Selective Attenuation by Perivascular Blood of Prostanoid-Dependent Cerebrovascular Dilation in Piglets

David W. Busija, PhD, and Charles W. Leffler, PhD

Cerebral hemorrhagic insults are common in neonates. However, the consequences of intracranial blood on cerebral hemodynamics are poorly understood. We examined the effects of perivascular blood on cerebrovascular dilator responses in 29 piglets. Fresh, autologous blood \((n=15)\) or cerebrospinal fluid \((n=14)\) was placed under the dura mater over the parietal cortex, and the piglets were allowed to recover from anesthesia. One to four days later, a closed cranial window was placed over the parietal cortex and pial arteriolar responses to arterial hypercapnia \((Paco_2>55\text{ mm Hg})\), hemorrhagic hypotension \((\text{mean arterial blood pressure}<35\text{ mm Hg})\), or topical application of \(10^{-6}\) and \(10^{-4}\text{ M isoproterenol}\) were determined. Pial arterioles in the cerebrospinal fluid group dilated 27±4\% \((n=11)\) in response to hypercapnia, 26±5\% \((n=9)\) in response to hypotension, and 26±3\% in response to \(10^{-4}\text{ M isoproterenol}\) \((n=11)\). In the group in which blood was placed on the parietal cortex, pial arterioles did not dilate significantly in response to hypercapnia \((8\pm3\%\text{, }n=11)\) or hypotension \((2\pm5\%\text{, }n=13)\) but dilated normally in response to isoproterenol \((25\pm5\%\text{ in response to }10^{-6}\text{ M and }36\pm7\%\text{ in response to }10^{-4}\text{ M, }n=13)\). We conclude that prolonged contact of pial arterioles with extravascular blood selectively attenuates cerebrovascular dilation in piglets. (Stroke 1991;22:484-488)

Intracranial hemorrhage (ICH) is a serious problem in preterm and full-term infants. The most common types are subdural, primary subarachnoid, intraparenchymal, and intraventricular hemorrhages. Depending on the location and extent of bleeding, the neurologic sequelae can be quite serious. Several mechanisms contribute to immediate and later derangements of cerebral blood flow (CBF). First, rupture of arteries and capillaries leads to edema in areas served by those vessels. Second, increased intracranial pressure due to blood and edema causes extraluminal compression of vessels, which can reduce CBF. Third, the presence of blood and/or macrophages leads to production of substances that could damage vessels and, consequently, alter cerebral vascular responsiveness. For example, we have shown that fresh, autologous blood placed on the brain surface generates superoxide anion in piglets, and free radicals have been implicated in the altered responsiveness of cerebral resistance vessels.

In adult animal models of ICH, the perivascular presence of blood alters cerebrovascular responsiveness. In particular, responses involving endothelium-derived relaxing factor (EDRF) and arterial hypercapnia can be reduced. On the other hand, little is known concerning the effects of perivascular blood on dilator responses of the cerebral circulation in neonates. We have recently shown that the chronic, but not acute, presence of perivascular blood selectively attenuates pial arteriolar constrictor responses to different neurotransmitters.

The purpose of this study was to examine the effects of perivascular blood on pial arteriolar dilation in newborn pigs. We tested two hypotheses based on our earlier findings with constrictor stimuli that the chronic, but not acute, presence of perivascular blood would attenuate cerebrovascular dilator responses and that attenuation of cerebrovascular dilation due to perivascular blood would occur in response to some, but not all, stimuli.

Materials and Methods

The animal protocols used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee, Memphis.
We divided 34 piglets into three groups: two in which autologous blood (ICH group) or sterile, artificial cerebrospinal fluid (CSF group) was placed on the parietal cortex 1–4 days before the day the experiment was performed and one in which autologous blood was placed on the cortex for 30 minutes approximately 1 hour prior to the beginning of the experimental protocol.

For the chronic placement of blood (n=15) or CSF (n=14), the piglets were anesthetized with halothane and nitrous oxide. Using aseptic procedures, a small burr hole was made in the skull over the frontal cortex (the left side for all 15 ICH and 10 of the CSF animals and the right side for four of the CSF animals). Then, a 29-gauge Teflon catheter was used to pierce the dura at an angle to the surface sufficient to prevent penetration into the brain. Following removal of the needle, the tip of the catheter was advanced 2 cm posteriorly under the dura to the parietal cortex. Then, 2 ml of either fresh, sterile, nonheparinized blood (removed via direct puncture of the precava) or sterile, artificial CSF (see below for composition) was injected over 1–2 minutes. The catheter was removed, the burr hole was filled with sterile bone wax, and the scalp was sutured. The piglets were treated with gentamicin and benzathine penicillin postoperatively. Following surgery, the piglets exhibited no apparent behavior abnormalities.

For the acute placement of blood in five piglets, the scalp, skull, and dura were removed and fresh autologous blood was placed on the cortical surface on the left side and allowed to remain there for 30 minutes. Then the clot was removed without touching the brain, and the brain surface was flushed gently with artificial CSF.

A cranial window was placed over the left parietal cortex (see below). The cranial window was flushed with artificial CSF several times. The diameter of one pial arteriole was measured in each piglet. Following a control period during which CSF containing no drug was infused under the window and blood gases and arterial blood pressure were within normal limits, the animals were exposed to several stimuli. These included 10^{-4} and 10^{-4} M topical isoproterenol in CSF, arterial hypercapnia (breathing 10% CO₂, 21% O₂, balance N₂), and hemorrhagic hypotension (withdrawal of venous blood). Measurements were taken during steady-state conditions: after 2–4 minutes’ exposure to isoproterenol, 12 minutes’ exposure to hypercapnia, and 9 minutes’ exposure to hypotension.

In the acute blood experiment, the cranial window was put in place after the clot was removed. In the chronic experiments, the window was placed over the parietal cortex at the site of injection of blood (n=15) or CSF (n=10); in the other four CSF piglets, the window was placed in the side opposite the CSF injection. Since responses were similar, the CSF animals were combined to give an overall group size of 14. For the CSF group, two, five, four, and three piglets were studied at days 1, 2, 3, and 4, respectively. For the ICH group, two, six, three, and four animals were studied at days 1, 2, 3, and 4, respectively.

For the cranial window experiments, the piglets were initially anesthetized with 33 mg/kg i.m. ketamine hydrochloride and 3.5 mg/kg i.m. acepromazine followed by α-chloralose (50 mg/kg i.v. initially plus 5–10 mg/kg/hr). Catheters were placed into a femoral artery to record blood pressure and to sample for gases and pH and into a femoral vein to inject drugs and fluids. The animals were intubated and ventilated with air. Body temperature was maintained at 37–38°C using a water-circulating rubber heating pad. After the scalp was removed, a 2-cm-diameter hole 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. In the ICH piglets, the clot was removed with forceps without touching the brain surface and the brain surface was gently flushed with CSF. A stainless steel and glass cranial window was placed in the hole and cemented to the skull with dental acrylic. The space under the window was filled, through needles incorporated into the sides of the window, with artificial CSF of the following milligram composition: 220 KCl, 132 MgCl₂, 221 CaCl₂, 7710 NaCl, 402 urea, 665 dextrose, and 2,066 NaHCO₃; the pH was 7.33, Pco₂ 46 mm Hg, and Po₂ 43 mm Hg. The volume of fluid directly under the window was approximately 500 µl and was contiguous with the subarachnoid space. Pial arterioles were observed with a Wild trinocular stereomicroscope (Rockleigh, N.J.). Pial arteriolar diameter was measured with a television camera mounted on the microscope, a video monitor, and a video microscler (model VPA-100, FOR-A-CORPORATION, Newton, Mass.).

All values are presented as mean±SEM. We examined the effects of stimuli on pial arteriolar diameter using repeated-measures analysis of variance followed by the Student-Newman-Keuls test (control condition versus 10^{-6} M or 10^{-4} M isoproterenol) or the paired t test (control condition versus hypercapnia or hypotension). We compared differences in responses between the CSF and ICH groups using the unpaired t test on the percentage change data (control−experimental+control×100); an arcsine transformation was used to normalize the percentage change values. We also used regression analysis to determine whether responses varied by the number of days following the placement of CSF or blood. An α level of p<0.05 was used in all statistical tests.

Results

In the ICH group, gross visual examination of the brain surface both by the unaided eye and on the television screen revealed the presence of blood around cerebral vessels on the brain surface even after the removal of any large clots in this area. Pial arterioles dilated in response to topical isoproterenol, but not in response to hypercapnia or hypotension (Table 1, Figure 1). Regression analysis indi-
TABLE 1. Effects of Intracranial Blood and Cerebrospinal Fluid on Cerebral Hemodynamics in Piglets

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hypercapnia</th>
<th>Hypotension</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>P&lt;0.05</td>
<td>Control</td>
</tr>
<tr>
<td>Intracranial blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td>146±10</td>
<td>157±11</td>
<td>172±12</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>60±4</td>
<td>56±5</td>
<td>56±3</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td>163±9</td>
<td>205±10*</td>
<td>173±11</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>64±2</td>
<td>63±3</td>
<td>58±2</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MABP, mean arterial blood pressure. *p<0.05 different from corresponding control. †p<0.05 different from 10^-6 M isoproterenol.

cated that the responses were independent of time following the placement of blood. Values of  were 0.22 for hypercapnia, 0.04 for hypotension, 0.01 for 10^-6 M isoproterenol, and 0.001 for 10^-4 M isoproterenol. Values of  for these comparisons were all nonsignificant. Arterial blood pressure did not change from control values during hypercapnia or topical application of isoproterenol (Table 1). However, arterial blood pressure was reduced during hypotension. During the control condition, arterial blood pH was 7.57±0.02, Paco2 was 33±1 mm Hg, and PaO2 was 86±2 mm Hg (n=15). During hypercapnia, pH was 7.36±0.02, Paco2 was 61±1 mm Hg, and PaO2 was 92±3 mm Hg (n=11).

In the CSF group, visual examination of the brain by the unaided eye and on the television screen indicated that the presence of blood around cerebral vessels was absent or minimal. Pial arterioles dilated in response to topical isoproterenol and to hypercapnia and hypotension (Table 1, Figure 1). Responses were significantly different between the ICH and CSF groups for hypercapnia and hypotension but not for isoproterenol. Regression analysis indicated that vascular responses were independent of time following the placement of CSF. Values of  were 0.25 for hypercapnia, 0.09 for hypotension, 0.31 for 10^-6 M isoproterenol, and 0.15 for 10^-4 M isoproterenol. Values of  for these comparisons were all nonsignificant. Arterial blood pressure did not change from control values during hypercapnia or topical application of isoproterenol (Table 1). However, arterial blood pressure was lower than control during hypotension. During the control condition, arterial blood pH was 7.58±0.05, Paco2 was 30±0.5 mm Hg, and PaO2 was 89±4 mm Hg (n=13). During hypercapnia, pH was 7.29±0.05, Paco2 was 63±2 mm Hg, and PaO2 was 100±4 mm Hg (n=10).

In the acute blood group, pial arterioles dilated in response to all three stimuli (p<0.05). Topical application of isoproterenol (n=4) increased diameters from 160±24 μm during the control condition to 217±34 μm at 10^-6 M (change=36±6%) and 218±33 μm at 10^-4 M (change=36±7%) (control<10^-6 M=10^-4 M). Hypercapnia (n=5) increased diameter from 167±17 to 202±14 μm (change=23±17%). Hypotension (n=3) increased diameter from 152±21 to 186±24 μm (change=23±2%). During the control condition, 10^-6 M isoproterenol, and 10^-4 M isoproterenol, arterial blood pressure was 61±1, 60±2, and 56±2 mm Hg, respectively (differences nonsignificant). Arterial blood pressure decreased (p<0.05) from 58±2 mm Hg during the control condition to 32±2 mm Hg during hypotension. Arterial blood pressure was 58±4 mm Hg during the control condition and 61±4 mm Hg during hypercapnia (difference nonsignificant). During the control condition, arterial blood pH was 7.53±0.03, Paco2 was 34±1 mm Hg, and PaO2 was 78±3 mm Hg. During hypercapnia, pH was 7.34±0.02, Paco2 was 65±3 mm Hg, and PaO2 was 83±4 mm Hg.

Discussion

The new finding of our present study is that the prolonged presence of perivascular blood selectively inhibits dilation in the piglet cerebral circulation.

![Figure 1. Comparison of mean±SEM percentage changes in arteriolar diameter to dilator stimuli in piglets treated with intracranial blood (filled bars) or cerebrospinal fluid (shaded bars). *p<0.05 different from blood group. Sample sizes are given in parentheses.](http://stroke.ahajournals.org/DownloadedFrom)
Thus, pial arteriolar dilation due to arterial hypercapnia or hemorrhagic hypotension is virtually eliminated, while dilation to topical isoproterenol is preserved. In contrast, acute exposure to blood does not immediately affect cerebrovascular dilator responses.

The duration of exposure of the cerebral arterioles to blood seems to be an important determinant of alterations of the responses. With acute exposure lasting only 30 minutes, pial arterioles retain the expected responses to arterial hypercapnia, hemorrhagic hypotension, and topical isoproterenol. However, exposure of the arterioles for 1–4 days is sufficient to eliminate selectively the dilator responses to the first two of these stimuli while not affecting that to the third stimulus. These results are similar to those of our previous studies in which total cerebral ischemia followed by reperfusion abolished postischemic dilator responses to topical hypercapnia and hypotension but not to isoproterenol in piglets. In contrast to our findings with perivascular blood, altered cerebrovascular responsiveness to dilator stimuli occurs soon after cerebral ischemia/reperfusion. The differences in results could be due to the magnitude or location of the damage. Clearly, ischemia followed by reperfusion immediately exposes all cerebral neurons, glial cells, and blood vessels to major changes in their environment, while topical application of blood exposes a more limited population of cells on the cortical surface to potentially damaging conditions. Thus, our results indicate that the presence of blood by itself is not detrimental to cerebrovascular function, at least in the short term. It is possible that damage to blood vessels begins to occur immediately after exposure to blood, but that at this time the effects are subtle enough to escape detection.

Our results confirm, in part, earlier in vitro studies in cerebral arteries of adult animals that indicate that the chronic presence of blood selectively alters vascular responses to dilator stimuli. Nakagomi et al. have reported that endothelium-dependent dilation of brain arteries in response to adenosine triphosphate, but not to acetylcholine, is inhibited by perivascular blood 2–6 days after placement via the single-injection method in rabbits. However, in a double-injection model, responsiveness to both stimuli was reduced. In addition, Kim et al. have reported that endothelium-dependent cerebrovascular relaxation in response to vasopressin and thrombin is reduced by the double-injection method in dogs, while other such responses to many other stimuli are preserved. Further, Svendgaard, et al. found that cerebrovascular dilation in response to arterial hypercapnia is attenuated in the presence of perivascular blood. To our knowledge, no other laboratory has investigated the effects of perivascular blood on cerebrovascular responses to arterial hypotension.

The mechanisms responsible for alterations in the cerebrovascular responses following ICH are not known. Since arterioles are still able to dilate in response to isoproterenol and constrict in response to neurotransmitters, it seems unlikely that blood caused extensive damage to smooth muscle cells. However, it is possible that blood caused subtle changes in the overall responses of pial arterioles, perhaps via mechanisms involving activated oxygen species or by disruption of the blood–brain barrier.

We have shown previously that piglet blood produces substantial amounts of superoxide anion in vivo and in vitro and that the immediate source of free radicals is due to activation of platelets. During clot formation, platelets generate thromboxane via the cyclooxygenase pathway. Later, free radical production may arise via the release of constituents such as hemoglobin and other proteins from hemolyzed erythrocytes and/or the activity of neutrophilic granulocytes attracted to the site of the clot. Since altered responsiveness was not apparent after short-term exposure to blood and since free radical generation probably occurs as an immediate burst after contact between blood and tissue, whatever changes that occur in vascular responsiveness must take time to happen. Oxygen free radicals could eliminate the dilator effects of arterial hypercapnia or hypotension by damaging the enzymes involved in prostanoid synthesis, or they could alter the receptor characteristics to prostanoids once they are formed. We and others have shown that arterial hypercapnia and hypotension but not isoproterenol dilate piglet arterioles via a prostanoid-related mechanism. A second possibility is that chronic exposure to perivascular blood leads to increased permeability of the blood–brain barrier and allows circulating substances to have access to cerebrovascular smooth muscle. Normally, plasma constrictor agents do not have ready access to cerebrovascular smooth muscle. However, following disruption of the blood–brain barrier, which has been reported to occur in response to perivascular blood, plasma agents could increase tone. We have found that plasma levels of catecholamines and vasopressin increase during arterial hypercapnia and hypotension in piglets. Since these are cerebrovascular constrictor agents, competing constriction may prevent dilation under these conditions. Topical application of isoproterenol would not be expected to affect plasma levels of catecholamines and vasopressin.

There are two important clinical applications of these findings for the treatment of neonates after ICH. First, although the actual rupture of cerebral vessels has immediate effects on the cerebral circulation and brain parenchyma, the presence of intracranial blood may have delayed, deleterious effects. This delay affords a therapeutic window of opportunity for the prevention of further impairment of cerebrovascular responses. If it turns out that oxygen free radicals play a part in the delayed damage to cerebral blood vessels, then appropriate drugs aimed at preventing the formation of and/or scavenging of free radicals could be administered. Second, potential disruption of the blood–brain barrier as a delayed consequence of intracranial blood could offer the
opportunity of administering drugs, such as calcium channel antagonists, to counteract the cerebrovascular effects of circulating constrictor agents.

In summary, the acute presence of perivascular blood does not alter pial arteriolar responses to several dilator stimuli in piglets. However, the chronic presence of perivascular blood selectively eliminates pial arteriolar dilation in response to arterial hypercapnia and hypotension but not to the topical application of isoproterenol.

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References

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