Endogenous prostanoids modulate the ACTH and AVP responses to hypotension in late-gestation fetal sheep

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Tong, Haiyan, Farahaba Lakhdir, and Charles E. Wood. Endogenous prostanoids modulate the ACTH and AVP responses to hypotension in late-gestation fetal sheep. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R735–R741, 1998.—We have previously reported that prostaglandin E2 and thromboxane A2 stimulate endocrine and cardiovascular responses similar to the responses to arterial hypotension. The present experiments were designed to test the hypothesis that prostanoids are involved in the generation of responses to hypotension induced by vena cava occlusion. Fetal sheep were either intact or subjected to a prior carotid sinus denervation and bilateral vagosympathetic nerve section. Indomethacin or vehicle was injected intravenously 90 min before the start of arterial hypotension. In intact fetuses treated with phosphate buffer, ACTH increased significantly from 83 ± 39 to 3,611 ± 774 pg/ml, arginine vasopressin (AVP) increased from 3.9 ± 0.5 to 1,079 ± 549 pg/ml, and cortisol increased from 4.7 ± 0.8 to 9.5 ± 1.7 ng/ml. Indomethacin treatment significantly reduced the magnitudes of the hormonal responses. Baroreceptor and chemoreceptor denervation attenuated the ACTH and AVP responses, but these responses were not further inhibited by indomethacin. We conclude that endogenous prostanoids partially mediate the reflex hormonal and hemodynamic responses to arterial hypotension in late-gestation fetal sheep.

adrenocorticotropic hormone; arginine vasopressin; cortisol; blood pressure; indomethacin

Prostanoids, including thromboxane A2 (TxA2), prostaglandin (PG) E2, and PGI2, are well known as locally generated mediators of vascular tone within the cerebral vasculature (9, 17, 24). We have hypothesized that, in addition to their role in the local control of cardiovascular function, prostanoids partially mediate hormonal and cardiovascular reflex responses to arterial hypotension in late-gestation fetal sheep. This hypothesis is based on the following observations: 1) arterial baroreceptors and chemoreceptors partially, but not completely, mediate the reflex ACTH and arginine vasopres-

in ACTH and arginine vasopressin (AVP) responses to hypotension (28); 2) PGE2 and TxA2, when infused into the blood perfusing the brain, stimulate endocrine and cardiovascular responses reminiscent of reflex responses to hypotension (15, 16); and 3) hypotension stimulates the local synthesis and release of PGE2 and TxA2 in the cerebral vasculature of the newborn piglet and therefore might have a similar effect in the late-gestation fetal sheep (10).

We designed the present study to test the hypothesis that moderate hypotension, reduction of fetal arterial blood pressure ~50% below normally controlled levels, stimulates endocrine responses that are mediated in part by endogenously generated prostanoids and in part by chemoreceptors and baroreceptors in the carotid sinus region and in areas innervated by afferent fibers in the vagosympathetic trunks. Specifically, this study was designed to identify effects of denervation alone and the effects of prostaglandin synthesis blockade alone and to identify any interactions between these two systems.

MATERIALS AND METHODS

Fifteen chronically catheterized fetal sheep between 124 and 136 days gestation were used in this study. The pregnant ewes were of mixed Western 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 in 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. Nine fetal sheep were subjected to catheterization only. Six fetal sheep were subjected to catheterization plus denervation of arterial baroreceptors and chemoreceptors. Fetal hindlimbs were identified and delivered through a small hysterotomy incision near the tip of one uterine horn. As previously described, we introduced polyvinyl chloride catheters into the tibial artery (0.050 in. ID, 0.090 in. OD) and saphenous vein (0.090 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, we sutured amniotic fluid catheters to the fetal skin and returned the hindlimb to the amniotic space. After placement of these vasculoc and amniotic fluid 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 retrogradely 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 (3, 32). In fetuses subjected to baro- and chemodenervation, the common carotid arteries and cervical vagosympathetic trunks were carefully exposed on both sides. The vagosympathetic trunks were cut bilaterally. 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 artery junction, were stripped of all visible nerve fibers. The carotid sinus denervation procedure used in this investigation was shown in a previous study to block the changes in fetal heart rate during hypoxia (33). Standard tests of the completeness of denervation were not possible in the present experiments because the severing of cervical vagosympathetic trunks interrupts the efferent limb of the reflex decrease in heart rate that would be observed after phenylephrine injection (28, 35). After these denervations were performed in the fetal neck, the fetal skin
was closed, the head was returned to the amniotic cavity, and the uterus was closed.

In some fetuses, we introduced a Swan-Ganz catheter (size 5-F) into the saphenous vein of one hindlimb and advanced the tip to the supradiaphragmatic inferior vena cava. In other fetuses, we placed an extravascular balloon occluder around the supradiaphragmatic inferior vena cava using a separate incision through the fourth intercostal space. Extravascular balloon occluders were either purchased (In Vivo Metric, Healdsburg, CA) or fabricated in the laboratory. Occluders fabricated in the laboratory were made from Penrose tubing (0.5 cm diameter), looped around the vena cava (between the diaphragm and right atrium), and connected to polyvinyl chloride tubing. Results from fetuses with intravascular versus extravascular balloon occluders were not distinguishable and therefore were combined. All catheters were exited via a small incision in the flank of the ewes.

Ampicillin trihydrate (500 mg Polylex; Aveco, Fort Dodge, IA) 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 two times daily for 5 days after surgery. All catheters were flushed and reheparinized at least one time 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 two times. Experiments consisted of a 90-min preocclusion control period (−90 to 0 min; Fig. 1), a 10-min occlusion period (0 to 10 min), and a 10-min postocclusion recovery period (10 to 20 min). In one experiment on each fetus, the vehicle for indomethacin (0.1 M phosphate buffer) 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. In each experiment, the intravascular or extravascular occluder was inflated for 10 min to produce arterial hypotension. Fetal arterial blood samples were drawn from tibial artery 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 start of occlusion. 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 of 0.5 M EDTA and 40 µg/ml indomethacin for measuring TxB2, 6-keto-PGF1, 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 AVP concentrations were measured by specific radioimmunoassay. ACTH was measured using 125I-labeled ACTH and rabbit anti-ACTH antiserum produced in this laboratory. Before assay, ACTH was extracted from plasma with powdered glass (Corning, Corning, NY). Cortisol was measured using [3H]cortisol purchased from Amersham (Arlington Heights, IL) and rabbit anticortisol antiserum produced in Dr. Wood’s laboratory. Before assay, cortisol was extracted from plasma using 20 volumes of ethanol. AVP was measured using anti-AVP antiserum also produced in Dr. Wood’s laboratory and 125I-labeled AVP purchased from Amersham. Before assay, AVP was extracted from plasma on bentonite (Sigma, St. Louis, MO). The ACTH, cortisol, and AVP assays have been completely described elsewhere (25, 32). PGE2, thromboxane B2 (TxB2), a stable metabolite of TxA2, and 6-keto-prostaglandin F1α (6-keto-PGF1α, a stable metabolite of PGF1) were measured using enzyme-linked immunoassay kits purchased from Cayman Chemical (Ann Arbor, MI). ACTH, cortisol, and AVP were extracted from acidified plasma with six volumes of ethyl acetate. The recovery with the use of this protocol averages ~60%, and the extracted prostanoids diluted parallel to the standard curves.

Fetal arterial blood pressure and amniotic fluid pressure were measured continuously during the 110-min experiments using a Grass recorder and Statham P23 ID pressure transducers. Not all hemodynamic variables were successfully measured in all experiments. These hemodynamic values were recorded, and analog-to-digital conversions were performed at 2-s intervals using an IBM PC computer. The data collection was achieved using Assistant+ software (Asyst Technologies, Rochester, NY). All fetal intravascular pressures were corrected by subtraction of amniotic fluid pressure.

Statistical analyses. Changes in the values of fetal hormonal and prostanoid variables over time and between groups were analyzed using two-way ANOVA corrected for unequal cell size and for repeated measures in one dimension, time (27). Multiple comparison of mean values was performed using the Student-Newman-Keuls test. The hormonal data were not distributed normally. All ANOVAs performed on hormonal data were calculated after logarithmic transformation to correct for heteroscedasticity of the data. 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

Prostanoids. The mean values of fetal PGE2, TxB2, and 6-keto-PGF1α concentrations are reported in Table 1. In intact fetuses, hypotension significantly increased plasma concentrations of PGE2 (compared with preocclusion value) but did not significantly change plasma concentrations of the other measured prostanoids. Indomethacin significantly decreased the plasma concentrations of PGE2, 6-keto-PGF1α, and TxB2 (Table 1).

Plasma concentrations of the prostanoids were reduced by denervation of arterial baroreceptors and chemoreceptors. In particular, plasma TxB2 concentrations were significantly lower before and during hypotension in denervated fetuses treated with phosphate buffer. Plasma PGF1α concentrations during hypotension were lower in (phosphate buffer treated) denerv-
Concentrations in the denervated fetuses. Indomethacin did not produce further decreases in plasma prostanoid concentrations in the denervated fetuses than in intact fetuses. Indomethacin did not produce further decreases in plasma prostanoid concentrations in the denervated fetuses.

Cardiovascular variables. In the intact group (n = 9), vena cava occlusion significantly decreased mean arterial blood pressure from 42.9 ± 2.2 to 24.8 ± 3.7 mmHg (mean ± SE) in the fetuses treated with phosphate buffer; mean arterial blood pressure decreased from 46.5 ± 1.8 to 26.0 ± 3.1 mmHg in fetuses treated with indomethacin (Fig. 2). The effect of the vena cava occlusion on blood pressure was not significantly different in intact versus denervated groups. The values of fetal blood gases and pH are reported in Table 2. There are no statistical differences between the intact and denervated groups and phosphate buffer and indomethacin treatment groups.

Endocrine variables. The initial values of ACTH, AVP, and cortisol (before the injection of phosphate buffer or indomethacin) were not significantly different between the intact and denervated groups. Compared with phosphate buffer-treated fetuses in the intact group, indomethacin treatment significantly attenuated the fetal plasma ACTH and AVP reflex responses to vena cava occlusion (Fig. 3). Vena cava occlusion increased fetal plasma ACTH concentration significantly from 83.2 ± 38.8 to 3,611.2 ± 774.2 pg/ml, increased AVP from 3.9 ± 0.5 to 1,079.3 ± 549.4 pg/ml, and increased cortisol from 4.7 ± 0.8 to 9.5 ± 1.7 ng/ml in fetuses treated with phosphate buffer in the intact group; in the indomethacin treatment fetuses, ACTH increased significantly from 156.0 ± 63.0 to 1,921.5 ± 673.8 pg/ml, AVP increased significantly from 5.2 ± 0.7 to 225.7 ± 181.1 pg/ml, and cortisol increased from 8.1 ± 1.8 to 9.9 ± 2.6 ng/ml. After conversion of data to common logarithms, analysis by ANOVA indicated that indomethacin attenuated the ACTH, AVP, and cortisol responses to the vena cava occlusion (significant interaction of time × group in 2-way ANOVA corrected for unequal cell size and for repeated measures in 1 dimension, time).

The ACTH, AVP, and cortisol responses to the vena cava occlusion were also significantly attenuated in the phosphate buffer-treated denervated fetuses compared with phosphate buffer-treated intact fetuses (significant main effect of group with significant interaction of time × group in 2-way ANOVA). Vena cava occlusion increased fetal plasma ACTH concentration from 201 ± 31.7 pg/ml in intact fetuses to 1,673.8 ± 181.1 pg/ml in indomethacin-treated denervated fetuses.

### Table 1. Plasma concentrations of prostanoids

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Intact</th>
<th>Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0</td>
<td>PB</td>
<td>Indo</td>
</tr>
<tr>
<td>0-10</td>
<td>PGE2</td>
<td>105.3 ± 29.7</td>
</tr>
<tr>
<td>10-20</td>
<td>TxB2</td>
<td>116.8 ± 31.7</td>
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<tr>
<td></td>
<td>PGF1α</td>
<td>151.1 ± 68.4</td>
</tr>
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</table>

*Values are means ± SE in pg/ml. Responses are grouped by time of vena cava occlusion and intact or denervated and indomethacin (Indo) or phosphate buffer (PB) treatments. Periods of occlusion were from 0 to 10 min. PGE2, prostaglandin E2; TxB2, thromboxane B2. *Statistically significant difference between Indo and PB treatment groups at P < 0.05 (Student-Newman-Keuls test); †statistically significant difference between intact and denervated groups; ‡statistically significant difference when compared within group to value at −10 min.

### Table 2. Fetal blood gases and pH before Indo or PB injections

<table>
<thead>
<tr>
<th>Gas</th>
<th>Intact</th>
<th>Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PB</td>
<td>Indo</td>
</tr>
<tr>
<td>PAO2, mmHg</td>
<td>19.70 ± 1.18</td>
<td>18.16 ± 1.18</td>
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<tr>
<td>PACO2, mmHg</td>
<td>51.51 ± 1.81</td>
<td>45.71 ± 1.94</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.01</td>
<td>7.36 ± 0.01</td>
</tr>
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</table>

*Values are means ± SE. PAO2 and PACO2, partial pressure of O2 and CO2, respectively, in arterial blood.
77 to 456 ± 135 pg/ml and AVP from 4.1 ± 0.5 to 15.8 ± 5.6 pg/ml in the phosphate buffer-treated denervated fetuses. In contrast to its effect in intact fetuses, indomethacin had no further effect on the ACTH, AVP, or cortisol responses to vena cava occlusion in the denervated fetuses (no significant main effect of group and time × group interaction). After indomethacin treatment, vena cava occlusion increased plasma ACTH concentration from 282 ± 66 to 1,246 ± 560 pg/ml and increased AVP from 8.3 ± 2.8 to 16.9 ± 5.7 pg/ml in the denervated fetuses.

These results therefore support our earlier conclusions that sinoaortic afferent fibers partially mediate these responses to hypotension. These data extend our earlier observations by demonstrating that the baro- and/or chemoreflex control of ACTH and AVP secretion is partially dependent on the endogenous production of prostanoids. There are several important implications of these results that we will address individually.

ACTH and AVP responses to hypotension in sheep fetus are not completely mediated by carotid sinus and vagal afferent fibers. In previous studies, we demonstrated that carotid sinus denervation attenuated the ACTH and AVP responses to systemic hypotension (produced by vena cava obstruction) or to carotid arterial hypotension (produced by bilateral carotid occlusion). Sinoaortic denervation impairs the ability of the fetus to maintain arterial blood pressure during progressive hemorrhage (12). On the other hand, bilateral cervical vagosympathetic nerve section has no apparent effect on the reflex hormonal responses to hemorrhage or on the ability of the fetus to maintain...
blood pressure when blood volume is reduced (31). We therefore strongly suspected that the carotid sinus afferent fibers were more important for reflex hormonal and cardiovascular responsiveness than afferent fibers in the vagosympathetic trunks carrying afferent information from cardiopulmonary receptors. It was possible, although perhaps not likely, that the ACTH and AVP responses to hypotension would be completely eliminated by total (carotid sinus and vagosympathetic nerve section) denervation. The present results prove that the ACTH and AVP responses to hypotension are not completely mediated by neural afferent fibers from these regions.

In the present study, we measured arterial blood gases only at the beginning of each experiment. In a previous study, we found that vena cava obstruction produced no statistically significant changes in the partial pressure of O2 and CO2 in fetal arterial blood (PaO2 and PaCO2, respectively) but did produce moderate decreases in pH (−0.04 pH units) (34). In another study, we found that such small changes in pH on their own were not sufficient to stimulate either ACTH or vasopressin secretion (30). Finally, we found that sinoaortic denervation did not alter this pattern of decreasing pH and unchanging PaO2 or PaCO2 (28). For these reasons, we do not think that the responses that we observed during vena cava obstruction were caused by changes in arterial blood gases or that baro- and chemodenervation altered arterial blood gases during this manipulation. Increases in plasma hormone concentrations can also be caused by hypotension-induced decreases in hormone clearance from plasma. Although this can be a complicating factor, even dramatic changes in clearance cannot account for the large changes in plasma concentrations we measured.

ACTH and AVP responses to hypotension are mediated, in part, by endogenous prostanoids. An important result of the present study is the demonstration that the ACTH and AVP responses to hypotension are partially inhibited by indomethacin in intact fetuses. We and others have previously demonstrated that exogenous PGE2 and TxA2 mimetic (U-46619) stimulate ACTH secretion (5, 6, 14, 15, 32). It is also well known that in newborn piglets the endogenous production of PGE2, PGI2, and TxA2 is stimulated during induced reductions in cerebral blood flow (10). These prostanoid responses are thought to be important for the autoregulation of blood flow when perfusion pressure is changed (11). Although the prostanoid generation and release into venous blood is often thought of as originating in the vasculature, it is known that the neurons contain the prostanandin G/H synthase (1) and thromboxane synthase (19). We designed the present experiments to test the hypothesis that endogenous production of prostanoids, whether in vasculature or in neurons, might partially mediate the reflex hormonal responses to hypotension. The results indicate that this hypothesis was correct.

The results reveal an interaction between the afferent fibers and the endogenous prostanoids. Indomethacin had a statistically significant effect only in the intact fetuses. The ACTH and AVP responses were attenuated by the denervation procedure but were not further attenuated by indomethacin. This suggests that the action of the prostanoids must be on the afferent pathways or on a central element that is activated by the afferent pathways. In other words, this selective action appears to rule out an effect solely on the anterior or posterior pituitary or on the nerve terminals of the median eminence, because an action on the so-called “final common pathway” would attenuate the response in both groups. The results therefore demonstrate that indomethacin is not simply a “nonspecific” inhibitor of ACTH and AVP secretion.

We and others have found that exogenous prostanoids may stimulate hormonal and hemodynamic responses by affecting the neuronal processing within the central nervous system. These studies have focused mainly on PGE2 and TxA2. Infusions of PGE2 into the carotid arteries of conscious adult sheep increase heart rate and blood pressure (2–4) and ACTH secretion (14). This effect is not attenuated by carotid sinus baro- or chemodenervation (16) and therefore is likely to be a direct effect on the brain. Infusions of PGE2 also stimulate increases in fetal blood pressure and heart rate (15, 16).

Intracerebroventricular (6) or intravenous (38) infusion of PGE2 can stimulate ACTH and cortisol secretion in fetal sheep, and treatment with indomethacin decreases ACTH release (26). In addition to its effect on the brain, PGE2 has a direct effect on the fetal sheep pituitary gland by enhancing AVP-stimulated, but not CRH-stimulated, ACTH secretion from dispersed fetal anterior pituitary cells in culture (5). TxA2 stimulates increases in arterial blood pressure and heart rate and the rate of ACTH secretion in both adult and fetal sheep (13, 32).

The influence of prostanoids on AVP secretion has been investigated in adult dogs and rats and in newborn piglets. Intraventricular administration of PGE2 (18, 36) or PGD2 (7) stimulates AVP secretion. Indeed, the stimulation of AVP secretion in response to intracerebroventricular injections of ANG II or acetylcholine is attenuated by indomethacin (20, 21), suggesting that central pathways affecting AVP secretion by hypothalamic magnocellular neurons are dependent on prostanoid generation. AVP responses to hypertonic saline are significantly inhibited by prior administration of medofenamate, indomethacin, or of flunixin meglumin (7, 18, 23). The effect of endogenous prostanoids on the AVP responses to hemorrhage were somewhat more variable. Medofenamate, but not indomethacin, attenuated the AVP response to hemorrhage in pentobarbital sodium-anesthetized dogs (7). Indomethacin augmented the AVP response to hemorrhage in morphine-sedated and urethan-chloralose-anesthetized dogs (8). These interesting experiments suggest that there might be a differential effect of indomethacin and medofenamate on the relative degrees of inhibition of the prostanoid biosynthetic enzymes (cyclooxygenase, thromboxane synthase, etc.) or, perhaps, that there might be an
effect of anesthesia on the degree to which prostanoids influence AVP secretion.

We measured plasma concentrations of PGE$_2$, 6-keto-PGF$_1$,$\alpha$, and TxB$_2$ before and after indomethacin injection to confirm our assumption that the production of prostanoids would be reduced. Overall, indomethacin did effectively reduce the plasma concentrations of all three prostanoids, although the effect was more pronounced in the intact fetuses. The denervated fetuses had lower plasma prostanoid concentrations initially, and suppression was therefore less pronounced. Although we used plasma concentrations as an index of overall prostanoid production, it is important to emphasize that these compounds are most likely not acting as hormones and that the local concentrations within the brain were inaccessible to us with the present experimental design. We are confident that the circulating prostanoids are generated either on the afferent nerves subserving arterial baroreceptors and chemoreceptors or that they are generated within the nucleus of the solitary tract other sites within the central nervous system receiving afferent input from baroreceptors and chemoreceptors.

Perspectives

Endogenous prostanoid generation is an important component of the reflex control of fetal ACTH and vasopressin secretion. The results of the present investigation do not identify the source of these prostanoids or the site of action. However, the results of these experiments do suggest the possibility that generation of prostanoids within the brain has potent effects on endocrine secretion. It is thus possible that local generation of prostanoids within the fetal brain might alter the sensitivity to changes in both blood pressure and volume and might also be involved in what are thought of as normal “ontogenetic” changes in fetal hormone secretion, such as the increase in fetal ACTH secretion which ultimately initiates parturition.

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REFERENCES


