Cerebral Vasoconstriction in Response to Hypocapnia Is Maintained After Ischemia/Reperfusion Injury in Newborn Pigs

Robert Mirro, MD†; Lisa Lowery-Smith, MD; William M. Armstead, PhD; Masaaki Shibata, PhD; Samuel L. Zuckerman, MD; and Charles W. Leffler, PhD

Background and Purpose: Hypocapnic cerebral vasoconstriction is used therapeutically to reduce elevated intracranial pressure caused by cerebral edema. Because cerebral ischemia/reperfusion injury causes a selective loss of prostanoid-dependent responses, including vasodilation to hypercapnia, we designed these experiments to examine the effect of ischemia/reperfusion on hypocapnic cerebral vasoconstriction.

Methods: Microvascular responses were studied in 10 newborn pigs (closed cranial window) in response to hyperventilation-induced hypocapnia (Paco₂, 22±2 mm Hg) both before and 45 minutes after 20 minutes of global cerebral ischemia. Responses to hypercapnia (Paco₂, 63±3 mm Hg), topical isoproterenol (10⁻⁷ M), and norepinephrine (10⁻⁴ M) were also studied before and after ischemia in the same animals for comparison.

Results: Before ischemia/reperfusion, pial arterioles vasoconstricted to hypocapnia (—17±2%) and norepinephrine (—35±4%) and vasodilated to CO₂ (37±7%) and isoproterenol (25±2%). After ischemia/reperfusion, the constriction of pial arterioles to hypocapnia (—19 ±2%) was similar to that before ischemia. This is in contrast to the loss of dilation to hypercapnia. Dilation to isoproterenol and constriction to norepinephrine were not affected by ischemia.

Conclusions: Hypocapnic cerebral vasoconstriction is maintained after ischemia/reperfusion. Since prostanoid-dependent responses, such as hypercapnic dilation, are lost following cerebral ischemia, these data suggest that hypocapnic constriction is not dependent on an intact prostanoid system and that cerebral vascular responses to CO₂ involve multiple mechanisms, depending on whether CO₂ is increasing or decreasing from baseline. (Stroke 1992;23:1613–1616)

KEY WORDS • cerebral circulation • cerebral ischemia • hypercapnia • pigs
The scalp was removed, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura and arachnoid membranes were cut without touching the brain, and all cut edges were reflected over the bone so that the periarachnoid space was not exposed to damaged tissue. A stainless steel and glass cranial window was placed in the hole and cemented into place with bone wax and dental acrylic. After implantation of the window and bolt, at least 20 minutes were allowed before experimentation was begun. Briefly, a manometer and aCSF reservoir were connected to the hollow bolt and the intracranial pressure increased to 15 mm Hg above mean arterial blood pressure. In addition, the animals had blood withdrawn to limit the Cushing response. This was usually 10–20 ml/kg blood to limit the blood pressure to a maximum of 100 mm Hg. This procedure produces zero cerebral blood flow as previously measured by microspheres. Cerebral ischemia was maintained for 20 minutes, the pressure was released to atmospheric, and the hollow bolt resealed. A reperfusion period of 45 minutes then followed, and the tests of vascular reactivity were repeated in random order.

Data were analyzed using an analysis of variance for repeated measures and Scheffe’s post hoc test. In all cases, a value of \( p < 0.05 \) was considered significant. Values are reported as mean±SEM.

### Results

Mean arterial blood pressures, arterial blood gases, and \( \mathrm{pH} \) values are shown in Table 1. During hyperventilation, \( \mathrm{pH} \) increased and \( \mathrm{Paco}_2 \) decreased. Conversely, during hypercapnia, \( \mathrm{pH} \) decreased and \( \mathrm{Paco}_2 \) increased. These changes were not different before and after ischemia. Arterial blood pressure and \( \mathrm{Pao}_2 \) were not significantly altered by any intervention.

Table 2 shows the changes in pial arteriolar diameters, and percent change in vessel size is depicted in Figure 1. The most important finding is that pial arterioles constricted similarly in response to hyperventilation (hypocapnia; \( \mathrm{Paco}_2 = 22 \) mm Hg) both before and after ischemia/reperfusion. In addition, greater hyperventilation (\( \mathrm{Paco}_2 = 15 \) mm Hg) produced similar pial arteriolar constriction before and after ischemia/reperfusion (28±2% versus 26±1% constriction before and after ischemia, respectively; \( n = 4 \)). In contrast, hypercapnic dilation was lost after ischemia/reperfusion, and the responses to isoproterenol and norepinephrine were unchanged by ischemia/reperfusion. Figure 1 illustrates constriction in response to \( 10^{-7} \) M norepinephrine. The dose–response relation between pial arteriolar diameter and norepinephrine was likewise unchanged by ischemia. Thus, constrictions to norepinephrine at \( 10^{-7} \)–

### Table 1. Mean Arterial Blood Pressure, Arterial Blood Gases, and \( \mathrm{pH} \) During Hyperventilation, Hypercapnia, Isoproterenol, and Norepinephrine Before and After Ischemia/Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HV(_1)</th>
<th>HV(_2)</th>
<th>CO(_2)</th>
<th>ISO</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before ischemia/reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>65±5</td>
<td>63±6</td>
<td>64±2</td>
<td>64±5</td>
<td>58±6</td>
<td>62±5</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.04</td>
<td>7.56±0.02*</td>
<td>7.63±0.02*</td>
<td>7.15±0.03*</td>
<td>7.43±0.03</td>
<td>7.43±0.04</td>
</tr>
<tr>
<td>( \mathrm{Paco}_2 ) (mm Hg)</td>
<td>41±3</td>
<td>22±1*</td>
<td>14±1*</td>
<td>63±3*</td>
<td>39±2</td>
<td>40±2</td>
</tr>
<tr>
<td>( \mathrm{Pao}_2 ) (mm Hg)</td>
<td>92±8</td>
<td>100±7</td>
<td>101±4</td>
<td>87±9</td>
<td>94±6</td>
<td>88±8</td>
</tr>
<tr>
<td><strong>After ischemia/reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>70±4</td>
<td>59±6</td>
<td>65±1</td>
<td>68±6</td>
<td>67±3</td>
<td>63±6</td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.03</td>
<td>7.50±0.06*</td>
<td>7.64±0.02*</td>
<td>7.16±0.04*</td>
<td>7.43±0.06</td>
<td>7.43±0.06</td>
</tr>
<tr>
<td>( \mathrm{Paco}_2 ) (mm Hg)</td>
<td>38±3</td>
<td>21±2*</td>
<td>14±1*</td>
<td>58±3*</td>
<td>38±1</td>
<td>39±3</td>
</tr>
<tr>
<td>( \mathrm{Pao}_2 ) (mm Hg)</td>
<td>86±6</td>
<td>97±8</td>
<td>97±3</td>
<td>81±7</td>
<td>92±10</td>
<td>92±8</td>
</tr>
</tbody>
</table>

Values are mean±SEM; \( n = 10 \). HV, hyperventilation (HV\(_1\), \( \mathrm{Paco}_2 = 22 \) mm Hg; HV\(_2\), \( \mathrm{Paco}_2 = 15 \) mm Hg); CO\(_2\), hypercapnia; ISO, isoproterenol; NE, norepinephrine; BP, blood pressure.

\* \( p < 0.05 \) different from control.
TABLE 2. Pial Arteriolar Diameters During Hyperventilation, Hypercapnia, Isoproterenol, and Norepinephrine Before and After Ischemia/Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HV</th>
<th>Control</th>
<th>CO₂</th>
<th>Control</th>
<th>ISO</th>
<th>Control</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia/reperfusion</td>
<td>86±5</td>
<td>73±4*</td>
<td>74±7</td>
<td>100±7*</td>
<td>75±7</td>
<td>95±9*</td>
<td>81±5</td>
<td>52±4*</td>
</tr>
<tr>
<td>After ischemia/reperfusion</td>
<td>93±7</td>
<td>75±6*</td>
<td>98±7</td>
<td>100±7</td>
<td>96±7</td>
<td>110±9*</td>
<td>95±6</td>
<td>67±6*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (in micrometers); n=10. HV, hyperventilation (PCO₂, 20 mm Hg); CO₂, hypercapnia; ISO, isoproterenol; NE, norepinephrine (10⁻⁴ M).
*p≤0.05 different from corresponding control value.

10⁻⁴, and 10⁻⁵ M were 9±1%, 16±2%, and 24±1% before and 9±1%, 15±1%, and 25±1% after ischemia, respectively (n=4).

Discussion

The new finding from the present experiment is that ischemia/reperfusion does not alter the cerebral vasocostriction induced by hypocapnia, in contrast to complete inhibition of hypercapnic cerebral vasodilatation. In addition, the dose–response relation between pial arteriolar diameter and norepinephrine was likewise unchanged by ischemia. The present study, therefore, extends the previous observation that pial arteriolar constriction in response to serotonin and angiotensin II was similarly unchanged after ischemia/reperfusion in cats. Impairment of cerebral vasodilation and preservation of cerebral vasocostriction could contribute to blunted reperfusion of the cerebral circulation after cerebral ischemia.

Control of regional perfusion by lowering PCO₂ is a therapeutic approach used to vasoconstrict the cerebral circulation, thus attenuating the elevated intracranial pressure caused by cerebral edema, and to vasodilate the pulmonary circulation in persistent pulmonary hypertension of the newborn. In both settings, hyperventilation decreases PCO₂ to levels at which cerebral blood flow is significantly decreased. In addition, others have observed that hyperventilation results in elevated cerebral lactate production indicating a cerebral blood flow insufficient to maintain the same level of aerobic metabolism. Because hyperventilation has been reported to adversely affect the outcome of patients with severe head injury, this therapeutic approach remains controversial.

Prostanoids play an important role in control of the cerebral circulation during the perinatal period. Dilation responses to hypercapnia and histamine seem to be dependent on prostanoid production. Constrictor responses can also be dependent on prostanoids, as observed with acetylcholine and endothelin. Alternatively, prostanoids can also attenuate constriction, as seen with norepinephrine. The present findings require that we consider different mechanisms for dilation and constriction in response to changes in PCO₂ from baseline in the newborn period.

The cellular mechanism by which PCO₂ influences the cerebral circulation remains unclear, especially in light of the apparently different mechanisms involved in vascular responses seen when CO₂ is increased or decreased from baseline. With the dilator response that accompanies hypercapnia, prostanoids increase and participate in dilation. A similar response is seen when acid is used to lower the pH of CSF. A possible hypothesis is that the interstitial acidosis that accompanies hypercapnia causes an increase in prostanoids, which act through an adenylate cyclase mechanism in smooth muscle, causing vasodilatation. An alternate mechanism, however, must be suggested to account for the vasocostriction seen with hypocapnia. We speculate that hypocapnia (increased pH) may act directly on vascular smooth muscle via alternate second messengers, such as inositol 1,4,5-trisphosphate, which have the capability of increasing cystolic calcium concentration to induce vasocostriction.

In summary, the present study shows that ischemia/reperfusion does not alter newborn cerebral vasocostriction in response to hypocapnia. These data support the hypothesis that prostanoids are not involved in hypocapnic cerebral vasocostriction and further the hypothesis that at least two mechanisms must be involved in PCO₂ influences on the newborn cerebral circulation. We suggest that prostanoids are required for vasodilation, and an as yet unidentified vasoconstrictor mechanism is invoked during hypocapnic vasocostriction.

Acknowledgments

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References


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**Editorial Comment**

Although the role of prostanoids in the regulation of the cerebral circulation in adult animals may be controversial, investigators have shown that prostanoids are important in the regulation of the cerebral circulation in newborns. Synthesis of prostanoids appears to be important in the maintenance of basal cerebral blood flow, autoregulation of cerebral blood flow during decreases in cerebrovascular perfusion pressure, and vasodilator responses of the cerebral circulation to hypercapnia, hypoxia, and vasoactive agonists.1–7

Cerebral ischemia followed by reperfusion produces a dramatic and selective inhibition of prostanoid-dependent dilatation of the cerebral circulation in newborns.8,9 The mechanism by which ischemia/reperfusion inhibits prostanoid-dependent dilatation is not clear but may be related to alterations in the synthesis, metabolism, and release of reactive oxygen radicals and arachidonic acid, and/or effects on cellular membranes/metabolism to inhibit the release of prostanoids.9

The goal of the present study by Mirro et al was to determine whether ischemia/reperfusion, in addition to altering prostanoid-dependent dilatation, affects cerebral vasoconstrictor responses of newborns to hypoxacapnia. Ischemia/reperfusion did not alter constrictor responses of the cerebral circulation in newborns to hypoxacapnia. The authors suggest that hypoxacapnia may affect vascular muscle via inositol 1,4,5-trisphosphate, thereby increasing intracellular calcium to induce vasoconstriction. This mechanism of hypoxacapnic-induced vasoconstriction presumably would be independent of the synthesis and release of prostanoids. Thus, the findings of Mirro et al in the accompanying article suggest distinct mechanisms for the effects of carbon dioxide on the cerebral circulation in newborn piglets following ischemia/reperfusion.

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**References**


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