Prostanoids in Cortical Subarachnoid Cerebrospinal Fluid and Pial Arterial Diameter in Newborn Pigs

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SUMMARY. These studies were designed to investigate the relationship between cerebral prostanoid synthesis and pial arterial caliber in chloralose-anesthetized newborn pigs with normal blood gases and pH and during combined arterial hypoxia and hypercapnia. Piglets less than 5 days old were equipped with closed cranial windows to allow direct observation of pial vessels, application of prostaglandin E₂ and sampling of cortical subarachnoid cerebrospinal fluid. We found that prostanoids accumulate in cerebrospinal fluid on the cortical surface. The only prostanoid detected in arterial blood was 6-keto-prostaglandin F₁α [442 ± 74 pg/ml (radioimmunoassay)]. Only small quantities of 6-keto-prostaglandin F₁α (214 ± 53 pg/ml) and thromboxane B₂ (122 ± 18 pg/ml) were found in cerebrospinal fluid from the cisterna magna. Higher concentrations of 6-keto-prostaglandin F₁α (1056 ± 159 pg/ml), thromboxane B₂ (229 ± 64 pg/ml), and prostaglandin E₂ (4235 ± 269 pg/ml) were found in cortical subarachnoid fluid. In contrast to arterial and cisternal concentrations, the concentrations of 6-keto-prostaglandin F₁α, thromboxane B₂, and prostaglandin E₂ in cortical subarachnoid fluid were increased reversibly by ventilation with 9% carbon dioxide, 10% oxygen (6-keto-prostaglandin F₁α 5436 ± 1576 pg/ml; thromboxane B₂ 694 ± 122 pg/ml; and, prostaglandin E₂ 12,455 ± 3688 pg/ml). Further, pial arteries dilated in response to topical application of prostaglandin E₂ at the concentration that was found in cortical subarachnoid fluid during combined hypoxia and hypercapnia. Systemic administration of indomethacin trihydrate (5 mg/kg) markedly reduced cortical subarachnoid fluid prostanoid concentrations and attenuated the pial artery vasodilation induced by combined hypoxia and hypercapnia. Our data are consistent with the hypothesis that cerebral prostanoids represent an integral component in cerebral hemodynamic control in the neonatal pig. (Circ Res 57: 689–694, 1985)

The role of prostanoids in cerebral hemodynamics in adult animals remains controversial. Investigators using adult primates, rats, and gerbils have found that systemic administration of indomethacin decreases cerebral blood flow during normocapnia and reduces the hyperemia induced by hypercapnia (Pickard and MacKenzie, 1973; Sakabe and Siesjö, 1979; Crockard et al., 1982). Conversely, several investigators using rabbits, dogs, and cats have been unable to detect alterations in resting cerebral blood flow and pial arterial diameter or in the responses to hypercapnia or hypoxia following systemic treatment with indomethacin (Wei et al., 1980; Busija, 1983; Busija and Heistad, 1983; Jackson et al., 1983), whereas others reported changes similar to those found in primates, rats, and gerbils after treatment of dogs (Rusczewski and Herbachynska-Cedro, 1978), cats (Vlahoo, 1976; Shigeno et al., 1983), and rabbits (Bill, 1979; Pinard, 1983) with indomethacin. Further, measurements of prostanoids in cerebral venous blood and brain tissue have not revealed changes in cerebral prostanoid synthesis during alterations in blood gases (Ellis et al., 1982; Eriksson et al., 1983; Jackson et al., 1983; McCalden et al., 1984).

Prostanoids appear to be more prominent in the cardiovascular physiology of the fetus and neonate than in the older mammal. For example, treatment of a fetus or neonate that has a patent ductus arteriosus with cyclooxygenase inhibitors causes constriction of that vessel (Friedman et al., 1976; Heymann and Rudolph, 1976; Heymann et al., 1976). Treatment of anesthetized, newly ventilated fetal goats with cyclooxygenase inhibitors causes more pronounced pulmonary vasoconstriction and a greater augmentation of hypoxic pulmonary vasoconstriction than does treatment of older newborns (Tyler et al., 1975), and prostacyclin contributes to the decrease in pulmonary vascular resistance that occurs at the time of birth (Leffler et al., 1978; Leffler and Hessler, 1979; Leffler et al., 1980; Leffler and Hessler, 1981; Leffler et al., 1984). Available evidence suggests that cyclooxygenase inhibitors can decrease cerebral blood flow in perinatal animals (Heymann and Rudolph, 1976; Ment et al., 1983; Ment et al., 1984). Finally, plasma prostacyclin concentrations are higher in the fetus and neonate than in the older baby or adult animal (Kappa et al., 1982; Leffler et al., 1982).

The present study was designed to investigate the potential significance of prostanoids in control of cerebral hemodynamics in the newborn pig. Thus, experiments were conducted to determine whether:

1) prostanoids accumulate in cerebrospinal fluid
(CSF) as the CSF passes over the cortical surface (cortical subarachnoid CSF); (2) prostanoid concentrations in cortical subarachnoid CSF are altered during acute, combined hypoxia/hypercapnia coincident with cerebral vasodilatation; (3) pial vessels dilate in response to prostanoids; (4) systemic indomethacin treatment reduces cortical subarachnoid CSF prostanoid concentrations; and (5) systemic indomethacin treatment constricts pial vessels and decreases the dilation in response to combined hypercapnia/hypoxia.

Methods

Newborn pigs (0.8–1.8 kg) of either sex, 1–5 days of age, were anesthetized with ketamine hydrochloride (33 mg/kg, im) and acepromazine (3.3 mg/kg, im) and maintained on α-chloralose (50 mg/kg, iv, initially, plus 10 mg/kg per hr). The animals were intubated percutaneously and ventilated with air and supplemental oxygen. Catheters were inserted in the femoral vein for injection of drugs and in the femoral artery to record blood pressure and to draw samples for blood gas, pH, and prostanoid analysis. The body temperature was maintained between 37°C and 38.5°C by wrapping the piglet in plastic film and a water-circulated rubber heating pad. The scalp was removed, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura and arachnoid membrane were cut without touching the brain, and all cut edges were reflected over the bone so that the subarachnoid space was not exposed to either damaged bone or membranes. A stainless steel and glass cranial window was placed in the hole and cemented into place with dental acrylic. The space under the window was filled with artificial CSF (220 mg KCl, 132 mg MgCl₂, 221 mg CaCl₂, 7710 mg NaCl, 402 mg urea, 665 mg dextrose, 2066 mg NaHCO₃ per liter, pH 7.33; Pco₂ = 46 mm Hg; Po₂ = 43 mm Hg) through needles incorporated into the sides of the window. The volume of fluid directly under the window was 500 μl and was continuous with the subarachnoid space. Following implantation of the window, at least 30 minutes were allowed for exchange and equilibration of fluid under the window with the subarachnoid fluid before experimentation was begun.

Pial arterioles were observed with a Wild trinocular stereo microscope. Pial arterial diameter was measured with a television camera mounted on the microscope, a video monitor, and a video microscaler (model VPA-1000, FOR-A-Corporation). Using a stage micrometer, we determined that the scaler was linear over the range from 0–1000 μm.

Collection of Samples

Arterial samples (2 ml each) were drawn from the femoral arterial line into a syringe containing 15 μg of indomethacin trihydrate and 15 U of heparin. The formed elements were removed by centrifugation (30,000 g for 20 minutes at 2°C) and the plasma was drawn off and stored at −60°C for later analysis. Cerebral surface CSF (300 μl) was collected by placing 1-ml syringes on the injection ports of the cranial window. CSF was collected by withdrawing CSF simultaneously with a slow infusion of an equal volume of artificial CSF at the opposite side of the window. In preliminary experiments, we discovered that exposing the subarachnoid space to atmospheric pressure during ventilation with high CO₂, low O₂ mixture (see below) allowed the brain to swell into the pocket formed under the cranial window. Therefore, the sample was drawn into the syringe during administration of hypoxia/hypercapnia, but the syringe was not removed until several minutes after the piglet had been returned to ventilation with oxygenated air. CSF from the cisterna magna (300 μl) was collected by direct cisternal puncture with a 22-gauge needle.

Experimental Design

The cranial window was flushed with fresh artificial CSF equilibrated with 6.5% CO₂, 6% O₂, and N₂. Twenty minutes later, an arterial sample was drawn for blood gas analysis and prostanoid determination, 300 μl of cerebral surface CSF from under the cranial window were collected, and pial arterial diameter was measured. The ventilation mixture was then changed to 9% CO₂, 10% O₂, and N₂. Twenty minutes later, arterial samples were drawn for blood gas analysis and prostanoid determination. CSF from beneath the window was collected, pial arterial diameter was measured, and the ventilation mixture was returned to oxygenated air. For measurements later, the CSF was flushed again from beneath the cranial window. The pial arterial diameter was measured 5 minutes later. After 20 minutes of ventilation with the oxygenated air, the arterial samples and cortical CSF were once again collected. The piglet was administered 5 mg/kg of freshly dissolved indomethacin trihydrate (Merck, Sharp, and Dohme) in saline (1 mg/ml, pH 6.9) as an intravenous infusion over a 1-minute period. The CSF from under the cranial window was flushed 3 times during the ensuing 30 minutes, and the procedures employed before indomethacin administration were repeated.

In a separate group of piglets, the diameter of a pial artery was determined: (1) during ventilation with oxygenated air, (2) at the end of 10 minutes of ventilation with 9% CO₂, 10% O₂, (3) 10 minutes after 5 mg/kg indomethacin trihydrate (iv) while continuing ventilation with 9% CO₂, 10% O₂, (4) 30 minutes after return to ventilation with oxygenated air, and (5) at the end of another 10 minutes of ventilation with 9% CO₂, 10% O₂.

After administration of indomethacin trihydrate (iv), we determined the ability of exogenously administered prostaglandin E₂ (PGE₂) to dilate the pial arteries of newborn pigs. PGE₂ was dissolved in artificial CSF, which was placed under the cranial window at concentrations of 10, 100, 1000, and 2000 ng/ml. Pial arterial diameter was determined when a steady state was reached (1–2 min).

Prostanoid Analysis

Prostanoids [6-keto-prostaglandin F₁α (6-keto-PGF₁α), thromboxane B₂ (TXB₂), and PGE₁] in plasma were determined by radioimmunoassay. All tubes contained an identical amount of pig plasma. The standard curves, knowns, blanks, and make-up for greater dilution, used plasma from the same piglets as the unknowns, following dialysis of plasma against 4 liters of Krebs bicarbonate buffer. Previously, we determined that results from extracted plasma and unextracted plasma against the dialyzed plasma matrix were not significantly different when extracted samples were corrected for recovery. CSF samples were analyzed by radioimmunoassay employing an artificial CSF matrix.

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 below 1%. The assays were performed in gelatin-Tris buffer using the appropriate tritiated prostanoid. After 24 hours of 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 prostanoid 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 prostanoid concentrations between 100 and 50,000 pg/ml.

### Statistical Analyses

All values are presented as means ± SEM. Comparisons between populations were made using analysis of variance for repeated measures (followed by pairwise tests with Bonferroni correction, when appropriate). Significance at the 5% level was required for inference that populations were different.

### Results

The concentrations of 6-keto-PGF_{1α}, TXB₂, and PGE₂ in the aortic plasma, cisterna magna CSF, and cortical subarachnoid CSF of chloralose-anesthetized neonatal pigs are shown in Table 1. The only detectable prostanoid in aortic plasma was 6-keto-PGF_{1α}. CSF from the cisterna magna contained both 6-keto-PGF_{1α} and TXB₂, but no detectable PGE₂. In contrast, high concentrations of both 6-keto-PGF_{1α} and PGE₂ were found in cortical subarachnoid CSF, and a concentration of TXB₂ higher than was found in the aorta or the cisterna magna was detected as well. Intravenous treatment with 5 mg/kg of indomethacin trihydrate markedly decreased the concentrations of all prostanoids examined in the aortic plasma and the cortical subarachnoid CSF (Table 1).

### Table 1

| Prostanoid Concentrations in Aorta, Cisterna Magna CSF, and Cortical Subarachnoid CSF of Chloralose-Anesthetized Pigs before and after Treatment with Indomethacin |
|---------------------------------|------------------|------------------|------------------|
| Prostanoid concentration (pg/ml) | 6-keto-PGF_{1α} | TXB₂ | PGE₂ |
| Aorta (n = 7)                   |                  |      |      |
| Control                         | 442 ± 74†        | ND‡  | ND   |
| Indomethacin*                   | ND               | ND   | ND   |
| Cisterna magna (n = 6)          |                  |      |      |
| Control                         | 214 ± 53         | 122 ± 18 | ND   |
| Cortical subarachnoid CSF (n = 7) |                  |      |      |
| Control                         | 1056 ± 159       | 229 ± 64 | 4235 ± 269 |
| Indomethacin                    | ND               | ND   | 424 ± 182 |

* 5 mg/kg indomethacin trihydrate, iv.
† Mean ± SEM.
‡ ND = not detectable.

The cisterna magna was sampled only one time in each of these piglets.

Ventilation of neonatal pigs with 9% CO₂/10% O₂ caused pial arterial diameters to increase from 176 ± 44 μm to 244 ± 63 μm without significantly affecting arterial pressure (56 ± 4 and 50 ± 2 mm Hg) (n = 5). Ten minutes after return to ventilation with oxygenated air, the pial arterial diameters (182 ± 42 μm) were not significantly different from the diameters before ventilation with 9% CO₂/10% O₂.

Ventilation of neonatal piglets with 9% CO₂/10% O₂ caused a pronounced increase in all three prostanoids examined in cortical subarachnoid CSF (Fig. 1). The concentration of 6-keto-PGF_{1α} increased approximately 5 times, while the concentrations of TXB₂ and PGE₂ both increased approximately 3 times during ventilation with a hypoxic/hypercapnic gas mixture. The increase in prostanoids in the cortical subarachnoid CSF was reversed following return to ventilation with oxygenated air. No increase in prostanoids was observed in the aortic plasma during ventilation with 9% CO₂, 10% O₂ (435 ± 119 pg/ml during normoxia/normocapnia, 386 ± 112 pg/ml during hypercapnia/hypoxia). In two additional piglets, CSF from the cisterna magna was examined before and during ventilation with 9% CO₂/10% O₂. Ventilation of neonatal piglets with 9% CO₂/10% O₂ caused a pronounced increase in all three prostanoids examined in cortical subarachnoid CSF (Table 1).
CO₂ and 10% O₂. Prostanoids in the cisterna magna were not changed greatly during ventilation with 9% CO₂/10% O₂ (means of 2: 6-keto-PGF₁α = 162 pg/ml, control and 234 pg/ml, hypoxia/hypercapnia; TXB₂ = 100 pg/ml, control and 91 pg/ml, hypoxia/hypercapnia).

PGE₂ in the artificial CSF placed under the cranial window caused a dose-dependent dilation of pial arteries up to a concentration of 100 ng/ml. Thus, pial artery diameter was increased 19 ± 3% by 10 ng/ml PGE₂, 34 ± 10% by 100 ng PGE₂/ml, 40 ± 16% by 1000 ng PGE₂/ml, and 39 ± 15% by 2000 ng PGE₂/ml (n = 4). The maximum dilation caused by exogenous PGE₂ (from 131 ± 13 to 182 ± 13 μm in diameter) was 74 ± 17% of that attained with hypoxia/hypercapnia in the same piglets. At a dose of 10 ng/ml PGE₂ caused a dilation of piglet pial arteries which was 38 ± 5% of the maximum dilation achieved with combined hypoxia/hypercapnia.

The effect of intravenous administration of indomethacin trihydrate on the pial artery diameter and on responses of pial arteries to ventilation with 9% CO₂/10% O₂ is shown in Figure 2. Intravenous administration of indomethacin during ventilation with 9% CO₂/10% O₂ caused a significant vasosconstriction of pial arteries within 10 minutes. Following return to ventilation with oxygenated air for 30 minutes, previous treatment with indomethacin significantly inhibited, but did not abolish, the pial artery vasodilation caused by ventilation with 9% CO₂/10% O₂.

**Discussion**

The present study in newborn pigs indicates that (1) the prostanoid concentration in cortical subarachnoid CSF is higher than the prostanoid concentration in either the cisterna magna or the arterial blood, (2) prostanoids in cortical subarachnoid CSF increase during acute hypoxia combined with hypercapnia coincident with dilation of the pial vessels, (3) exogenous PGE₂ placed in CSF over the pial vessels at physiological concentrations causes dilation of the pial vessels, (4) systematically administered indomethacin crosses the blood-brain barrier in sufficient quantities to reduce cortical subarachnoid CSF prostanoid concentrations, and (5) systemic indomethacin decreases pial artery dilation in response to combined hypoxia and hypercapnia.

Application of PGE₂ to pial arteries, at approximately the same level as is achieved during combined hypercapnia and hypoxia, produced about 40% of the dilation that is caused by the combined hypoxia and hypercapnia. Therefore, the physiological levels of PGE₂ in the cortical surface CSF are inadequate to account for the pial artery dilation produced by combined hypoxia/hypercapnia. However, systemic treatment with indomethacin decreased cortical subarachnoid CSF prostanoid concentrations to near or below detectable levels, but only reduced the vasodilation produced by the severe blood gas alteration approximately 50%. Further, if the tissues responsible for the increased prostanoid synthesis are vascular, then the prostanoid concentrations in and around the blood vessels near the site of synthesis might be much higher. The prostacyclin (PGI₂) synthesis by the blood vessels also might be important in the cerebral vasodilation. Meaningful assessment of the ability of prostacyclin to dilate the cerebral vessels was not possible using the techniques presently employed because of the rapid hydrolysis of PGI₂ at physiological pH. The large increase in 6-keto-PGF₁α concentration in the cerebral spinal fluid on the brain surface that occurred during combined hypoxia/hypercapnia suggests that high rates of prostacyclin synthesis by vascular endothelial and/or smooth muscle might result in very high concentrations in and around the vascular smooth muscle which could produce vasodilation.

Cerebrospinal fluid is produced by the choroid plexus in the lateral ventricles, the 3rd ventricle, and the 4th ventricle, as well as by extrachoroidal tissue. CSF leaves the ventricular system from the roof of the 4th ventricle into the subarachnoid cisterna magna and from the lateral recesses of the 4th ventricle. The CSF moves throughout the subarachnoid space of the brain and spinal cord, exiting into venous sinuses. In the adult human, the half-time...
for CSF renewal is about 200 minutes (Maren, 1974). Thus, the very rapid decline in prostanooid levels seen in the cerebral surface CSF upon return to ventilation with oxygenated air following hypoxia combined with hypercapnia is surprising. However, the subarachnoid fluid on the cerebral surface forms a very thin film, the turnover of which is certainly much greater than that of the greater bulk of the CSF in the ventricles and cisterna. Thus, if the rate of prostanooid synthesis were to decline, the increased prostanooid level could fall rapidly as the prostanooids are carried away in the venous blood. Nevertheless, the low flow of CSF compared to that of blood would make even considerable prostanooid concentrations in the CSF added to cerebral venous blood undetectable when diluted in the larger volume of venous blood. Prostaglandins can be transported actively from the CSF to the blood by the choroid plexus (Bito and Davson, 1974; Davson, 1976). Thus, the low prostanooid concentration in cisternal CSF compared to cortical subarachnoid CSF is not surprising.

Our results in the neonatal piglet are consistent with those of others in perinatal animals which suggest a role for prostanooids in perinatal cerebral hemodynamics. For example, Bedard et al. (1983) reported that, although cerebral blood flow was not altered significantly by indomethacin in pentobarbitol-anesthetized puppies 3-27 days of age, indomethacin appeared (not significant with small sample size) to reduce cerebral blood flow in puppies less than 3 days old. In addition, Ment et al. (1983, 1984) found, using carbon-14 autoradiography, that indomethacin and ethamsylate decreased cerebral blood flow of newborn puppies. Further, Heymann and Rudolph (1976) found that aspirin treatment of fetal lambs in utero decreased cerebral blood flow.

In contrast, a role for the prostanooid system in cerebral hemodynamics of adult animals is controversial. On the one hand, investigators studying adult baboons, rats, and gerbils found that indomethacin reduced cerebral blood flow during normocapnia and virtually abolished the increase in cerebral blood flow during hypercapnia (Pickard and MacKenzie, 1973; Sakabe and Siesjo, 1979; Crockard et al., 1982). In addition, some investigators observed similar results using cats, rabbits, and dogs (Vlahov, 1976; Ruszczewski and Herbaczyńska-Cedro, 1978; Bill, 1979; Shigeno et al., 1983; Pinard, 1983). On the other hand, others have found that indomethacin, at doses that block vasodilatory responses of pial vessels to exogenous arachidonic acid, did not affect cerebral blood flow, pial artery diameter, or responses of cerebral arteries to hypercapnia in adult cats, rabbits, or dogs (Wei et al., 1980; Busija, 1983; Busija and Heistad, 1983; Jackson et al., 1983). Further, in adult animals, the cerebral vasodilator response to arterial hypoxia was consistently unaltered by intravenous administration of indomethacin (Sakabe and Siesjö, 1979; Wei et al., 1980). The conflicting data in adult animals could involve species differences. The more consistent finding in perinatal animals might indicate that the prostanooid system is a more integral component of circulatory control in the perinatal animal than in the adult animal.

A greater contribution of prostanooids to perinatal hemodynamics than to hemodynamics of adult animals is suggested by several lines of evidence, including: (1) the elevated concentration of the prostacyclin hydrolysis product, 6-keto-PGF1α, in fetal and neonatal arterial plasma when compared to the adult (Kaapa et al., 1982; Leffler et al., 1982); (2) the ability of cyclooxygenase inhibitors to close the ductus arteriosus in the fetus or neonate (Friedman et al., 1976; Heymann and Rudolph, 1976; Heymann et al., 1976), suggesting that prostanooids are important in the maintenance of patency of the ductus arteriosus in the fetus; (3) data supporting the hypothesis that pulmonary prostacyclin production contributes to the decline in pulmonary vascular resistance at the onset of ventilation at birth (Leffler et al., 1978; Leffler and Hessler, 1979; Leffler et al., 1980; Leffler and Hessler, 1981; Leffler et al., 1984); and (4) the transient decline in renal function that typically accompanies treatment of neonates with cyclooxygenase inhibitors (Cifuentes et al., 1979; Friedman and Kirkpatrick, 1980), in contrast to the negligible effects of cyclooxygenase inhibition upon renal function in adults. However, since the effect of indomethacin upon pial arterial diameter or cerebral blood flow in adult pigs has not been examined, it is not known whether the response of the newborn pig pial vessels to indomethacin treatment is greater than that of the adult pigs.

In conclusion, our data are consistent with the hypothesis that the prostanooid system is an integral component of cerebral hemodynamics in the neonatal pig.

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