increased cortical periarachnoid cerebrospinal fluid prostanoid concentrations. However, neither norepinephrine nor hypotension altered cerebral prostanoid production 2–3 or 24 hours after cerebral ischemia. Therefore, we conclude that after cerebral ischemia, autoregulatory pial arteriolar dilation in response to hypotension is absent, while vasoconstriction in response to norepinephrine is intact. (Stroke 1989;20:541–546)

Impairment of cerebral autoregulation after cerebral ischemia has been described in adult animals and humans. In newborn babies and lambs, similar impairment after severe hypoxia or asphyxia has been reported. Prostanoids provide an important vasodilator mechanism in the newborn pig brain and mediate cerebral vasodilator responses to hypotension and asphyxia. Dilator prostanoids also attenuate constrictor responses to norepinephrine. Ischemia could affect the prostanoid dilator component of cerebral blood flow regulation, but it is not known whether cerebral ischemia alters the cyclooxygenase pathway in newborn pigs. Therefore, our experiments in newborn pigs address the hypothesis that cerebral ischemia alters the response of cerebral resistance vessels to hypotension and that this failure of autoregulation is in part due to impairment of the vasodilator prostanoid system.

Materials and Methods

The animal protocols used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee, Memphis. Under 0.7–1.5% halothane and 80% nitrous oxide anesthesia administered by mask, hollow stainless steel bolts were implanted aseptically in the skulls of 19 1-day-old piglets without damaging the dura. Catheters were also placed in the aortae for blood withdrawal and blood pressure monitoring. Three days later, 20 minutes of total brain ischemia was produced by increasing the intracranial pressure. Artificial cerebrospinal fluid (CSF) at 37°C was infused into the hollow bolt in the skull of each mechanically ventilated, unanesthetized piglet to maintain intracranial pressure at 15 mm Hg above mean arterial blood pressure. Arterial blood was withdrawn as necessary to maintain mean arterial blood pressure at ≤100 mm Hg. Within 5 seconds of increasing the intracranial pressure, all piglets were unresponsive to tactile and auditory stimuli and their pupils were fixed and dilated. There was no evidence of pain and no apparent awareness of the procedure before loss of consciousness. In preliminary experiments on eight piglets, we found
that this procedure reduces blood flow throughout the brain and spinal cord to levels that are not detectable using radiolabeled microspheres. Spontaneous ventilation resumed after 30-60 minutes of reperfusion.

Piglets were divided into three groups for examination of the pial microcirculation using cranial window techniques. There were two subgroups of sham-operated (control) piglets treated identically to those in the other two groups except that intracranial pressure was increased to approximately 100 mm Hg for only 1-2 seconds, returned to baseline, and the cranial window was implanted 24 hours later (n=4) or the bolt was inserted but intracranial pressure was not increased (n=3). Since data from these two subgroups of sham-operated piglets were virtually identical and were similar to control values obtained in other experiments, these seven piglets were considered the sham-operated control group for statistical purposes. In the second group of six piglets (2-3 hours postischemia), surgery for implantation of the cranial window was begun after 50 minutes of reperfusion, allowing the microcirculatory studies to begin after 2 hours of reperfusion. In the third group of six piglets (24 hours postischemia), the cranial window was implanted after 24 hours of brain ischemia. Piglets in the 24 hours postischemia group were fed pig milk substitute by gavage since they did not regain consciousness.

Newborn pigs were anesthetized with 33 mg/kg i.m. ketamine hydrochloride and 3.3 mg/kg i.m. acepromazine and maintained on a-chloralose (50 mg/kg i.v. initially, then 10 mg/kg/hr). The piglets were intubated and ventilated with air. Catheters were inserted in the femoral vein and artery. Body temperature was maintained at 37-38° C. The scalp was retracted, and a 2-cm-diameter hole was made in the skull over the parietal cortex at a site remote from the hollow bolt. 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 damaged bone or damaged membranes. A stainless steel-and-glass cranial window was placed in the hole and cemented into place with dental acrylic. Through needles incorporated into the sides of the window, we filled the space under the window with artificial CSF of the following composition (meq/l): 150 Na⁺, 3 K⁺, 2.5 Ca²⁺, 1.2 Mg²⁺, 132 Cl⁻, 25 HCO₃⁻, with 3.7 mM glucose and 6 mM urea at a pH of 7.33, a PCO₂ of 46 mm Hg, and a PO₂ of 43 mm Hg, at 37° C. The volume of fluid directly under the window was 500 μl and was contiguous with the periarachnoid space. After implanting the window, we allowed 20 minutes for exchange and equilibration of fluid under the window with the periarachnoid fluid before beginning the experiment.

We observed pial arterioles with a trinocular stereomicroscope. We measured pial arteriolar diameter with a television camera mounted on the microscope, a video monitor, and a video microscanner (Model VPA-1000, FOR-A, Los Angeles, California).

Cerebral surface CSF (300 μl) was collected by placing a 1-ml syringe on an injection port of the cranial window. We collected CSF by slowly infusing artificial CSF into one side of the window and allowing the CSF under the window to drip freely into a collection tube on the opposite side.

The experimental design consisted of measurements of pial arteriolar diameter, arterial blood pressure, arterial blood gases and pH, and collection of cortical periarachnoid CSF. After 20 minutes of stabilization, we flushed the window with artificial CSF and collected CSF 10 minutes later. Then 10⁻⁴ M norepinephrine dissolved in artificial CSF was injected under the cranial window. After a 10-minute period, CSF under the cranial window was collected for prostanoid analysis. We then flushed the window with artificial CSF twice at 5-minute intervals, followed by another 10-minute collection period. The arterial blood pressure of the piglet was rapidly decreased by phlebotomy to 25-35 mm Hg. After another 10-minute period, final measurements and collection of cortical periarachnoid CSF were made. We analyzed arterial blood gases and pH from samples taken at the beginning and end of each microcirculatory experiment.

Prostanoids (6-ketoprostaglandin F₁₀ [6-keto-PGF₁₀], thromboxane B₂ [TXB₂], PGE₂, and PGF₂α) in cortical periarachnoid CSF were assayed by radioimmunoassay against an artificial CSF matrix as described previously. All unknowns were assayed at three dilutions, with parallelism between the unknown dilution curve and the standard curve required before the result was used. The sample dilutions we used allowed analysis of prostanoid concentrations between 100 and 50,000 pg/ml. Using this assay previously, we demonstrated large proportional increases in the concentrations of prostanoids after topical application of arachidonic acid and >90% decreases in the concentrations of all prostanoids examined in the cortical periarachnoid CSF after treatment with 10 mg/kg i.v. indometh-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pHa</th>
<th>Paco₂ (mm Hg)</th>
<th>Paco₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>End</td>
<td>Beginning</td>
</tr>
<tr>
<td>Sham operation</td>
<td>7</td>
<td>7.54±0.06</td>
<td>34 ±2</td>
</tr>
<tr>
<td>24 hr postischemia</td>
<td>6</td>
<td>7.49±0.06</td>
<td>33 ±2</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
TABLE 2. Effects of Topical Norepinephrine on Pial Arterioles and Arterial Pressure of Newborn Pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>185±18</td>
<td>135±19*</td>
</tr>
<tr>
<td>Postischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3 hr</td>
<td>164±21</td>
<td>111±15*</td>
</tr>
<tr>
<td>24 hr</td>
<td>206±22</td>
<td>162±18*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. *p<0.05 different from sham-operated controls.

Acin under baseline conditions and when stimulated with exogenous arachidonic acid,10 Our antibodies cross-react minimally (<1%) with the other prostanoids studied and with other eicosanoids examined (arachidonic acid, 5-hydroxyicosatetraenoic acid [5-HETE], 12-HETE, 15-HETE, leukotriene B4 [LTB4], LTC4, LTD4, LTE4, lipoxin A4, lipoxin B4, and PGD2).

All values are presented as mean±SEM. Two groups were compared using paired t tests. More than two groups were compared using analysis of variance followed by t tests with Bonferroni’s correction. For inference that groups were different, p<0.05 was required.

Results

There were no differences in blood gases or pH between the groups or from the beginning to the end of the microcirculatory experiment (Table 1).

Topical application of 10^-4 M norepinephrine caused similar constrictions of pial arterioles regardless of treatment group (Table 2, Figure 1). In contrast, we observed the pial arteriolar dilator response to hypotension only in the sham-operated piglets (Table 3, Figure 2). Two–three and 24 hours after cerebral ischemia, systemic hypotension caused a decrease in pial arteriolar diameter similar to that obtained by topical application of 10^-4 M norepinephrine (Figures 1 and 2).

Two–three and 24 hours after cerebral ischemia, arterial blood pressure appeared to be less than that in the sham-operated piglets (Table 3). Therefore, it was possible that the apparently (but not significantly) reduced arterial blood pressures in the hypotensive postischemia piglets compared with the sham-operated piglets could contribute to the disparity in responses. To address this possibility, we compared the three piglets with the lowest blood pressures in the sham-operated group with the two piglets with the highest blood pressures in each postischemia group combined; the arterial blood pressures in these two subgroups were almost identical (Table 4). Nevertheless, we observed the disparate responses of pial arteriolar diameter to hypotension.

Cortical periarachnoid 6-keto-PGF1α and PGE2 concentrations appeared to be increased 2–3 hours after ischemia compared with those in sham-operated piglets, but the differences were not significant (Figure 3); TXB2 and PGF2α concentrations also were not different. In contrast, 24 hours after ischemia 6-keto-PGF1α and PGE2 concentrations in cortical periarachnoid CSF were significantly decreased compared with those in sham-operated piglets (Figure 3).

In sham-operated piglets, topical application of 10^-4 M norepinephrine stimulated cortical prostanoid synthesis, as indicated by an increase in cortical periarachnoid prostanoid concentrations (Figure 3). In contrast, norepinephrine failed to
stimulate cortical prostanoid synthesis either 2–3 hours or 24 hours after cerebral ischemia (Figure 3).

Systemic hypotension in sham-operated piglets caused a pronounced increase in cortical periarachnoid 6-keto-PGF₁α and PGE₂ concentrations, with a smaller but significant increase in TXB₂ concentration (Figure 4). In contrast, hypotension did not alter cortical prostanoid production in piglets 2–3 hours or 24 hours after cerebral ischemia (Figure 4).

**Discussion**

Our results demonstrate that while cerebral ischemia does not alter the cerebral microcirculatory constrictor effects of topical norepinephrine, pial arteriolar vasodilation is abolished in response to systemic hypotension. In addition, after cerebral ischemia, cerebral prostanoid stimulation by both norepinephrine and hypotension is absent, which could contribute to the abnormal response.

Prostanoids are an integral component of the regulation of cerebral hemodynamics in the perinatal period. In particular, cerebral vasodilation in response to systemic hypotension is accompanied by an increase in cerebral vasodilator prostanoid production, which produces cortical periarachnoid concentrations near the top of the vasodilatory concentration-response curve of pial arterioles to exogenous prostanoids. These vasodilator prostanoids appear to be critical to the ability of neonatal pigs to maintain cerebral blood flow at low arterial blood pressures since treatment of hypotensive piglets with 5 mg indomethacin/kg produces a marked decline in cerebral blood flow, which leads to coma. Thus, one would predict the coupling of a lack of prostanoid response to hypotension with a failure of autoregulatory vasodilation, as we observed.

Arachidonic acid metabolism can be involved in ischemia/reperfusion-associated vascular and metabolic abnormalities. Ischemia results in release of free fatty acids, arachidonate in particular, which accumulate throughout the ischemic period when oxygen is not available. Upon reperfusion, arachidonic acid metabolism can occur, resulting in cyclooxygenase products, lipoxygenase products, and activated oxygen. In newborn pigs, during reperfusion after cerebral ischemia there is a large generation of superoxide anion, which can be blocked by previous treatment with indomethacin. Superoxide anion is dismutated to hydrogen peroxide, and the extremely reactive hydroxyl radical is produced via iron-catalyzed reactions. Hydroxyl...


radical could inhibit the activity of cyclooxygenase, and cyclooxygenase could self-deactivate, thereby greatly reducing the generation of cyclooxygenase products after large bursts of activity. In addition, 15-HETE, which would be produced by 15-lipoxygenase metabolism of arachidonic acid, is a potential inhibitor of cyclooxygenase. Thus, during the immediate reperfusion period the potential exists for an increased rate of arachidonic acid metabolism to greatly depress subsequent prostanoid synthesis. Although there was no significant increase in cortical periarachnoid prostanoid concentrations by 2 hours of reperfusion, the apparently elevated levels coupled with the greatly decreased levels of these prostanoids at 24 hours of reperfusion suggest that prostanoid synthesis could have been markedly stimulated earlier during reperfusion. Of course, alterations occurring during ischemia and reperfusion that affect cellular metabolism could result in a decrease in arachidonic acid release in response to stimuli, either because of damage to stimulus-effector coupling or diminished phospholipase A2 activity. Further, it is possible that lipid peroxidation in membranes caused by activated oxygen species could attenuate release of free arachidonic acid in response to stimuli.

Sympathetic vasoconstriction in the pial circulation of newborn pigs appears to be opposed by vasodilator prostanoids. Our results using norepinephrine indicate that after cerebral ischemia sympathetic vasoconstriction remains functional in the pial circulation but that the inhibitory vasodilator prostanoid component is eliminated. Therefore, one might expect vasoconstriction in response to topical application of norepinephrine to be augmented after cerebral ischemia, but it was not. In a previous study of newborn pigs, we found that indomethacin treatment uncovered a constrictor response at a previously subthreshold dose of norepinephrine ($10^{-6}$ M). It is possible that the higher dose of norepinephrine ($10^{-4}$ M) used in our present study overwhelmed the counteracting prostanoid effect in the sham-operated piglets. In fact, in piglets not subjected to ischemia, we found that indomethacin did not potentiate constriction to $10^{-4}$ M norepinephrine (unpublished observations).

Newborn babies may experience impaired cerebral blood flow or severe cerebral asphyxia due to difficulties associated with labor, delivery, and ventilation. It has been reported that, subsequent to such episodes, control of cerebral blood flow is compromised. Such compromised cerebral vascular control may further reduce the infant's ability to respond to added stresses. These include repeated episodes of hypotension or hypoxia, which are common occurrences with such newborns. The mechanisms behind these cerebrovascular abnormalities in newborn babies have not been elucidated but could involve arachidonic acid metabolism, as suggested by our results.

In summary, 20 minutes of total cerebral ischemia in newborn pigs produces abnormalities in cerebral vascular control. These abnormalities are observed 2–3 and 24 hours after the ischemic episode. Specifically, while vasoconstrictor responses to exogenous norepinephrine remain intact, vasodilator responses to systemic hypotension are abolished. Neither stimuli, which in the intact cerebral circulation stimulate prostanoid synthesis, produce any changes in cerebral prostanoid synthesis after cerebral ischemia. Thus, cerebral ischemia appears to eliminate an important vasodilator system from the cerebral circulation.

Acknowledgments

We acknowledge the excellent technical assistance of D. Hardy, M. Jackson, L. Doty, T. Nilson, J. Giddens, and N. Leffler.

References


**KEY WORDS**  
- cerebral ischemia  
- microcirculation  
- norepinephrine  
- pigs
Postischemic cerebral microvascular responses to norepinephrine and hypotension in newborn pigs.
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Stroke. 1989;20:541-546
doi: 10.1161/01.STR.20.4.541

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