Maintenance of Cerebral Circulation During Hemorrhagic Hypotension in Newborn Pigs: Role of Prostanoids

Charles W. Leffler, David W. Busija, Donathan G. Beasley, and Anthony M. Fletcher

The possibility that the prostanoid system contributes to the capability of the newborn piglet to maintain cerebral blood flow and cerebral metabolic rate during hypotension was investigated. The effect of hemorrhage on net (arterial-to-venous) cerebral prostacyclin production and the effects of indomethacin on cerebral hemodynamic response to hemorrhage and on the cerebral oxygen utilization following hemorrhage were determined in chronically instrumented, unanesthetized newborn pigs. Hemorrhage decreased arterial pressure about 35% but did not affect cerebral blood flow or cerebral O$_2$ consumption. Hemorrhage was accompanied by an increase in net cerebral 6-keto-PGF$_{1a}$ production from $4.0 \pm 1.1$ to $15.3 \pm 4.9$ ng/100 g min (mean ± SEM). Indomethacin treatment of piglets following hemorrhage inhibited the net cerebral production of 6-keto-PGF$_{1a}$ and caused a decrease in blood flow (≈40%) to all brain regions within 20 minutes. The decrease in cerebral blood flow was the result of an increase in cerebral vascular resistance of 57 and 180%, 20 and 40 minutes post treatment, respectively. Cerebral O$_2$ consumption was reduced from $2.5 \pm 0.3$ ml/100 g min to $1.5 \pm 0.3$ ml/100 g min 20 minutes following treatment of hemorrhaged piglets with indomethacin and to $1.1 \pm 0.3$ ml/100 g min 40 minutes after treatment. Six of 8 piglets for whom the data were recorded that were administered indomethacin following hemorrhage became comatose with cerebral O$_2$ consumption of $0.4 \pm 0.1$ ml O$_2$/100 g min by 40 minutes after treatment. These data are consistent with the hypothesis that the prostanoid system contributes to the maintenance of cerebral blood flow and cerebral metabolic rate during hypotension in the newborn. (Circulation Research 1986;59:562–567)

A characteristic feature of the cerebral circulation is that blood flow is maintained relatively constant over a wide range of perfusion pressure. In adults, autoregulatory mechanisms prevent cerebral blood flow from changing appreciably over the arterial pressure range of approximately 60–150 mm Hg.$^{1,2}$ Cerebral autoregulation in fetuses and newborns extends to lower arterial pressures than in adults.$^{3,4}$ and may reflect adaptations of the cerebral circulation of fetuses and neonates to lower resting arterial pressure.

Various mechanisms have been suggested to account for cerebral autoregulation, including neural, myogenic and metabolic.$^5$ However, intriguing evidence suggests a possible role of prostanoids in maintenance of cerebral blood flow following hemorrhage.$^7$

The prostanoid system could contribute to maintenance of cerebral blood flow at low arterial pressures in the newborn since the prostanoid system appears to be more prominent in the neonate than in the older individual. For example, the plasma levels of the hydrolysis product of prostacyclin, 6-keto-PGF$_{1a}$, in the fetus and newborn are much higher than in adults.$^8,9$ Treatment of newborn human infants with prostanoid cyclooxygenase inhibitors to close the patent ductus arteriosis frequently causes transient renal dysfunction,$^{10,11}$ in contrast to negligible effects on adults. A role for prostanoids in perinatal pulmonary vascular transition has been established.$^{12-14}$ Recently, we have presented evidence to support a role for prostanoids in neonatal cerebral hemodynamics during control conditions and during asphyxia.$^{15-17}$

The present study was undertaken to investigate the possibility that the prostanoid system contributes to the capability of the newborn to maintain cerebral blood flow and cerebral metabolic rate during hypotension. The effect of hemorrhage on net cerebral prostacyclin production and the effect of indomethacin on the cerebral hemodynamic response to hemorrhage and on the cerebral oxygen utilization following hemorrhage were determined in newborn pigs.

Materials and Methods

Surgical and experimental procedures employed were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee, Memphis.

Newborn pigs (1.5 ± 0.1 kg, mean ± SEM) were instrumented prior to 36 hours of age. All surgery was performed under aseptic conditions. Piglets were anesthetized with a mixture of halothane, nitrous oxide, and oxygen. Polyurethane catheters were placed in the
Blood Chemistry Determinations and Cerebral O$_2$ Consumption

Blood pH, Pco$_2$, and Po$_2$ were determined using an Instrumentation Laboratories Blood Gas Analyzer. Percent saturation of the hemoglobin in the arterial and sagittal sinus blood were determined using an American Optical Reflection Oxymeter. Blood hemoglobin was determined using a Reichert Hemoglobinometer. We assumed the oxygen capacity of the hemoglobin to be 1.39 ml O$_2$/g of hemoglobin. Blood oxygen content was then calculated as $C^2 = (g$ hemoglobin/ml $\times 1.39$ ml O$_2$/g hemoglobin $\times \%$ saturation of hemoglobin with O$_2$) + dissolved O$_2$. Cerebral metabolic rate for oxygen was calculated as (arterial $C^2 -$ venous $C^2$) $\times$ cerebral blood flow.

Radioactive Microsphere Determination of Cerebral Blood Flow

A known amount of radioactivity in 15 $\mu$m microspheres (300,000–800,000 microspheres) was injected into the left ventricle and the injection line flushed with 1 ml saline. Withdrawal of reference blood samples (1.03 ml/min from the descending aorta) was begun 15 seconds prior to microsphere injections and continued for 2 minutes after the injection. Blood withdrawn for the initial blood flow determination began the hemorrhage period. Thereafter, withdrawn blood was replaced with blood removed when the piglet was hemorrhaged. Following the experiment, the piglet was killed by injecting euthanasia solution (T-61) into the left ventricle and the brain removed. The brain was subdivided into major regions and tissues. Samples were counted in a well-type gamma counter. The energy from each nuclide was separated by differential spectroscopy. Aliquots of the actual microsphere solutions injected were used for overlap calculations. The lungs were counted to detect extensive arterio-venous shunting of microspheres. "Lung blood flow" (comprised of bronchial flow and whole body arterio-venous shunt flow) averaged 2% of cardiac output, indicating that no extraordinary shunting of microspheres occurred. Cardiac output was calculated as cardiac output = (reference withdrawal rate) $\times$ (counts injected) $\times$ (counts in reference withdrawal)$^{-1}$. Blood flow to each brain region at the time the microspheres were injected was calculated by using the formula: $Q = C \times R \times CR^{-1}$, where $Q =$ organ blood flow in ml/min $\times 100$, $C =$ counts/100 g tissue, $R =$ rate of withdrawal of reference blood sample in ml/min, and CR = total counts in reference arterial blood sample. Cerebral vascular resistance was calculated by dividing mean arterial pressure by cerebral blood flow. We assumed cerebral venous pressure to be negligible because we measured sagittal sinus pressure in several piglets and found it to be between 1 and 2 mm Hg and relatively constant over time.

Prostanoid Analysis

6-keto-PGF$_{1\alpha}$ in plasma was determined by radioimmunoassay as described previously. All tubes contained an identical amount of pig plasma. The standard curves, knowns, blanks, and make-up for greater dilutions used plasma from the same piglet as the unknowns, following dialysis of the plasma against 4 l of Krebs bicarbonate buffer. Antisera used were produced in rabbits immunized with 6-keto-PGF$_{1\alpha}$ coupled to thyroglobulin using the mixed anhydride method. Cross-reactivities of the antibody with other known, biologically relevant pros-tanoids, leukotrienes, and arachidonic acid were all below 1%. The assays were performed in gelatin-Tris buffer using tritiated 6-keto-PGF$_{1\alpha}$. Following 24-hour incubation at 4°C, the free fraction was separated from
the fraction bound to antibody by precipitating the rabbit antibodies with anti-rabbit gamma globulin and 60% saturated ammonium sulfate solution. Data were handled by computer, with determination of second-order regression of free tracer over tracer bound to antibody by least squares. All unknowns were assayed at 3 dilutions with parallelism between the unknown dilution curve and the standard curve required before using the results.

Statistical Analysis

All values are presented as means ± SEM. Comparisons between two values were made using t tests (for paired or unpaired observations, as appropriate), and comparisons among three or more values were made using analysis of variance, followed by pair-wise tests, when appropriate). Significance at the 95% confidence level was required for inference that populations were different.

Results

The effects of removal of 30 ml of blood/kg of piglet upon the arterial pressure, gasses, pH, hemoglobin concentration, and hemoglobin saturation with O₂ are shown in Table 1. Hemorrhage produced significant decreases in arterial pressure, hemoglobin concentration, and pH. Even though the piglets were ventilated mechanically, hemorrhage caused an increase in ventilation over the ventilator that produced a significant decrease in PaO₂. Arterial Po₂ and hemoglobin saturation with O₂ were not affected by the hemorrhage. There were no differences in the responses of the animals in the two groups to the hemorrhage prior to treatment with either indomethacin or vehicle. Sixty minutes following the hemorrhage, PaO₂ had increased, and PacO₂, pH, and hemoglobin concentration had all decreased from values obtained immediately following the hemorrhage, whether the piglet was treated with indomethacin or vehicle. There were no differences in any of the above variables between indomethacin and vehicle control animals 60 minutes after hemorrhage.

The effects of hemorrhage upon net cerebral 6-keto-PGF₁₀ production (arterial minus sagittal sinus concentration times cerebral blood flow) are shown in Figure 1. Prior to hemorrhage, there was addition to 6-keto-PGF₁₀ to blood passing through the cerebral circulation (i.e., net production). This production was increased about fourfold 60 minutes after hemorrhage. Indomethacin treatment (5 mg/kg, administered 20 minutes after hemorrhage) inhibited the cerebral production of 6-keto-PGF₁₀ so that net cerebral 6-keto-PGF₁₀ was less following hemorrhage and indomethacin treatment than it was before hemorrhage and treatment with indomethacin. The hemorrhage also caused a small but significant increase in arterial 6-keto-PGF₁₀ concentration from 123 ± 33 pg/ml to 216 ± 56 pg/ml 60 minutes after hemorrhage.

Hemorrhage of 30 ml/kg did not decrease blood flow to any brain region (Figure 2). In the group to be treated with vehicle, hemorrhage produced a significant increase in flow to the medulla. In the group to be treated with indomethacin, an apparent increase in flow to the medulla was seen also, but this was not significant. There were no significant differences between the piglets in the group to be treated with indomethacin and those to be treated with vehicle either during the control period or following hemorrhage.

Indomethacin treatment of piglets following hemorrhage caused a decrease in blood flow to all brain regions within 20 minutes, which was maintained 20

<table>
<thead>
<tr>
<th>Table 1. Arterial Pressure and Blood Chemistry of Newborn Pigs</th>
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<td>Time (min)</td>
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<tr>
<td><strong>Indomethacin-treated group</strong></td>
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<tr>
<td>Arterial pressure (mm Hg)</td>
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<td>Hb (g/100 ml)</td>
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<td>% saturation with O₂-art.</td>
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| **Vehicle-treated group** |
|-------------------------|----------------|---------------|----|----|
| Arterial pressure (mm Hg) | 57 ± 5*         | 36 ± 3         | 35 ± 3 | 33 ± 2 |
| PaO₂ (mm Hg)            | 71 ± 6          | 76 ± 5         | —   | 80 ± 5* |
| PacO₂ (mm Hg)           | 29 ± 2*         | 23 ± 1         | —   | 24 ± 2* |
| pHₐ                     | 7.47 ± 0.03    | 7.44 ± 0.04    | —   | 7.34 ± 0.08 |
| Hb (g/100 ml)           | 8.3 ± 0.3*      | 6.6 ± 0.4      | —   | 5.7 ± 0.4* |
| % saturation with O₂-art. | 89 ± 1        | 89 ± 1         | 88 ± 1 |

n = 13, indomethacin group.  
n = 6, vehicle group.  
*p < 0.05 compared to 20 minutes posthemorrhage. (There are no significant differences between indomethacin and vehicle control piglets.)
minutes later (Figure 2). The decrease in flow was uniform and averaged about 40%. Following treatment with vehicle, there were no declines in blood flow to any brain regions. In fact, cerebral blood flow tended to increase following vehicle treatment after hemorrhage, although the increases were significant only in the pons and medulla at 40 minutes posthemorrhage.

Figure 3 shows the calculated cerebral vascular resistances for the animals in the two groups prior to and following hemorrhage. Hemorrhage caused a decline in cerebral vascular resistance in animals from both groups. The decline in cerebral vascular resistance following hemorrhage in the group to be treated with indomethacin was not significant because 2 of 13 animals had unusual responses. Treatment with the vehicle following hemorrhage had no effect on the cerebral vascular resistance 20 or 40 minutes later. In contrast, following indomethacin treatment of hemorrhaged piglets, cerebral vascular resistance increased 57% in 20 minutes and 180% by 40 minutes post treatment.

Hemorrhage of 30 ml/kg had no effect on cerebral metabolic rate for oxygen (Figure 4). Indomethacin treatment of hemorrhaged piglets caused a marked decline in cerebral O$_2$ consumption (40% at 20 minutes, 55% at 40 minutes after treatment).

Vehicle control piglets typically slept in the sling throughout most of the experiments but could be awakened easily. On the other hand, 6 of 8 piglets for whom the data were recorded that were administered indomethacin following the hemorrhage became comatose by 40 minutes after treatment. The mean cerebral O$_2$ consumptions of these 6 comatose piglets was $0.4 \pm 0.1$ ml O$_2$/100 g·min.
Discussion

The present investigation establishes the ability of the untreated newborn pig to maintain cerebral O₂ consumption even when arterial pressure is decreased below 40 mm Hg by hemorrhage. Cerebral O₂ consumption rate can be maintained because cerebral blood flow is unaffected by hemorrhage due to reduction in cerebral vascular resistance. The decline in cerebral vascular resistance is accompanied by an increase in net cerebral prostacyclin production (detected as 6-keto-PGF₁α). Treatment of hemorrhagic hypotensive piglets with indomethacin inhibited cerebral prostacyclin production and reversed the cerebral autoregulatory response, thereby causing a decrease in cerebral metabolic rate sufficient to induce coma in 75% of piglets so treated.

The ability of the fetal and newborn lamb and newborn puppy to maintain cerebral blood flow at arterial pressures as low as 30 mm Hg, far below the adult autoregulatory range, has been established. This ability was not affected by lactic acidosis in the puppy but was abolished by hypoxia in the fetal lamb. In contrast, hemorrhage of the newborn pig anesthetized with nitrous oxide to 40 mm Hg caused a considerable decrease in blood flow throughout the brain initially, although flows to the brain stem and cerebellum recovered by 17 minutes while the flow to the cerebral remained depressed. The same authors found that hemorrhage of acutely instrumented (1 hour prior to experimentation) piglets caused considerable decreases in cerebral blood flow at pressures below 50 mm Hg, with the greatest decrease in the cerebral and the least in the brain stem. In contrast, in the present study of chronically instrumented piglets, the cerebral blood flow appeared to be higher 60 minutes after hemorrhage in the vehicle group when arterial pressure was below 35 mm Hg then prior to hemorrhage. We do not know the reasons for the differences between the present study and those of Laptook et al but speculate that the anesthetic agents may have interfered with the cerebral vascular responses.

Hemorrhagic hypotension and incomplete cerebral ischemia have been reported to increase brain concentrations of dilator prostanooids in a variety of animal models. An increase in brain vasodilator prostanooid synthsis is consistent with the increase in net cerebral vascular prostacyclin synthesis and the marked vasoconstrictor effect of indomethacin observed in the present study of piglets subjected to hemorrhage.

In adult animals, effects of indomethacin on cerebral hemodynamics following hemorrhage have been reported. Pickard et al found in anesthetized baboons that, although indomethacin decreased resting cerebral blood flow, the relative cerebral vascular response to hemorrhage was unaltered. However, the absolute cerebral blood flow was reduced about 30% at all arterial pressures between 60 and 130 mm Hg resulting in a decline in cerebral oxygen consumption at a higher arterial pressure in the indomethacin-treated than in the control baboons. In adult dogs, in refractory hemorrhagic shock, indomethacin caused a marked increase in cerebral vascular resistance in contrast to control dogs in which it had no effect on cerebral vascular resistance.

Previous studies have found that prostanoid synth-
sis inhibitors can decrease cerebral blood flow in perinatal animals in conditions other than hemorrhagic hypotension. Treatment of fetal lambs in utero with aspirin 27 or indomethacin 28 decreases resting cerebral blood flow. Indomethacin has been reported to decrease cerebral blood flow of newborn puppies. 28,29 Finally, indomethacin decreased cerebral blood flow and attenuated the cerebral vascular response to asphyxia in unanesthetized newborn pigs. 15

In conclusion: The present study indicates that indomethacin treatment of newborn pigs following hemorrhage causes a marked decrease in cerebral blood flow and cerebral O2 consumption. These data are consistent with the hypothesis that the prostanoid system contributes to the capability of the newborn to maintain cerebral blood flow and cerebral metabolic rate during hypotension.

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References

Key Words • prostanooids • hemorrhage • cerebral circulation • cerebral metabolism • newborn
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