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Role of Prostanoids in the Regulation of Cerebral Blood Flow During Normoxia and Hypoxia in the Fetal Sheep

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ABSTRACT: The fetal cardiovascular responses to hypoxia include decreased peripheral blood flow and increased cerebral, cardiac, and adrenal blood flow. Prostanoids, metabolites of cyclooxygenase enzyme activity, have potent effects on vascular tone in both the adult and the fetus. To examine the role of prostanoids in the regulation of fetal cerebral blood flow (CBF) during acute hypoxic stress, eight near term fetal sheep were studied after infusing vehicle or diclofenac, a cyclooxygenase inhibitor, followed by a 30-min period of hypoxia (arterial Po_2 12 Torr). In the control experiments, CBF, measured continuously with laser Doppler flowmetry, increased to 148% of baseline values ($p < 0.01$) and cerebral vascular resistance decreased to 70% of baseline values after 30 min of hypoxic stress. During diclofenac infusion, hypoxia resulted in a CBF increase to only 129% of baseline, a significant attenuation ($p < 0.05$), accompanied by decreased plasma prostanoid concentrations. Increases in mean arterial blood pressure during hypoxia were also attenuated by diclofenac infusion. Flow and pressure responses were not accompanied by changes in cerebral vascular resistance. These results indicate that prostanoids indirectly modulate fetal CBF responses to hypoxia, but that their effects are mediated through modulation of systemic rather than cerebral vascular tone. **(*Pediatr Res* 60: 524–529, 2006)**

The fetus is often subjected to hypoxic insult during gestation and labor. In defense against hypoxic injury, mechanisms come into play that optimize the balance between oxygen delivery and consumption in vital organs such as the brain, heart, and adrenals. In the fetal brain, this is accomplished in part by an increase in CBF (1–3), a regulated reduction in oxidative metabolism (4–6), and an increase in anaerobic metabolism. This increase in CBF is accomplished in part by redistribution of blood flow during hypoxic stress resulting in increased blood flow to vital organs and decreased blood flow to peripheral organs (1). The redistribution of flow to these organs is the result of increased systemic arterial blood pressure and decreased local cerebral vascular resistance (1,7). Previous work by our group and others has shown that both adenosine and nitric oxide are important mediators of this effect (5,6). However, the changes in CBF and vascular resistance during hypoxia cannot be completely attributed to these systems. The studies presented here focus on the role of prostanoids in mediating these changes in blood flow to the fetal brain during hypoxia.

Prostanoids, a subclass of eicosanoids that includes prostaglandins and thromboxanes, are found throughout biologic tissues and are important modulators of vascular tone. Prostanoids are synthesized by oxidation of free arachidonic acid, a reaction catalyzed by cyclooxygenase enzymes cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2). In adult vascular tissues, COX-1 is a constitutive enzyme, whereas COX-2 is an inducible enzyme, both of which are involved in the production of precursors of the vasoconstrictor thromboxane [thromboxane A_2 (TxA_2)], the vasodilator prostaglandin I_2 (PGI_2), and prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which can induce either vasoconstriction or vasodilation (8). The activity of both COX enzymes can be selectively antagonized by diclofenac (9).

Prostanoids play an important role in the regulation of basal CBF of adults (10–13), newborns (14–16), and fetuses (17,18), although there is at least one report of no effect of COX inhibition in adults (19). Data regarding the role of prostanoids in the regulation of vascular responses to hypoxia are less clear, with some reports concluding they do play a role (20) and some concluding they do not (15,21). In the fetus, although prostanoids have been suggested to play a role in the regulation of CBF responses to hypotension (22) and in metabolic responses to hypoxia (17,23), we found no reports on the role of prostanoids in the regulation of fetal CBF responses to hypoxia.

In recent work, we have shown important roles for adenosine and nitric oxide in cerebral vasodilation during hypoxia in the near term fetal sheep (2,5,6). A number of reports in adults and newborns suggests that a complex interaction exists between these modulators and prostanoids in the vascular response to hypoxia (24,25). Therefore, we hypothesized that prostanoids would also be involved in mediating fetal CBF

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Abbreviations: CBF, cerebral blood flow; **COX 1,** cyclooxygenase 1; **COX 2**, cyclooxygenase 2; **PGE_2**, prostaglandin E_2; **$PGF_{2\alpha}$**, prostaglandin $F_{2\alpha}$; **PGI_2**, prostaglandin I_2; **TxA_2**, thromboxane A_2; **TxB_2**, thromboxane B_2

responses to hypoxia. The present study was designed to test this hypothesis by examining the effects of i.v. infusion of diclofenac on the chronically instrumented fetal sheep before and during a period of acute hypoxic stress.

METHODS

Animal preparation. Animal protocols were approved by the Loma Linda University Animal Care Research Committee. Fourteen Western pregnant ewes obtained from Nebeker Ranch (Lancaster, CA) were used for the study. Ewes and fetuses were instrumented at 124 to 127 d of gestation. Anesthesia was induced with 0.5 g thiopental i.v. and maintained by inhalation of 1.5–2.5% halothane through a tracheal tube. A polyvinyl catheter was placed in the maternal femoral vein for administration of fluids and euthanasia solution at a termination of the experiments. Under aseptic conditions, through a midline abdominal incision, the uterus was incised and the fetal head and upper torso were exposed. Polyvinyl catheters were placed in brachial arteries bilaterally for arterial blood sampling and blood pressure measurement, in the right subclavian vein for drug administration, and in the amniotic fluid for pressure measurement and administration of antibiotics. A thermocouple was placed in a brachial artery for fetal temperature measurement. The fetal skull was exposed, and a polyvinyl catheter was inserted into the sagittal sinus for cerebral venous blood sampling. Bur holes 0.5 mm in diameter were made bilaterally 5 mm lateral to the sagittal sinus suture and 15 mm posterior to the coronal suture. A thermocouple was inserted in one bur hole for brain temperature measurement. A composite laser Doppler flow and tissue Po_2 probe (Oxford Optronix, Oxford, UK) was inserted through the other into the parasagittal parietal lobe cortex to a depth 5 mm below the dura. The probe was then fixed to the skull with Germicidal Pink (Western Medical Supply, Arcadia, CA). The fetal skull was closed with tissue glue, the fetal upper torso was returned to the uterus and incisions sutured closed in layers. The catheters, probes, and thermocouple leads were passed to the ewe's flank and secured there in a nylon pouch. The ewe was given 900,000 U penicillin i.m. for 3 d, and the fetus was given 40 mg gentamicin and 500 mg ampicillin in the amniotic fluid and 50 mg cefotaxime i.v. once daily. Experiments were carried out beginning 4 d after the surgery with at least 48 h separating control and diclofenac protocols.

Experimental procedures. Two experiments were performed in random sequence in each fetal sheep. Each consisted of a 30-min baseline period, a 90-min i.v. infusion period, and a 30-min recovery period following the end of the infusion. In the control protocol, only the vehicle was given. Diclofenac was dissolved in a vehicle of 100 mM sodium carbonate on the same day of the experiment. The drug was infused as a bolus of 1.33 mg kg^{-1} estimated fetal weight of 2 kg followed by a continuous infusion of 0.44 $mg \cdot kg^{-1} \cdot h^{-1}$, a dose effective in reducing hypoxia-induced vasodilation in the rat hind limb (24,26). Hypoxia was administered for 30 min beginning one half hour after the start of the infusion by having the ewe breathe 10–12% O_2 in nitrogen administered with a flow rate of 30 $L \cdot min^{-1}$ into a bag placed over the ewe's head.

Blood sampling. Fetal arterial and sagittal sinus blood samples (0.3 mL each) were collected at 0, 15, and 30 min during baseline period, at 40, 50, and 60 min during the prehypoxia infusion period, at 65, 70, 80, and 90 min during the infusion and hypoxia periods, at 100, 110, and 120 min during the posthypoxia infusion period, and at 135 and 150 min during the recovery period. These samples were analyzed for blood gases (ABL3, Radiometer, Copenhagen, Denmark), and values were corrected to the body temperature of the fetal sheep. Hemoglobin concentration and oxyhemoglobin saturation were measured spectrophotometrically (OSM2 Hemoximeter, Radiometer).

Plasma glucose and lactate concentrations were measured using an YSI 2700 analyzer (Yellow Springs Instruments, Dayton, OH). At 15 and 30 min during the baseline period, at 50 and 60 min during the prehypoxia infusion period, and at 65, 80, and 90 min during hypoxia period, additional fetal arterial blood samples (0.7 mL each) were collected for prostanoid assay. The samples were centrifuged at 4°C for 5 min at $1000 \times g$, and the plasma was removed and stored at −70°C. At a later date, the samples were thawed and pooled by animal and protocol stage, resulting in pooled arterial and sagittal sinus samples from the baseline, prehypoxia infusion, and the hypoxia periods for each animal studied. The pooled samples were analyzed *via* EIA by Cayman Chemical (Ann Arbor, MI) for PGE_2, PGI_2, and TxA_2 concentrations. PGE_2 concentrations were determined by conversion of all major PGE_2 metabolites into a single stable derivative measurable *via* electroimmunoassay. PGI_2 concentrations were determined by measurement of 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2). TxA_2 concentrations were determined by measurement of TxB_2 (a stable metabolite of TxA_2).

Electronic data acquisition and handling. Measurements of local CBF were performed continuously with a laser Doppler flowmeter (Oxyflow, Oxford Optronix, Oxford, UK), whose results have been found in satisfactory agreement with microsphere determinations (27). Analogue outputs were digitized (sampling rate 100 Hz) and stored using an analogue-to-digital converter (Powerlab 16/SP, ADInstruments, Colorado Springs, CO) and data acquisition software (Chart v.5, ADInstruments). Because laser Doppler flowmetry provides only a relative measure of blood flow, all values calculated using CBF are presented as a percentage change from baseline values. CBF, cortical tissue Po_2, arterial blood pressure corrected for amniotic fluid pressure, heart rate, and sagittal sinus pressure were calculated and expressed as 3-min averages. Cerebral vascular resistance was calculated as the difference between arterial and sagittal sinus pressure divided by CBF. Due to loss of sagittal sinus catheter patency, arterial blood pressure was assumed to be equivalent to cerebral perfusion pressure in four of the sheep studied under both the control and diclofenac protocols. Cerebral vascular resistance and mean arterial blood pressure were also expressed as a percentage of baseline measurements.

Western blotting. Previously unstudied near-term pregnant ewes whose vessels were to be used for other studies were killed with an overdose of pentobarbital. Fetal middle cerebral artery segments were collected for Western immunoblotting to determine COX-1 and COX-2 protein abundances. Total cellular proteins were obtained by glass-on-glass homogenization of the freshly frozen artery segments at a fixed buffer-to-tissue ratio of 60 μL/mg wet weight. The homogenization procedure (28) used a Laemmli buffer (0.125 mM TrisHCl, pH 6.8, containing 10% glycerol, 2.5% SDS, 0.006% bromophenol blue, and 0.1 M dithiothreotol) followed by boiling for 10 min at 100°C. Proteins (30 μg protein/lane) were separated by 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked overnight with 5% milk in triethanolamine buffered saline and 0.1% Tween 20. The membranes were probed for 2 h with COX-1 or COX-2 human monoclonal antibody (Cayman Systems, Stoneham, MA at 1:500 dilution), followed by a horseradish peroxidase goat anti-mouse IgG (HRP Pierce at 1:15,000 dilution). The immunoreactive bands were visualized using an AlphaInnotech ChemiImager and quantified by digital densitometry. Known amounts of human recombinant standards (Cayman Systems, Stoneham, MA) were included in each gel to prepare a standard curves from which the absolute masses of COX-1 and COX-2 were determined.

Statistical methods. Data are presented as means ± the standard error of the mean (SEM) [standard error (SE)]. For statistical analysis of continuously measured parameters, mean values for each consecutive 15-min period of the experiment were used. The significance of changes over time was evaluated using a one-way analysis of variance (ANOVA) with repeated measures and Dunnett's *post hoc* test when a significant difference was detected by ANOVA. The significance of differences between the control and diclofenac results was evaluated using a two-way ANOVA with a Bonferroni *post hoc* test. Comparison of COX-1 and COX-2 protein was performed using an unpaired *t* test with Welch's correction. Graphpad Prism 3.0 (Graphpad Software Inc., San Diego, CA) was used for all statistical analyses.

RESULTS

Blood gases, pH, oxyhemoglobin saturation, glucose, and lactate values. Table 1 provides the results of fetal arterial blood gases, pH, and plasma glucose and lactate values. The similar fall in arterial Po_2 and oxygen content during the period of hypoxia indicates a comparable hypoxic stress in the two study groups. During hypoxia, arterial Po_2 decreased 23 to 12 Torr, Pco_2 decreased from 49 to 42 Torr (attributable to maternal hyperventilation), and a mild metabolic acidosis developed. Blood gas values returned to baseline levels during the hour following hypoxia.

Cortical Tissue Po_2. Cortical tissue Po_2 measurements are shown in Figure 1. Tissue Po_2 decreased markedly from baseline values to comparable levels in both groups, again indicating a comparable level of hypoxic stress. During hypoxia, cortical tissue Po_2 ranged from 2 to 3 Torr.

Cortical blood flow and vascular resistance. Figure 2 shows changes in cortical blood flow and cortical resistance to blood flow for the control and diclofenac experiments. In the

Table 1. *Arterial blood gases, oxygen content (O2 ct), pH, glucose (gluc), and lactate (lact) in response to drug infusion and hypoxia*

	Baseline	Infusion	Hypoxia	Recovery
Po_2 (Torr)				
Control	22 ± 1	23 ± 1	12 ± 1*	25 ± 1*
Diclofenac	23 ± 1	22 ± 1	12 ± 1*	24 ± 1
O_2 ct (mM)				
Control	7.1 ± 0.2	7.2 ± 0.2	3.2 ± 0.1*	7.0 ± 0.3
Diclofenac	7.1 ± 0.3	7.1 ± 0.2	3.3 ± 0.2*	6.7 ± 0.3
Pco_2 (Torr)				
Control	49 ± 1	49 ± 1	43 ± 1*	48 ± 2
Diclofenac	50 ± 1	50 ± 1	43 ± 1	51 ± 1
pH				
Control	7.34 ± 0.01	7.34 ± 0.01	7.35 ± 0.01	7.24 ± 0.02*
Diclofenac	7.34 ± 0.01	7.33 ± 0.01	7.34 ± 0.01	7.22 ± 0.0*
Gluc (mM)				
Control	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1*
Diclofenac	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1*	1.6 ± 0.1*
Lact (mM)	1.0 ± 0.1	1.0 ± 0.1	2.7 ± 0.2*	4.7 ± 0.4*
Control				
Diclofenac	1.0 ± 0.1	1.1 ± 0.1	3.0 ± 0.2*	5.4 ± 0.4*

All values expressed as mean ± SEM.
* Significant difference from baseline ($p > 0.01$).

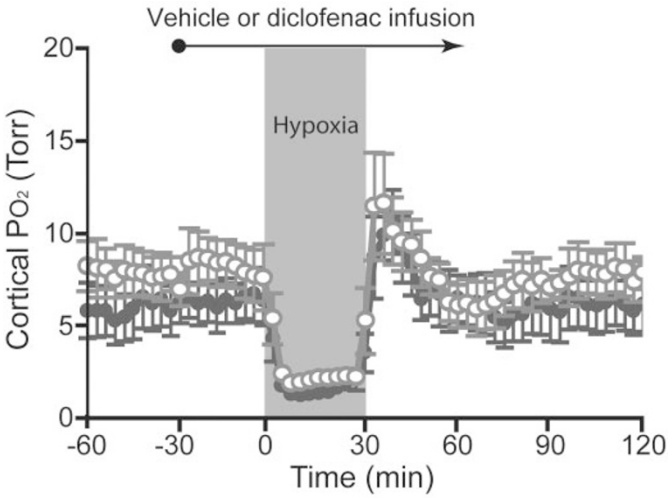

Figure 1. Cerebral cortical tissue Po_2 levels during baseline, hypoxia, and recovery periods in control ($n = 9$) and diclofenac ($n = 7$) experiments.

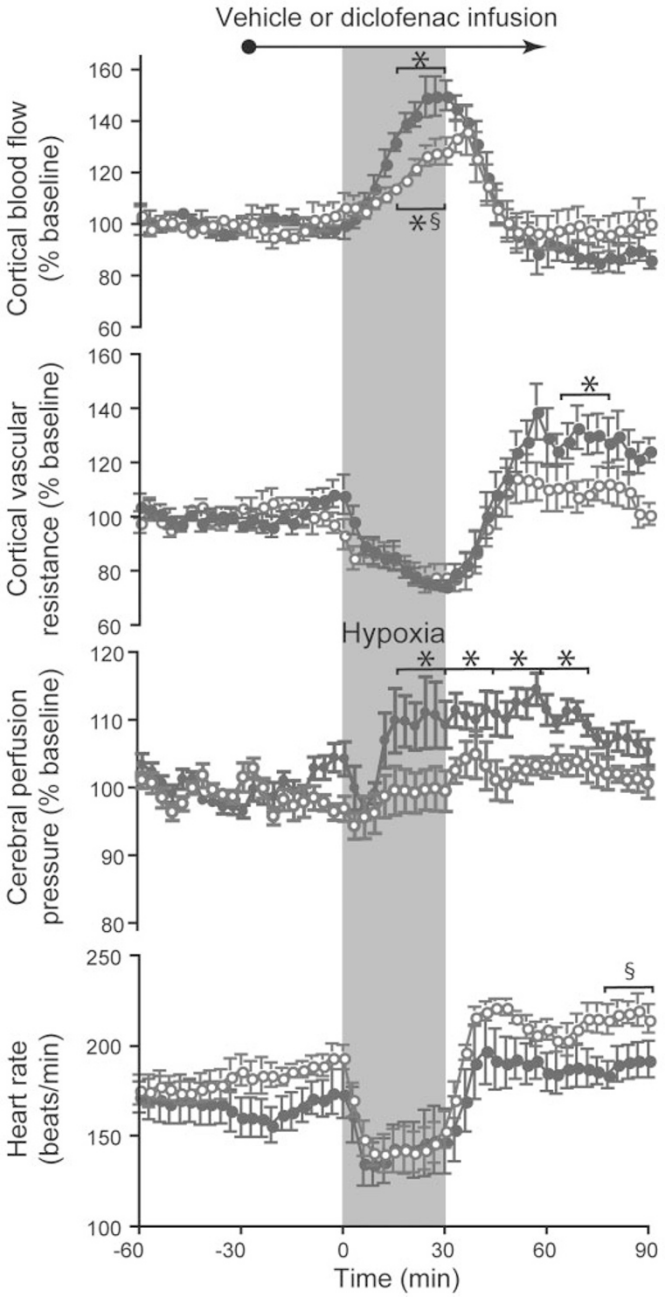

Figure 2. Changes in CBF, cerebral vascular resistance, mean arterial blood pressure, and heart rate during baseline, hypoxia, and recovery periods in control and diclofenac groups. Effect of hypoxia on CBF ± SEM measured by laser Doppler flowmetry and cortical vascular resistance ± SEM during the whole experiment in control ($n = 8$) and diclofenac ($n = 7$) groups. Effect of hypoxia on mean arterial blood pressure ± SEM and heart rate ± SEM during the whole experiment in control ($n = 9$) and diclofenac ($n = 8$) groups. §Significant difference between control and diclofenac ($p < 0.01$). *Significant difference from baseline period ($p < 0.01$). Two-way ANOVA revealed a significant difference between control and diclofenac experiments ($p < 0.01$), although posttest did not identify a significant difference between treatments for any specific 15-min segment of the experiment.

control experiments, cortical blood flow increased progressively during the first 20 min of hypoxia, reaching a peak of 148 ± 5% of baseline values at the end of the hypoxic period, a value significantly greater than baseline ($p < 0.01$). Flow returned to baseline values within 15 min after the end of hypoxia and remained stable during the remainder of the experiment. During hypoxia, resistance to flow decreased rapidly, reaching a nadir of 73 ± 2% of baseline during the final 10 min of hypoxia. After the termination of hypoxia, resistance gradually increased to a peak of 137 ± 10% of baseline, then gradually came back to baseline and remained stable during the recovery period.

When diclofenac was infused during the prehypoxic period, cortical blood flow did not change measurably. After induction of hypoxia, cortical blood flow increased significantly from baseline to reach a peak of 129 ± 5%. This increase was significantly less than that of the control group. CBF remained elevated during the first 5 min of recovery from hypoxia and then gradually returned to baseline values for the remainder of the experiment. There were no significant differences in cerebral vascular resistance to blood flow between the control and diclofenac groups at any point during the study.

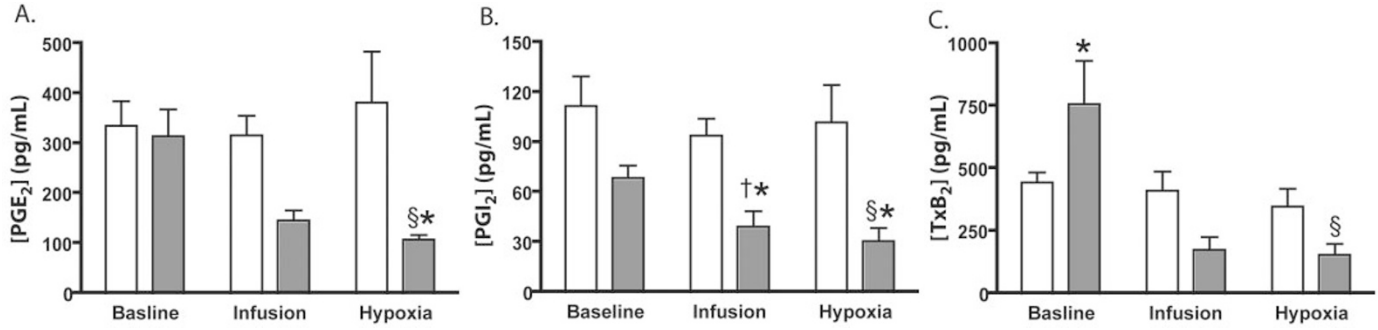

Figure 3. Arterial prostanoid concentrations during baseline, prehypoxia infusion (infusion), and hypoxia + infusion (hypoxia) periods in control (*open columns*) and diclofenac groups (*solid columns*). (*A*) Changes in arterial PGE_2 content ($n = 5$). (*B*) Changes in arterial 6-keto $F_1\alpha$ (PGI_2) content ($n = 5$). (*C*) Changes in arterial TxB_2 (TxA_2) content ($n = 4$). Treatment has a significant effect on all three metabolites ($p < 0.01$). *Post hoc* analyses are indicated by † (significant difference from controls) ($p < 0.05$), §significant difference from controls ($p < 0.01$), and *significant difference from baseline ($p < 0.01$).

Mean arterial blood pressure and heart rate. Figure 2 shows mean arterial blood pressure and heart rate for both experimental groups. In control experiments, mean arterial blood pressure increased significantly from baseline values during the hypoxic period and did not return to baseline until approximately 45 min after the end of the hypoxic insult. In contrast, there were no significant changes in mean arterial blood pressure during hypoxia following diclofenac infusion. Two-way ANOVA detected a significant difference between control and diclofenac blood pressures, although the posttest did not find significance for any 15-min segment of the experiment. Other than a significant increase in heart rate during the last 15 min of the diclofenac experiments compared with baseline values, there were no significant changes in the diclofenac results compared with baseline or controls.

Plasma PGE_2, PGI_2, and TxA_2 values. Plasma concentrations of PGE_2, PGI_2, and TxA_2 were measured to determine the effect of hypoxia on prostanoid synthesis and to establish the effectiveness of diclofenac blockade of prostanoid synthesis. The results are shown in Figure 3. In the control group, there were no significant changes in arterial PGE_2, PGI_2, or TxA_2 throughout the course of the experiment. During the prehypoxic period in diclofenac experiments, PGE_2, PGI_2, and TxA_2 all decreased, but the change only reached significance for PGI_2. By completion of the hypoxic insult, the arterial concentrations of all three analytes had decreased measurably in diclofenac experiments despite large interanimal variability in absolute concentrations.

COX-1 and COX-2 concentrations in fetal middle cerebral arteries. To establish the presence or absence of COX-1 and COX-2 enzymes in the cerebral vasculature, Western blot analyses were conducted on middle cerebral arteries of fetal sheep. The results are shown in Figure 4. COX-1 was detected at a concentration of 0.05 ± 0.004 $\mu g/\mu L$, whereas COX-2 was found at a concentration of 0.15 ± 0.029 $\mu g/\mu L$. It may be noted that each microliter of the COX sample represented an equivalent wet weight mass of artery tissue (60 μL buffer per milligram of wet weight).

DISCUSSION

This study has shown that blockade of prostanoid synthesis by diclofenac attenuates the normal increase in cortical blood flow that occurs in response to hypoxia in near term fetal sheep. This attenuation occurred despite no measurable effect of diclofenac on cortical resistance to blood flow. These results indicate that, although prostanoids may play a systemic role in changes in blood flow distribution during hypoxia, they do not appear to act locally on resistance vessels in the brain. This conclusion is in accord with that reached earlier using the pial window technique in newborn piglets (16).

The prostanoids are produced by cyclization and then oxygenation of arachidonic acid to PGH_2 (prostaglandin H_2) by the COX-1 and COX-2 enzymes. PGH_2 is subsequently converted to PGE_2, TxA_2, PGI_2, PGD_2 (prostaglandin D_2), or $PGF_{2\alpha}$ by one of five different enzymes. Diclofenac has been shown to inhibit COX activity by competing with arachidonic acid for the active site (8). Unlike many COX inhibitors that exhibit greater inhibition of either COX-1 or COX-2, diclofenac is a relatively equipotent COX-1 and COX-2 inhibitor (8,9). In addition, diclofenac has been reported to be effective in blockade of sheep COX enzymes and has fewer known nonspecific effects compared with many other COX inhibitors such as indomethacin (9).

Prostanoids are potent regulators of vascular tone, their effects being mediated by activation of G protein–coupled receptors on the plasma membrane (8,29). PGE_2, PGI_2, TxA_2, and $PGF_{2\alpha}$ are known to be vasoactive in the fetal brain (22). In the present study, arterial plasma concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) were measured as known potent vasodilators (15,30,31). TxB_2 (a stable

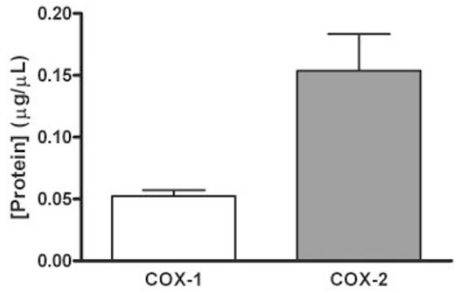

Figure 4. Middle cerebral artery concentrations of COX-1 and COX-2 as determined by Western blot analysis. Absolute COX-1 and COX-2 mass was determined based on a standard curve of mass *vs*. optical density for known amounts of human recombinant COX-1 and COX-2.

metabolite of TxA_2) was measured as a known potent vasoconstrictor (16,32,33). Although hypoxia may induce tissue-specific changes in prostanoid production, arterial plasma concentrations of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 were not affected by hypoxia in this study, a finding consistent with previous reports (15,23). The magnitude of reductions in plasma concentrations of PGE_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 following diclofenac infusion were also similar to those observed in previous reports in which COX inhibitors were systemically infused, most of which reported significant physiologic effects (22,34,35).

Nonsteroidal antiflammatory drugs such as diclofenac and indomethacin have been shown to selectively antagonize COX enzymes (9). In adults and newborns, indomethacin, for example, has either no effect (36) or an attenuation of increased CBF in response to hypoxia (37). In the present study, increases in CBF were significantly attenuated, with no significant difference in cerebral resistance to flow between diclofenac and control experiments. Because arterial blood pressure was significantly lower in the diclofenac group compared with controls, the data suggest that the attenuation of increased CBF by diclofenac was related to systemic effects rather than direct effects on the cerebral vasculature. Thus, diclofenac results suggest that prostanoids do not bring about significant changes in cerebrovascular tone during hypoxia, although the prostanoids may be important in mediating systemic responses to hypoxia, which in turn indirectly alter cortical blood flow in the fetus (1,7).

Although the results of the present studies indicate that prostanoids are not necessary for regulation of fetal cerebral vascular tone during hypoxia, Western blotting data indicate that both COX-1 and COX-2 are indeed present in the cerebral vasculature. The finding that COX-2 concentrations were appreciably higher than those of COX-1 may represent a significant difference between the fetus and adult, as Schonbeck *et al.* (38) have reported COX-1 but undetectable levels of COX-2 in normal adult human vascular smooth muscle, whereas Parfenova *et al.* (28) have measured constitutive expression of both COX-1 and COX-2 in the newborn. The potency of diclofenac against COX-1 is nearly equivalent to that of COX-2 in the sheep (9). It is worth noting that quantification of COX enzymes in these experiments was performed using sheep tissues but human recombinant protein standards. In addition, the quantification of COX protein concentrations does not provide an accurate indication of enzyme activity *in vivo*. Thus, although these findings indicate that COX enzymes are present in fetal cerebral vasculature, because of differences across species and age, the relative physiologic contributions of the two enzymes cannot be determined from the present experiments. There also remains the possibility that vascular tone did not change because of a balanced reduction of vasoconstrictor and vasodilator prostanoids by diclofenac administration.

In recent years our work has focused on the mechanisms regulating fetal CBF during hypoxia. We have established a predominant role for adenosine (2,6), a lesser role for nitric oxide (5), and now a limited and largely systemic role for the prostanoids. It is likely that these mediators and others such as carbon monoxide (39) interact and compensate for one another in a complex and redundant system to mediate responses to hypoxia. Indeed, there are many reports of a variety of mediators stimulating production or activity of one or more others (24,25).

In summary, administration of diclofenac does not affect cortical blood flow during a normoxic baseline period, but rather attenuates the normal increases in cortical blood flow that occur in response to hypoxic. The attenuation is not brought about locally, but rather by peripheral actions, perhaps principally by reductions in blood pressure, cardiac function and by inducing changes in other mediators.

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