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Postasphyxial Increases in Prostanoids in Cerebrospinal Fluid of Piglets

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ABSTRACT. The dilator stimuli that contribute to postasphyxial increases in cerebral blood flow in the neonate are unclear. To assess the possible role of cyclooxygenase products in these responses, we measured pial arteriolar diameter in six piglets and determined levels of prostaglandin (PG) E_2 and 6-keto-PG $F_{1\alpha}$ (hydrolysis product of PGI_2) in cerebrospinal fluid (CSF) bathing the parietal cortex during control conditions, after 4–10 min of complete respiratory arrest (asphyxia), and after 5–12 min of reventilation. Pial arterioles are important resistance vessels in the cerebral circulation. Baseline pial arteriolar diameter was 220 ± 40 μm (mean ± SEM) and increased to a maximum of 252 ± 49 and 267 ± 56 μm after asphyxia and reventilation, respectively. During control conditions, CSF PGE_2 ($n = 6$) and 6-keto-$PGF_{1\alpha}$ ($n = 4$) levels were 1947 ± 310 and 794 ± 147 pg/ml, respectively. During asphyxia, CSF levels of PGE_2 did not increase, whereas 6-keto-$PGF_{1\alpha}$ increased modestly. During reventilation, CSF PGE_2 increased to 3576 ± 499 pg/ml, and 6-keto-$PGF_{1\alpha}$ increased to 2846 ± 123 pg/ml. In other experiments, we determined that these CSF levels of PGE_2 and PGI_2 (as 6-keto-$PGF_{1\alpha}$) were within the vasodilator range for pial arterioles. We conclude that postasphyxial increases in pial arteriolar diameter are associated with a rise in CSF levels of dilator prostanoids. (*Pediatr Res* 24: 229–232, 1988)

Abbreviations

PGE_2, prostaglandin E_2
6-keto-PG $F_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$
CSF, cerebrospinal fluid
CBF, cerebral blood flow
PGI_2, prostaglandin I_2

Birth asphyxia and postnatal apneic episodes and their consequences are major concerns of physicians. While it is clear that CBF increases dramatically during and after asphyxia (1–3), the mechanisms underlying cerebrovascular dilation are poorly understood. Several mechanisms, including cerebral acidosis or accumulation of vasodilator metabolites such as prostanoids or adenosine could be involved. We have provided evidence that the prostanoid system plays an important role in regulation of neonatal cerebral hemodynamics under several conditions. In particular, in piglets cerebral vasodilation during combined arterial hypoxia and hypercapnia (4, 5), hemorrhagic hypotension (6, 7), and increase in airway pressure (8) is accompanied by increased levels of prostacyclin and PGE_2 in the CSF surrounding cerebral resistance vessels. Further, inhibition of prostanoid synthesis by administration of indomethacin severely blunts cerebrovascular dilation under these conditions. If prostanoids participate in cerebral vasodilation during a more severe perturbation of arterial blood gases such as accompanies asphyxia and reventilation is unclear. Allen *et al.* (9) found that asphyxia in neonatal guinea pigs increases brain levels of prostanoids. However, in that study, prostanoid levels in whole brain were measured, and prostanoid changes were not correlated with cerebral hemodynamics. Brain levels of prostanoids are difficult to interpret, because the bulk of whole brain tissue is cytoplasm, and prostanoids are in the extracellular fluid.

The purpose of our study was to examine the effects of asphyxia and reventilation on cerebral hemodynamics and levels of CSF prostanoids in neonatal pigs. We tested the hypothesis that cerebrovascular dilation after asphyxia is accompanied by increases in CSF levels of prostacyclin and PGE_2.

MATERIALS AND METHODS

Thirteen newborn pigs (1.2–1.8 kg) of either sex, 1–5 days of age, were anesthetized with intramuscular ketamine-hydrochloride and acepromazine (33 and 3.3 mg/kg, respectively). Six of 13 piglets were used in the asphyxia experiments and seven were used to determine the cerebrovascular responsiveness to PGE_2 and I_2 (see below). A catheter was inserted into the femoral vein for injection of α-chloralose, 50 mg/kg initially, followed by 10 mg/kg/h to maintain the desired level of anesthesia. Another catheter was inserted into the femoral artery to record blood pressure and to sample blood. The animals were intubated and ventilated with air using a Harvard piston-type ventilator.

Body temperature was maintained between 37 and 38° C by wrapping the piglet in plastic wrap and a water-circulated heating pad. Then, a closed cranial window (4) was implanted over the left parietal cortex so the pial arteriolar diameter could be measured and CSF sampled. To implant the cranial window, the scalp was cut and reflected from the skull. A hole approximately 2 cm in diameter was made on the skull. Incision was made through the dura and arachnoid membranes, and these membranes then were reflected over the edge of the bone, into which a stainless steel ring with a premounted glass coverslip was inserted. The window was cemented in place with dental acrylic. Three needles pierce the ring and allow injection of artificial CSF under the window and sampling of CSF from under the window. The space under the window was filled with artificial CSF (Na^+ 150 mEq/liter, K^+ 3 mEq/liter, Ca^{2+} 2.5 mEq/liter, Mg^{2+} 1.2 mEq/liter, Cl^- 132 mEq/liter, glucose 3.7 mM, urea 6 mM, HCO_3^- 25 mEq/liter, pH = 7.33, PCO_2 = 46 mm Hg, PO_2 = 43 mm Hg). The volume directly below the window was approximately 500

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μl. After the implantation of the window, 30 min was allowed for equilibration of CSF under the window.

Pial arterioles were observed with a Wild trinocular stereomicroscope. Pial arteriolar diameter was measured with a television camera mounted on the microscope, a videomonitor, and a video microscaler. Briefly, the image of an arteriole was displayed on the television monitor and the sides of the vessels were bracketed by parallel lines projected by the microscaler. As the arteriolar diameter changed, the lines were moved manually to correspond with the vessel walls. Precalibration of the distance between the lines allowed determination of arteriolar diameter.

EXPERIMENTAL DESIGN

The asphyxia experiment was carried out in a group of six piglets (1.2–1.8 kg) of either sex, 1–5 days old. After flushing the cranial window with artificial CSF, the following baseline measurements were taken: arterial blood pressure, arterial blood gas values, and pial arteriolar diameter. Additionally, 300 μl of CSF were collected from under the cranial window. CSF was collected by slowly infusing artificial CSF through one needle and allowing the CSF under the window to drip freely from another needle on the opposite side of the window. The respirator then was turned off for 4–10 min based on animal's tolerance to asphyxia and the tubing to the ventilator clamped. Pial arteriolar diameter and blood pressure were measured during asphyxia, and CSF was sampled again at the end of the asphyxia. We terminated the asphyxial period when the animals had exhibited severe bradycardia and arterial blood pressure fell to ≤ 20 mm Hg. After the respirator was turned on, measurements were repeated, and CSF sampled after 5–12 min of reventilation.

In the dose response experiment, seven piglets comparable to previous group in regard to age, sex, and weight were studied. The piglets were pretreated with indomethacin (10 mg/kg, intravenous), 30 min earlier in order to stop endogenous prostaglandin production. We have shown previously that this dose of indomethacin crosses the blood brain barrier in sufficient quantities to drastically reduce synthesis of brain prostaglandins in piglets (10). PGE_2, 200 and 2000 pg/ml and PGI_2, 500 and 1000 pg/ml concentrations were used for this dose response experiment. To slow the degradation of PGI_2 that occurs in water, this prostanoid was dissolved in (Tris)-buffer, which by itself has no effect on pial arteriolar diameter. Before application of the first prostanoid, artificial CSF with no prostanoid was injected under the window and pial arteriolar diameter was measured (control value). Then the lower concentration of the first prostanoid was topically applied and maximal diameter recorded. Increased concentration of the same prostanoid was then added and maximal diameter was again recorded. Then the artificial CSF was injected into the window two to three times, 5 min apart until the pial arteriolar diameter has returned to control value. The desired concentrations of the second prostanoid were then applied, also measuring the maximal diameter with each concentration.

Prostanoid analysis. Prostanoids (6-keto-$PGF_{1\alpha}$ and PGE_2) were determined by radioimmunoassay (4). Antibodies to prostanoids were produced in rabbits immunized with prostanoids coupled to thyroglobulin using the mixed anhydride method. Cross-reactivities of our antibodies with other known biologically relevant prostanoids tested were all less than 1%. The assays were performed in gelatin (Tris) buffer using the appropriate tritiated prostanoid. After 24 h incubation at 4° C, the free fraction was separated from the fraction bound to antibody by precipitating the rabbit antibodies with anti-rabbit γ-globulin and 60% saturated ammonium sulfate. Data were handled by computer with determination of second order regression of free tracer over tracer bound to antibody against unlabeled prostanoids by the method of least squares. All unknowns were assayed at three dilutions with parallelism between the unknown dilution curve and the standard curve required before the result was used. Sample dilutions used in the present study allowed analysis of prostanoids concentrations between 100 and 50,000 pg/ml.

Statistical analyses were carried out using Friedman analysis of variance with the different periods as repeated measure. All values are presented as mean ± SEM.

RESULTS

During the control period, the mean arterial pressure was 57 ± 5 mm Hg ($n = 6$), and the mean arterial blood gases ($n = 6$) were: pH = 7.42 ± 0.48, $PaCO_2$ = 32 ± 2 mm Hg, and PaO_2 = 78 ± 4 mm Hg. During respiratory arrest, pial arterioles dilated (Fig. 1). At the end of the asphyxia period, the mean arterial pH was 7.17 ± 0.94, $PaCO_2$ was 67 ± 4 mm Hg, PaO_2 was 7 ± 2 mm Hg ($n = 3$), and arterial pressure was 30 ± 4 mm Hg ($n = 6$). During reventilation, dilation was maintained, and arterial pressure returned toward control (61 ± 3 mm Hg). The maximal increase in pial arteriolar compared to control diameter during reventilation was about 25%.

The CSF concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ during the control, asphyxia and reventilation periods are shown also in Figure 1. PGE_2 did not increase significantly above control during asphyxia, but was increased 111 ± 56% (value during asphyxia or reventilation-control value ÷ control value × 100) with reventilation at levels significantly higher than control. Levels of 6-keto-$PGF_{1\alpha}$ increased 111 ± 47% with asphyxia and continued to increase further during reventilation (244 ± 100%).

In another group of piglets, we found that PGE_2 dilated pial arterioles in the range of 200–2000 pg/ml, and PGI_2 dilated vessels over the range of 500–1000 pg/ml (Fig. 2). These levels were comparable to the changes in endogenous CSF levels of PGE_2 and PGI_2 during this experiment.

DISCUSSION

The major findings of our study in newborn pigs are that asphyxia and reventilation are accompanied by dilation of pial arterioles, and this change is associated, especially with reventilation, with a rise in CSF levels of PGI_2 (represented by 6-keto-$PGF_{1\alpha}$) and PGE_2. Further, CSF levels of prostanoids during these conditions were in the vasoactive range. Thus, it appears that prostanoids contribute to the cerebrovascular dilation observed during reventilation.

Postasphyxial cerebral hyperemia has been documented in animals of different ages and species. In neonatal dogs, sheep, and pigs, CBF increases dramatically during reventilation after asphyxia (1–3, 11). For example, CBF increases 250% in puppies (1), 191% in lambs (3), and 167% in piglets (2). In contrast, cerebrovascular responses during asphyxia can be quite variable. Hernandez *et al.* (11) found that blood flow to rostral areas of the puppy fell during 5 min of asphyxia, whereas blood flow to the pons and medulla increased. In contrast, in preliminary studies we have found that in newborn pigs blood flow to cerebrum and brainstem increased by the first minute of asphyxia (2). However, at 6 min of asphyxia, blood flow to cerebrum had returned to control levels, whereas brainstem blood flow was still elevated. In our study, pial arterioles remained dilated compared to control values at the end of the asphyxial period, indicating that resistance through this segment of the cerebral circulation fell under these conditions. Examination of dilator prostanoid levels in subarachnoid CSF and pial arteriolar tone during reventilation suggests that PGI_2 and PGE_2 are important determinants of cerebrovascular tone under these conditions. Thus, our dose-response curves for PGI_2 and PGE_2 indicate that the increase in CSF levels of these prostanoids with reventilation is sufficient to account for much but probably not all of the dilation. However, the lesser dilator response during asphyxia is probably not due to prostanoids, because the CSF levels of 6-keto-$PGF_{1\alpha}$ increase only moderately, and PGE_2 levels do not increase. Other possible mediators of vasodilation under these two conditions may be

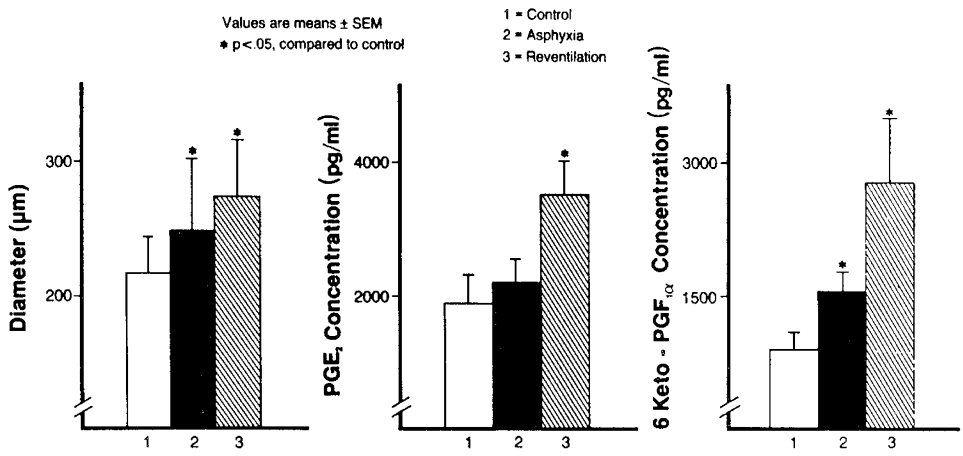

Fig. 1. Pial arteriolar diameter (n = 6) and CSF levels of PGE_2 (n = 6) and 6-keto-$PGF_{1\alpha}$ (n = 4) during control conditions, asphyxia, and reventilation. Diameters during asphyxia, and reventilation are maximal values.

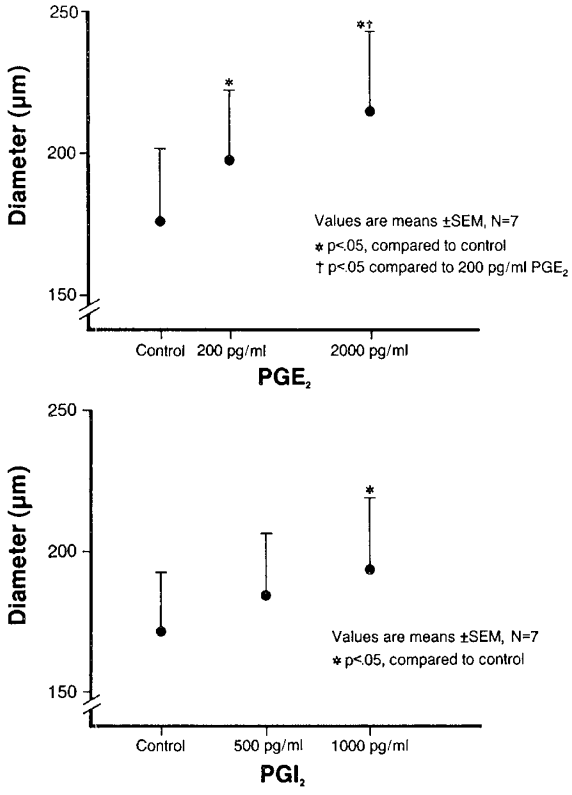

Fig. 2. Pial arteriolar responses to PGE_2 and PGI_2 in seven piglets.

increases in concentrations of brain adenosine (12), K^+ (13), or H^+ (14).

We are aware of only one other study that has examined cerebral prostanoid synthesis during asphyxia in the neonatal period. Allen *et al.* (9) exposed awake guinea pigs to 100% N_2 until they stopped breathing (3–4 min). Then, some of the animals were decapitated immediately, whereas others were resuscitated and then decapitated. During asphyxia, brain levels of PGE_2 but not $PGF_{2\alpha}$ were elevated over control values. After resuscitation, brain $PGF_{2\alpha}$ but not PGE_2 levels were higher than control values. In those animals, arterial blood pressure, gases, and cerebral hemodynamics were not measured. These results are difficult to interpret with regard to the cerebral circulation. Prostanoids are located extracellularly and are not stored in cells. Whole brain samples contain predominantly cytoplasm, and consequently it is difficult to say if whole brain levels of prostanoids reflect the concentrations of prostanoids around cerebral resistance vessels. In our experiments, we sampled CSF from the surface of the parietal cortex where the CSF is in immediate contact with cerebral vessels. We cannot be certain that a similar relationship exists between extracellular fluid and cerebrovascular tone in other parts of the brain. However, during arterial hypoxia or hypercapnia, all cerebral resistance vessels dilate, and blood flow to all areas of the brain increases (15).

The source of prostanoids in our CSF samples was probably the underlying parietal cortex. Our experiments do not allow us to distinguish the relative contribution of other tissues, *i.e.* lungs, or of the cortical neurons, glial cells, and cerebral vessels to prostanoid levels in CSF. However, on the basis of evidence from other studies, 6-keto-$PGF_{1\alpha}$ most likely is derived from vessels, whereas PGE_2 probably is produced by neurons or glial cells (16, 17).

In summary, cerebrovascular dilation during asphyxia and reventilation is associated with an increase in CSF levels of 6-keto-$PGF_{1\alpha}$ and PGE_2. It seems likely that a major part of the vascular response during reventilation is caused by dilator prostanoids.

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