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^-6 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

Cerebral blood flow decreases in response to hypocapnia.1-3 The mechanism appears to involve changes in extracellular fluid pH,1 which could act directly or by inducing the production of an as yet unidentified vasoconstrictor substance.

In newborn piglets, prostanoids play an important role in the control of the cerebral circulation,4 and hypercapnia-induced vasodilation is accompanied by an increase in cerebrospinal fluid (CSF) prostanoids. Furthermore, hypercapnic cerebral vasodilation is inhibited by indomethacin,5,6 whereas hypocapnic vasoconstriction is not affected by indomethacin.7

See Editorial Comment, p 1616

Cerebral ischemia/reperfusion elicits a profile of selective blockade of prostanoid-dependent responses (hypercapnia and hypotension) that closely resemble the action of indomethacin.4,8 Therefore, we designed these experiments to study the effects of cerebral ischemia/reperfusion on hypoxic vasoconstriction. In addition, known responses to hypercapnia, isoproterenol, and norepinephrine were also tested for comparison.

Materials and Methods

All protocols were approved by the Animal Care and Use Committee of the University of Tennessee, Memphis.

Ten newborn pigs (1–4 days old) were anesthetized with ketamine hydrochloride (33 mg/kg i.m.) and acepromazine (3.3 mg/kg i.m.) and maintained on alpha-chloralose (50 mg/kg initially followed by 5 mg/kg per hour i.v.). Catheters were placed into a femoral vein and artery. The venous catheter allowed for fluid and drug administration, and the arterial catheter was used for continuous blood pressure monitoring and for withdrawal of arterial blood for measuring gases and pH. The trachea was intubated with a 3.0-mm (i.d.) straight endotracheal tube, and the animals were ventilated with...
The window was placed in the hole and cemented into place. Vessel diameter was measured with a video microscope, a television camera (digital model CT 2081 Y, Panasonic), and was contiguous with the periarachnoid space. Fluid directly under the window was approximately 500 μm thick. A stainless steel and glass cranial microscaler (model VPA 1000, For-A-Corp, Los Angeles, Calif.) was incorporated into the sides of the window. The volume of fluid under the window was replaced with aCSF, flushed with aCSF after each treatment and the arterioles were observed for a 10-minute period. The window was sealed 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.8 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 pH values are shown in Table 1. During hyperventilation, pH increased and PacO₂ decreased. Conversely, during hypercapnia, pH decreased and PacO₂ increased. These changes were not different before and after ischemia. Arterial blood pressure and PacO₂ 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; PacO₂ = 22 mm Hg) both before and after ischemia/reperfusion. In addition, greater hyperventilation (PacO₂ = 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, 10⁻⁶, and 10⁻⁵ M were 8±2, 14±1, and 20±1 μm, respectively, before ischemia, and 7±1, 12±1, and 18±1 μm, respectively, after ischemia.
by guest on January 26, 2018 http://stroke.ahajournals.org/ Downloaded from

Values are mean±SEM (in micrometers); n=10. HV, hyperventilation (PaCO₂, 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 vaso-constriction induced by hypocapnia, in contrast to complete inhibition of hypercapnic cerebral vasodilation.⁶⁶ 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.⁹ Impairment of cerebral vasodilation and preservation of cerebral vasoconstriction could contribute to blunted reperfusion of the cerebral circulation after cerebral ischemia.⁹

Control of regional perfusion by lowering PaCO₂ is a therapeutic approach used to vasoconstrict the cerebral circulation, thus attenuating the elevated intracranial pressure caused by cerebral edema,¹⁰ and to vasodilate the pulmonary circulation in persistent pulmonary hypertension of the newborn.¹¹ In both settings, hyperventilation decreases PaCO₂ 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.¹² Because hyperventilation has been reported to adversely affect the outcome of patients with severe head injury, this therapeutic approach remains controversial.¹³

Prostanoids play an important role in control of the cerebral circulation during the perinatal period.⁴ Dilation responses to hypercapnia¹ and histamine¹⁴ seem to be dependent on prostanooid production. Constrictor responses can also be dependent on prostanooids, as observed with acetylcholine and endothelin.¹⁵¹⁶ Alternatively, prostanoids can also attenuate constriction, as seen with norepinephrine.¹⁷ The present findings require that we consider different mechanisms for dilation and constriction in response to changes in PaCO₂ from baseline in the newborn period.

The cellular mechanism by which PaCO₂ 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.⁴ A similar response is seen when acid is used to lower the pH of CSF.⁶ A possible hypothesis is that the interstitial acidosis that accompanies hypercapnia causes an increase in prostanoids, which act through an adenylylate cyclase mechanism in smooth muscle, causing vasodilation.¹⁸ An alternate mechanism, however, must be suggested to account for the vasoconstriction seen with hypocapnia. We speculate that hypercapnia (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 cytosolic calcium concentration to induce vasoconstriction.

In summary, the present study shows that ischemia/reperfusion does not alter newborn cerebral vasoconstriction in response to hypocapnia. These data support the hypothesis that prostanoids are not involved in hypocapnic cerebral vasoconstriction and further the hypothesis that at least two mechanisms must be involved in PaCO₂ 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 vasoconstriction.

Acknowledgments

The authors thank Alex Fedinic, Shiwei Tong, and Mildred Jackson for excellent technical assistance during the performance of the experiments.

References


FIGURE 1. Bar graph showing percent change in pial arteriolar diameter before and after ischemia. Vascular reactivity tested with hyperventilation (HV, PaCO₂=22 mm Hg), hypercapnia (CO₂), topical isoproterenol (ISO, 10⁻⁷ M), and topical norepinephrine (NE, 10⁻⁴ M). Values are mean±SEM; n=10. *p<0.05 different from value before ischemia.

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>

⁴ Leffler CW, Busija DW: Arachidonic acid metabolites and perinatal cerebral hemodynamics.
Although the role of prostanoids in the regulation of the cerebral circulation in adult animals may be controversi- 


eal, investigators have shown that prostanoids are 


apart during cerebral reperfusion. Synthesis of prostanoids appears to be im-


potent in the maintenance of basal cerebral blood flow, 


autoregulation of cerebral blood flow during decreases 


cerebrovascular perfusion pressure, and vasodilator 


takes 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-depen-


dilatation of the cerebral circulation in new-


borns.8,9 The mechanism by which ischemia/reperfusion inhibits prostanoid-dependent dilatation is not clear but 


may be related to alterations in the synthesis, meta-


bolism, and release of reactive oxygen radicals and arachi-


donic acid, and/or effects on cellular membranes/metabo-


lism 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 cere-


bral vasoconstrictor responses of newborns to hypoca-


pnia. Ischemia/reperfusion did not alter constrictor re-


ponses of the cerebral circulation in newborns to hypoca-


pnia. The authors suggest that hypoxia may affect vascular muscle via inositol 1,4,5-trisphosphate, thereby increasing intracellular calcium to induce vaso-


constriction. This mechanism of hypocapnic-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.

**Wireless G. Mayhan, PhD, Guest Editor**

*Department of Physiology and Biophysics*

*University of Nebraska Medical Center*

*Omaha, Neb.*

**References**


Cerebral vasoconstriction in response to hypocapnia is maintained after ischemia/reperfusion injury in newborn pigs.
R Mirro, L Lowery-Smith, W M Armstead, M Shibata, S L Zuckerman and C W Leffler

Stroke. 1992;23:1613-1616
doi: 10.1161/01.STR.23.11.1613
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/11/1613

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/