Indomethacin attenuates the cerebral blood flow response to hypotension in late-gestation fetal sheep

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Tong, Haiyan, and Charles E. Wood. Indomethacin attenuates the cerebral blood flow response to hypotension in late-gestation fetal sheep. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1268–R1273, 1999.—Previous studies by us and others have demonstrated that PGE2 and thromboxane (Tx) B2 are produced in the fetal and neonatal brain during cerebral hypoperfusion. The present study was to test the hypotheses that indomethacin would alter the cerebral blood flow (CBF) response to reduced cerebral perfusion pressure in late-gestation fetal sheep by inhibiting the local prostanoid production. We studied eight chronically catheterized, sinoaortically denervated, 126- to 136-day gestation fetal sheep. The cyclooxygenase inhibitor indomethacin (0.2 mg/kg) or its vehicle phosphate buffer was injected intravenously 90 min before the start of a 10-min period of cerebral hypoperfusion produced by brachiocephalic artery occlusion (BCO). We found that BCO decreased fetal regional CBF (rCBF) by 65–79% in the phosphate buffer group and by 45–57% in the indomethacin-pretreated group. The decrease in fetal rCBF during BCO after indomethacin was 30–49% less than after phosphate buffer. Plasma PGE2 and TxB2 concentrations were significantly reduced by indomethacin treatment. BCO increased plasma ACTH and arginine vasopressin (AVP) concentrations; but these responses were not affected by indomethacin. These data suggested that endogenous prostanoid production is involved in the regulation of fetal CBF but, in the absence of intact baro-or chemoreflexes, not in the regulation of ACTH or AVP responses to BCO. We conclude that indomethacin has a beneficial effect on CBF during cerebral ischemia in late-gestation fetal sheep.

METHODS AND MATERIALS

Eight chronically catheterized fetal sheep between 126 and 136 days gestation were used in this study. The pregnant ewes were of mixed Western and Florida native breeds. These experiments were approved by the University of Florida Institutional Animal Care and Use Committee and were performed in accordance with the Guiding Principles for the Care and Use of Animals of the American Physiological Society.

Surgical preparation. Aseptic surgery was performed at least 5 days before the start of experiments in each animal. The fetuses were chronically instrumented with tibial artery and saphenous vein catheters and amniotic fluid catheters as previously described. Fetal hindlimbs were identified and delivered through a small hysterotomy incision near the tip of one uterine horn. We introduced polyvinyl chloride catheters into the tibial artery (0.050 in. ID, 0.090 in. OD) and saphenous vein (0.030 in. ID, 0.050 in. OD) bilaterally and advanced the tips to the abdominal aorta and inferior vena cava, respectively. After closing the skin incisions in the fetal hindlimb, amniotic fluid catheters were sutured to the fetal skin, and the hindlimb returned to the amniotic space. After placement of these catheters and closure of the hysterotomy,
the fetal head was located, the uterus was incised, and the head was delivered. After a single midline incision in the skin of the neck was made, lingual arteries were identified, ligated, and catheterized with polyvinyl chloride catheters (0.030 in. ID, 0.050 in. OD), with the catheter tips advanced retrograde to the lumen of the common carotid arteries. As previously described, this catheterization technique allows measurement of common carotid arterial pressure without interruption of carotid arterial blood flow (25). The carotid sinus denervation was performed using methods described by Wood (24). The carotid sinus nerves were identified bilaterally and cut. The walls of the common carotid arteries in this area, as well as the lingual arteries and common carotid arteries extending 0.5–1 cm rostral from the lingual-carotid arterial junction, were stripped of all visible nerve fibers. After performing these denervations in the fetal neck, the fetal skin was closed. An extravascular balloon occluder (In Vivo Metric, Healdsburg, CA) was placed around the brachiocephalic artery by means of a separate incision through the fourth intercostal space on the left side of chest. Then the head was returned to the amniotic cavity, and the uterus was closed. All catheters exited via a small incision in the flank of the ewes.

Ampicillin trihydrate (Polyflex; Aveco Co, Fort Dodge, IA; 500 mg) was administered to the fetus via the amniotic fluid and to the mother (500–750 mg) intramuscularly at the time of surgery and again each time the fetus was studied or the catheters were flushed. Ampicillin (500–750 mg) was administered to the mother intramuscularly twice daily for 5 days after the surgery. All catheters were flushed and reharpoineitized at least one every 3 days.

Experimental protocol. Sheep were transported to the procedure room from their pens within the Health Center Animal Resources Department at least 1 h before the start of each experiment. Each fetus was studied twice. Experiments consisted of a 90-min preocclusion control period (−90 to 0 min), a 10-min occlusion period (0–10 min) and a 10-min postocclusion recovery period (10–20 min). In one experiment on each fetus, the vehicle of indomethacin, 0.1 M phosphate buffer (PB) was injected intravenously, and in the other experiment 0.2 mg/kg indomethacin (an inhibitor of cyclooxygenase) was injected intravenously 90 min before the 10-min period of hypotension. PB and indomethacin were randomly administered to the fetal sheep. In each experiment, the extravascular occluder was inflated for 10 min to produce arterial hypotension. Fetal arterial blood samples were drawn from the umbilical arterial catheters (descending aorta) at 90 and 10 min before the start of the period of occlusion, at the end of the 10 min period of occlusion, and 20 min after the onset of occlusion. A 1-ml arterial blood sample was drawn before the experiment to measure blood gases. Blood samples (3 ml) were collected into chilled polystyrene tubes containing 150 µL of 0.5 M EDTA. Separate blood samples (1 ml) were collected into chilled polypropylene tubes containing 50 µL 0.5 M EDTA and 40 µg/ml indomethacin for measuring thromboxane (TX) B2 and PGE2. Tubes were kept on ice until the end of the experiment and then centrifuged for 20 min at 3,000 g at 4°C. Plasma was separated and stored in separate aliquots at −20°C.

Plasma ACTH, cortisol, and arginine vasopressin (AVP) concentrations were measured by specific radioimmunoassay. These assays have been described in detail elsewhere (19, 25). PGE2 and TXB2, a stable metabolite of TXA2, were measured with enzyme-linked immunosassay kits purchased from Cayman Chemical. Before assay, the prostanooids were extracted from acidified plasma with six volumes of ethyl acetate. The recovery with this protocol averages ~60%, and the extracted prostanooids dilute parallel to the standard curves.

Fetal arterial blood pressure and amniotic fluid pressure values were measured continuously during the 110-min experiments with a Grass recorder and Statham P23 1D pressure transducers. These hemodynamic variables were sampled, and analog-to-digital conversions were performed at 2-s intervals with an IBM PC computer. The data collection was achieved using ASYSTANT+ software (Asyst Technologies, Rochester, NY). All fetal intravascular pressure measurements were corrected by subtraction of amniotic fluid pressure.

Immediately before and 5 min after the onset of hypotension, colored microspheres (15 µm; Dye-Track; Triton Technologies, San Diego, CA) were injected through the venous catheter, and simultaneous reference blood samples were drawn from the lingual arterial catheters at the rate of 3 ml/min for 1 min, 30 s. Four colors of microspheres were used in each animal for measuring CBF. These techniques have been described in detail (4).

At the end of the second experiment, the pregnant ewes (and the fetus) were euthanized with an overdose of pentobarbital sodium. The fetal brains were dissected for microsphere extraction.

Estimation of CBF with colored microspheres. The fetal brains were dissected into cerebral cortex, brain stem, hippocampus, hypothalamus, cerebellum, and pituitary. The dissected tissues were dissolved in 4 ml of 4 M potassium hydroxide and 0.2% Tween 80 for 48 h and filtered through an 8-µm-thick polyester membrane filter. The membrane containing the microspheres was placed in a microcentrifuge tube to air dry overnight. Dimethylformamide (150 µl) was added to the tube to leach the color microspheres from the membrane. Then the tube was centrifuged, and 80 µl of the supernatant was pipetted to a cuvette. The absorbance spectra was quantified with use of a spectrophotometer and the appropriate software for least-squares solution of absorbance of each dye. The reference blood samples were processed similarly, except that 16 M potassium hydroxide was used.

CBF was calculated by the following equation

\[ CBF = \left( \frac{\text{tissue ABS}}{\text{reference ABS}} \right) \times \text{reference flow rate} \]

where ABS is absorbance.

Statistical analyses. Changes in mean arterial blood pressure over time were analyzed by t-test. Changes in the values of fetal hormonal and prostanoid variables over time and between groups were analyzed with two-way ANOVA. A multiple comparison of mean values was performed with Duncan's test. The hormonal data were not distributed normally. All ANOVAs performed on hormonal data were calculated after logarithmic transformation to correct heteroscedasticity of the data. Fetal CBF was analyzed with three-way ANOVA (23) followed by Duncan's test. A significance level of \( P < 0.05 \) was used to reject the null hypothesis in all tests. Analyses were performed using SigmaStat software (Jandel Scientific, San Rafael, CA).

RESULTS

Blood gases and pressure. The blood gases measured before PB and indomethacin injections indicated that all fetuses were healthy during the experiments (Table 1). Injection of indomethacin did not alter fetal blood gases. Fetal mean arterial blood pressure before, during, and after brachiocephalic occlusion are presented in Fig. 1. Lingual arterial blood pressure decreased significantly \((P < 0.001)\) during the brachiocephalic...
occlusion in both PB- and indomethacin-treated groups. In the PB group, mean lingual arterial blood pressure decreased from 41.9 ± 3.1 to 16.5 ± 3.3 mmHg. In the indomethacin group, it decreased from 41.6 ± 2.5 to 14.6 ± 3.0 mmHg. Blood pressure returned to baseline level after the occluder was released. Femoral arterial blood pressure increased significantly (\(P < 0.05\)) during the brachiocephalic occlusion in PB-treated group, from 42.9 ± 3.7 to 64.9 ± 7.0 mmHg. In the indomethacin-treated group, mean femoral arterial blood pressure increased during occlusion to 51.1 ± 4.2 mmHg. This was higher (\(P < 0.05\)) than that after occlusion (39.3 ± 1.7 mmHg).

Plasma prostanoid concentrations. The mean values of fetal plasma PGE\(_2\) and TxB\(_2\) concentrations are summarized in Table 2. There was no difference in the initial values of prostanoid concentrations in the two groups before PB and indomethacin injections. After indomethacin injection, plasma PGE\(_2\) and TxB\(_2\) concentrations decreased significantly (\(P < 0.05\)), indicating that indomethacin effectively inhibited cyclooxygenase activity. Compared with the preclosure value, hypotension increased plasma PGE\(_2\) and TxB\(_2\) concentrations in the PB-treated group, but the apparent changes were not statistically significant.

Regional CBF. The mean values of fetal regional CBF (rCBF) are reported in Table 3. In the PB-treated group, brachiocephalic occlusion significantly (\(P < 0.05\)) decreased rCBF in cerebral cortex, brain stem, hippocampus, hypothalamus, and cerebellum. Indomethacin pretreatment (\(n = 7\)) slightly decreased but did not significantly change basal rCBF. After indomethacin treatment, hypotension decreased rCBF in all of the regions studied, especially in cerebral cortex, brain stem, and cerebellum (\(P < 0.05\)). Hypotension produced somewhat smaller changes in rCBF after indomethacin treatment. Thus the induced changes in rCBF in cerebral cortex, brain stem, hippocampus, hypothalamus, and cerebellum were 49%, 30%, 36%, 18%, and 42% less than after PB treatment. Three-way ANOVA was performed revealed that there was a significant interaction between drug (PB or indomethacin) and blood pressure (±occlusion) treatments (\(F = 5.224, P < 0.05\)).

Plasma hormone concentration. Plasma ACTH concentration was increased significantly from 73.8 ± 14.2 to 1,811.3 ± 1,009.3 pg/ml in the fetuses treated with PB and from 200.4 ± 51.9 to 1,489.6 ± 482.0 pg/ml in those treated with indomethacin by the brachiocephalic occlusion. Plasma AVP concentration was increased significantly (\(P < 0.05\)).

| Table 1. Arterial blood gases before PB and indomethacin injections |
|-----------------------------|-----------------------------|
|                            | PB             | Indomethacin |
| pH                         | 7.35 ± 0.01    | 7.35 ± 0.01  |
| PaO\(_2\), mmHg            | 19.28 ± 0.91   | 20.42 ± 0.68 |
| PaCO\(_2\), mmHg           | 51.88 ± 2.43   | 49.53 ± 1.72 |

Values are means ± SE. PB, phosphate buffer.

<table>
<thead>
<tr>
<th>Table 2. Fetal plasma prostanoid concentrations</th>
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<tbody>
<tr>
<td>Time, min</td>
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<tr>
<td>-----------</td>
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<tr>
<td>PGE(_2), pg/ml</td>
</tr>
<tr>
<td>-90</td>
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<tr>
<td>-10</td>
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<tr>
<td>10</td>
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<tr>
<td>20</td>
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<tr>
<td>TxB(_2), pg/ml</td>
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<tr>
<td>-90</td>
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<td>-10</td>
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<td>10</td>
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<td>20</td>
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</tbody>
</table>

Values are means ± SE. Time is relative to start of hypotension (t = 0 min). *P < 0.01, indicating difference due to time difference within indomethacin group (Duncan's test). **P < 0.05, indicating difference between PB and indomethacin group (Duncan's test).
Table 3. Fetal cerebral blood flow after PB and indomethacin treatments

<table>
<thead>
<tr>
<th></th>
<th>PB</th>
<th>Indomethacin</th>
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<tbody>
<tr>
<td></td>
<td>Normotension (n=5)</td>
<td>Hypotension (n=6)</td>
</tr>
<tr>
<td>Cortex</td>
<td>1.42±0.22</td>
<td>0.30±0.09*</td>
</tr>
<tr>
<td>Brain stem</td>
<td>2.43±0.39</td>
<td>0.86±0.25*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.37±0.18</td>
<td>0.49±0.17*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>2.20±0.33</td>
<td>1.05±0.40*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.85±0.27</td>
<td>0.41±0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml·g⁻¹·min⁻¹. *P < 0.05, indicating difference between normotension and hypotension in PB-treated group. †P < 0.05, indicating difference between normotension and hypotension in indomethacin group. All multiple-range test procedures were done with Duncan’s test.

Did find that the reduction in rCBF during the period of hypotension was ~30% less in indomethacin-treated fetuses compared with PB-treated fetuses. We believe that this difference was secondary to the inhibition of synthesis of constrictor prostanoids by indomethacin. Furthermore, we believe that the inhibition of prostanoids involved in the control of CBF is likely to be within the cerebral vasculature itself.

We designed our study to avoid the initial transient constriction produced by indomethacin injection. At the time of study, CBF was not altered in the normotensive condition. In one sense, our results contrast with those

DISCUSSION

The present study tested the effect of a therapeutic dose of indomethacin (0.2 mg/kg) on the CBF response during cerebral ischemia. It has been observed that prostanoids are important in modulating perinatal cerebral hemodynamics in human beings and in other species (11). Our previous study demonstrated that TxB₂ secretions increased significantly in sagittal sinus blood during cerebral hypoperfusion, and PGE₂ concentrations increased significantly in fetal brain stem and hypothalamus interstitial fluids. We tested the hypothesis that indomethacin may compromise fetal CBF by inhibiting the production of these or other prostanoids in the brain during cerebral ischemia.

Papile and co-workers (17) demonstrated CBF autoregulation in the preterm fetal lamb and reported that the range of autoregulation was narrowed in the preterm lamb. They speculated that autoregulatory mechanisms in the fetal brain under normal physiological conditions in utero may not protect against acute fluctuations in blood pressure, particularly hypotension (17). In the present study, brachiocephalic occlusion decreased carotid arterial blood pressure to the same degree in both PB- and indomethacin-treated fetuses. Although the induced decrease in arterial blood pressure was similar in both groups, the induced changes in rCBF were not. We cannot conclude that indomethacin shifted the autoregulation curve toward higher pressures, because we did not generate an autoregulation curve in the present study. However, we
of Leffler et al. (12), who found that indomethacin decreased CBF in newborn piglets by inhibiting dilator prostaglandins. We acknowledge that our design ignores this transient phase of the response. However, indomethacin can also produce transient effects that are not directly related to prostagland production (3, 9). Because the initial response to indomethacin likely includes both prostaglandin-dependent and -independent changes in CBF, we waited 90 min after injection of the indomethacin to allow establishment of a new steady state. Although we acknowledge that, after 90 min, other compensatory responses might be involved, we argue that prostagland synthesis within the vasculature is still reduced, a conclusion consistent with the reduction in circulating prostagland concentrations.

In a separate study, we found that brachiocephalic occlusion in carotid sinus-denervated fetuses caused the release of TxB2 into the sagittal sinus blood (21). The appearance of TxB2, the stable metabolite of TxA2, in sagittal sinus blood suggests that during hypoperfusion the concentration of TxA2 in the cerebral microvasculature is high. The source of the TxA2 is unknown but could be platelets or vascular endothelium. Thromboxane synthase, the enzyme that converts PGH2 to TxA2, is found in both sites in addition to neurons within the brain. Although it is theoretically possible that the TxB2 in the vasculature derives from neurons, we argue that in this case it is more likely to derive from a source on the blood side of the blood-brain barrier. We base this argument on our own previous observation that cerebral hypoperfusion decreases the concentration of TxB2 in the extracellular fluid in hypothalamus and brain stem, whereas it massively increases the concentration of TxB2 in sagittal sinus blood (21). Our results are consistent with a regulatory role of TxA2 (or another constrictor prostanoid) within the cerebral vasculature and agree with those of Chemtob et al. (5), who demonstrated in newborn piglets that inhibition of TxB2 synthesis was associated with an extension of the lower blood pressure limit of CBF autoregulation and an attenuation in the decrease in CBF below this blood pressure. Together, the results are consistent with the notion that TxA2 production within the cerebral microvasculature might be protective because it delays reperfusion and therefore limits oxidative stress.

In this experiment design, all fetuses were carotid sinus-denervated to eliminate complicating variables introduced by the presence of carotid arterial baroreceptors and chemoreceptors. The fetal rCBF was comparable to that reported by other investigators in control animals. Itskovitz and Rudolph (8) demonstrated that sinoaortic denervation of the preterm fetal lamb does not alter resting heart rate or blood pressure and had no effect on CBF. This is consistent with the conclusion that the autonomic nervous system does not appear to have a major role in the control of cerebral circulation (22). Nevertheless, we cannot rule out the possibility that a portion of the change in CBF flow might have derived from residual reflex responses in these fetuses.

The hormonal responses to cerebral hypoperfusion of this study were consistent with our previous experiments. In other experiments, we found that indomethacin reduced the magnitude of the ACTH and AVP responses to hypotension in late-gestation fetal sheep (20). Interestingly, we found that the effect of indomethacin on the ACTH and AVP responsiveness to hypotension was dependent on the presence of intact baroreceptor and chemoreceptor afferent fibers. In the present experiments, in which fetuses were subjected to carotid sinus denervation and indomethacin treatment, the endocrine results were much the same. We are assuming that indomethacin reduces prostaglandin biosynthesis on both sides of the blood-brain barrier, although this assumption is somewhat controversial. Indomethacin is highly lipid soluble (it is also highly protein bound in plasma). Indomethacin crosses the intact meninges by simple diffusion (2); it can be found in the brain within 30 min of systemic administration, and the concentrations of indomethacin in cerebrospinal fluid are similar to the unbound concentrations in plasma. Collectively, these studies suggest an effect of brain prostaglandins as modulators of endocrine responses to hypotension that requires intact afferent fibers from arterial receptors.

In conclusion, this study demonstrated that indomethacin had a beneficial effect on fetal rCBF in response to cerebral ischemia in late-gestation fetal sheep. We therefore speculate that, during cerebral hypoperfusion, endogenous production of vasoconstrictor prostanoids limits reperfusion.

Perspectives

Prostanoids have many actions, especially within the brain. A great deal of interest has been focused on the vasoregulatory actions of prostanoids in various vascular beds, and a great deal of important information has resulted from studies of prostanoid control of CBF. Our interest is in the involvement of prostanoids in the reflex control mechanisms within the brain as well as in the direct control of CBF. The present study does not address the generation of prostanoids within the brain, although our results in previous studies have demonstrated that hypotension stimulates prostanoid generation on both sides of the blood-brain barrier. Our data are consistent with the view that the generation of prostanoids within the vasculature directly influences vascular tone and therefore blood flow, and that local generation of prostanoids within neurons in cardiovascular and endocrine-controlling brain regions (e.g., paraventricular nucleus, nucleus of the solitary tract, and ventrolateral medulla) alters the activity of reflex pathways.

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