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

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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 O₂ consumption. Hemorrhage was accompanied by an increase in net cerebral 6-keto-PGF₁₀ production from 4.0 ± 1.1 to 15.3 ± 4.9 ng/100 g min (mean ± SEM). Indomethacin treatment of piglets following hemorrhage inhibited the net cerebral production of 6-keto-PGF₁₀ 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% at 20 and 40 minutes post treatment, respectively. Cerebral O₂ consumption was reduced from 2.5 ± 0.3 ml/100 g min to 1.5 ± 0.3 ml/100 g min 20 minutes following treatment of hemorrhaged piglets with indomethacin and to 1.1 ± 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 O₂ consumption of 0.4 ± 0.1 ml O₂/100 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.6

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-PGF₁₀, in the fetus and newborn are much higher than in adults.8,9 Treatment of newborn human infants with prostanoïd 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
descending aorta (via an umbilical artery) for blood sampling and reference withdrawal in microsphere experiments and in the left ventricle (via the right carotid artery) for microsphere injections. In piglets, ligation of one carotid artery has no detectable effect on cerebral blood flow.\textsuperscript{15,16} Unlike most animals, ligation of a femoral or brachial artery in a piglet results in dysfunction of the dependent limb. The trachea was exposed and the muscles sutured together dorsal to it to create a tracheal loop directly below the incision. This procedure allowed exposure of the trachea when the skin sutures were removed so that tracheal cannulation could be performed without general anesthesia on the day of the experiment.

Following surgery, the piglets were given benzathene penicillin, gentamicin, and colloidal iron and placed in cages warmed by overhead lamps. They were provided a continual supply of pig milk substitute and water. Experimentation was performed on the third postoperative day. On the morning of experimentation, the skin on the neck was anesthetized with lidocaine, the sutures removed, and the skin retracted slightly to expose the trachea. Following tracheal cannulation, the piglet was mechanically ventilated (peak tracheal pressure, 10 cm water) at a rate at which voluntary breathing stopped, using a Baby Bird ventilator. In preliminary experiments, we found that artificial respiration was necessary because hemorrhaged piglets frequently stopped breathing following treatment with indomethacin. The midline of the scalp was anesthetized with lidocaine and a 2-cm incision made to expose the cranial suture. The sagittal sinus was cannulated with a 22-gauge Angiocath for collection of cerebral venous blood. The Angiocath was secured with Superglue. The piglet was warmed with an overhead lamp. The piglet was allowed to rest in the sling for approximately 30 minutes, and then radioactive microsphere determinations of cerebral blood flow and cardiac output were made and blood samples were drawn from the aorta and sagittal sinus. Blood, 30 ml/kg, was withdrawn from the aorta over a 10-minute period and a second microsphere injection and sample collection made 10 minutes later. Indomethacin trihydrate (5 mg/kg) was administered intrarreticularly and further microsphere blood flow determinations and blood samplings made 20 and 40 minutes later. Vehicle control animals received saline injections instead of saline-containing indomethacin trihydrate.

**Blood Chemistry Determinations and Cerebral O\textsubscript{2} Consumption**

Blood pH, P\textsubscript{CO\textsubscript{2}}, and P\textsubscript{O\textsubscript{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 Oxyimeter. Blood hemoglobin was determined using a Reichert Hemoglobinometer. We assumed the oxygen capacity of the hemoglobin to be 1.39 ml O\textsubscript{2}/g of hemoglobin. Blood oxygen content was then calculated as C\textsubscript{O\textsubscript{2}} = (g hemoglobin/ml x 1.39 ml O\textsubscript{2}/g hemoglobin x % saturation of hemoglobin with O\textsubscript{2}) + dissolved O\textsubscript{2}. Cerebral metabolic rate for oxygen was calculated as (arterial C\textsubscript{O\textsubscript{2}} - venous C\textsubscript{O\textsubscript{2}}) x cerebral blood flow.

**Radioactive Microsphere Determination of Cerebral Blood Flow**

A known amount of radioactivity in 15 \textmu 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) x (counts injected) x (counts in reference withdrawal)\textsuperscript{-1}. Blood flow to each brain region at the time the microspheres were injected was calculated by using the formula: Q = C x R x CR\textsuperscript{-1}, where Q = organ blood flow in ml/min x 100 g, 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\textsubscript{1a} in plasma was determined by radioimunoassay as described previously.\textsuperscript{15,16} 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 41 of Krebs bicarbonate buffer. Antisera used were produced in rabbits immunized with 6-keto-PGF\textsubscript{1a} coupled to thyroglobulin using the mixed anhydride method. Cross-reactivities of the antibody with other known, biologically relevant prostanoids, leukotrienes, and arachidonic acid were all below 1%. The assays were performed in gelatin-Tris buffer using tritiated 6-keto-PGF\textsubscript{1a}. 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 against unlabeled 6-keto-PGF$_{1 \alpha}$ by the method of 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, gases, pH, hemoglobin concentration, and hemoglobin saturation with O$_{2}$ 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 PaO$_{2}$. Arterial Po$_{2}$ and hemoglobin saturation with O$_{2}$ 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, PaO$_{2}$ had increased, and PacO$_{2}$, 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-PGF$_{1 \alpha}$ production (arterial minus sagittal sinus concentration times cerebral blood flow) are shown in Figure 1. Prior to hemorrhage, there was addition to 6-keto-PGF$_{1 \alpha}$ 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-PGF$_{1 \alpha}$ so that net cerebral 6-keto-PGF$_{1 \alpha}$ 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-PGF$_{1 \alpha}$ 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

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<th>Table 1. Arterial Pressure and Blood Chemistry of Newborn Pigs</th>
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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.)

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**Figure 1.** Prior to hemorrhage, there was addition to 6-keto-PGF$_{1 \alpha}$ 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-PGF$_{1 \alpha}$ so that net cerebral 6-keto-PGF$_{1 \alpha}$ 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-PGF$_{1 \alpha}$ concentration from 123 ± 33 pg/ml to 216 ± 56 pg/ml 60 minutes after hemorrhage.

**Figure 2.** 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
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 O₂ 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 O₂ consumptions of these 6 comatose piglets was 0.4 ± 0.1 ml O₂/100 g-min.

Figure 1. Net cerebral 6-keto-PGF₁α production prior to and 60 minutes following hemorrhage (30 ml/kg) in unanesthetized newborn pigs. Vehicle (n = 6) or indomethacin (5 mg/kg, ia) (n = 6) was administered 20 minutes after hemorrhage. * = p < 0.05 compared to control, † = p < 0.05 compared to vehicle.

Figure 2. Regional cerebral blood flows (CBF) prior to and following hemorrhage (30 ml/kg) in unanesthetized newborn pigs. Treatment with indomethacin (5 mg/kg, ia) (n = 13) or vehicle (n = 6) was administered where indicated. (* = p < 0.05, compared to posthemorrhage-pre-treatment value (20 min). † = p < 0.05, indomethacin treated compared to vehicle.)
Discussion

The present investigation establishes the ability of the untreated newborn pig to maintain cerebral O\textsubscript{2} consumption even when arterial pressure is decreased below 40 mm Hg by hemorrhage. Cerebral O\textsubscript{2} 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-PGF\textsubscript{1α}). 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.

An increase in brain vasodilator prostanooid synthesis is consistent with the increase in net cerebral vascular prostacyclin synthesis and the marked vasodilator 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\textsuperscript{25} 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 prostanooid synthe-
sis inhibitors can decrease cerebral blood flow in perinatal animals in conditions other than hemorrhagic hypotension. Treatment of fetal lambs in utero with aspirin27 or indomethacin28 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.13

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 prostanooid 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 • prostaglandins • hemorrhage • cerebral circulation • newborn
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