Effects of long-term hypoxemia on pituitary-adrenal function in fetal sheep

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Departments of Obstetrics/Gynaecology and of Physiology, University of Western Ontario,
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Murotsuki, Jun, Robert Gagnon, Stephen G. Matthews, and John R. G. Challis. Effects of long-term hypoxemia on pituitary-adrenal function in fetal sheep. Am. J. Physiol. 271 (Endocrinol. Metab. 34): E678–E685, 1996.—To test the hypothesis that long-term hypoxemia causes premature activation of the fetal pituitary-adrenal function, we embolized the fetal side of the placenta in pregnant sheep and examined the changes in concentrations of immunoreactive adrenocorticotropic hormone (irACTH), cortisol, and prostaglandin E₂ (PGE₂) in fetal plasma, and levels and localization of proopiomelanocortin (POMC) mRNA in the pars distalis and the pars intermedia of the fetal pituitary. Twelve fetal sheep were studied (6 embolized and 6 control) for 21 days between 0.74 and 0.88 of gestation. Daily injections of radiolabeled microspheres were given into the fetal abdominal aorta to decrease fetal arterial oxygen content by 40–50% of the preembolization values. In the embolized group, concentrations of irACTH, PGE₂, and cortisol in fetal plasma increased gradually and were significantly (P < 0.05) elevated above those of controls after day 10, day 16, and day 20, respectively. POMC mRNA levels in the pars distalis were not different from those of controls but were significantly reduced in the pars intermedia (P < 0.05). Therefore, to test the hypothesis that chronic fetal stress causes premature activation of pituitary-adrenal function, we examined the effect of 21-day hypoxemia induced by chronic fetal placental embolization on levels of POMC mRNA in different regions of the fetal pituitary and on concentrations of immunoreactive (ir) ACTH, cortisol, and PGE₂ in fetal plasma.

MATERIALS AND METHODS

Surgical preparation. Twelve pregnant sheep of Western cross breed and a singleton fetus of the same flock were surgically prepared between 104 and 106 days of gestation. Anesthesia was induced by intravenous thiopental sodium (Abbott, Montreal, Quebec, Canada) and then maintained on a closed-circuit anesthesia system with 0.5–1.0% halothane (Halocarbon, North Augusta, SC) in oxygen. The uterus was exposed, and a hindlimb was exteriorized through a uterine incision. A polyvinyl catheter (V4, Bolab, Lake Havasu City, AZ) was implanted via the fetal femoral artery into the descending abdominal aorta. The catheter tip was below the renal arteries and ~1–2 cm above the common umbilical artery. A second catheter was introduced into the fetal inferior vena cava through the femoral vein. The correct position of the fetal catheters was confirmed at postmortem examination. A polyvinyl catheter (V11, Bolab) was sutured to the exterior of the fetal hindlimb and left freely floating in the amniotic cavity to record amniotic pressure. Stainless steel Teflon-coated wire electrodes (Cooner, Chatsworth, CA) were sewn into the myometrium for continuous recording of uterine electromyographic (EMG) activity. All catheters were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters (V11, Bolab) were also placed into a femoral artery and a vein of the ewe. At the time of surgery and for 3 days postoperatively, the ewe was

HYPOXEMIA in utero is a major stressor for the fetus and is associated with various adaptive endocrine responses. The hypothalamic-pituitary-adrenal (HPA) axis in the near-term fetal sheep responds to acute hypoxemia with release of adrenocorticotropic hormone (ACTH), cortisol, and prostaglandin E₂ (PGE₂) in fetal plasma, and levels and localization of proopiomelanocortin (POMC) mRNA in the pars distalis and the pars intermedia. Fetal placental embolization; adrenocorticotropic hormone; proopiomelanocortin; fetal growth retardation
received 800,000 IU of procaine penicillin G and 1 g of streptomycin (Riker, London, Ontario, Canada) daily by intramuscular injections, and the fetus received 1,000,000 IU sodium penicillin G (Ayerst, Montreal, Quebec, Canada) into the femoral vein and the amniotic cavity.

After surgery, the sheep were housed in individual metabolic cages, with hay and water available ad libitum. Ewes were maintained on a 12:12 h light-dark cycle and were allowed ≥4 days to recover from surgery before the experiments began. This study was approved by the Animal Care Committees of St. Joseph’s Health Centre and the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care.

**Experimental protocol.** On the 4th day postrecovery (range 108–110 days), animals were assigned randomly to either an embolized (n = 6) or a control (n = 6) group. After a 2-h recording period (8 A.M. to 10 A.M.), nonradiolabeled carbonized latex microspheres, suspended in dextran and diluted with sterile saline, were injected every 15 min into the experimental fetuses over a 2-h period (10 A.M. to 12 noon) through the descending abdominal aorta. Occlusion of the placental arteries and arterioles by fetal placental embolization decreases the number of perfused villi and umbilical blood flow (7) and thus reduces the area available for gas exchange, leading to fetal hypoxemia with mild metabolic acidosis. The size of the microspheres was 15 μm on days 1–8 but was changed to 50 μm on days 9–21 because during preliminary experiments we had determined that after day 9 the smaller size of microspheres became less effective at inducing fetal hypoxemia. The number of microspheres injected was adjusted to decrease the fetal arterial oxygen content (CaO2, in mmol/l) by 40–50% of the preembolization value. Control fetuses were injected over the same period, with the vehicle diluted in sterile saline. Paired maternal and fetal femoral artery blood samples were taken daily at 9 A.M. for measurement of CaO2, P02, Pco2, pH, and hemoglobin.

A fetal arterial blood sample (3 ml) was taken at 9 A.M. (1 h postembolization on days 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 21) to measure arterial gases and oxygen content until the fetal CaO2 was 40–50% below control values. Fetal blood gases were measured in the absence of uterine EMG activity. The animals were then allowed to recover until the next day. The ewe was killed on day 21 at 4 P.M., and the fetuses were delivered and their pituitaries removed rapidly. Pituitaries were frozen (−80°C) using dry ice and were stored at −70°C until tissue sectioning.

**Analytic measurements.** Maternal and fetal arterial blood samples were drawn into heparinized syringes and placed on ice. Fetal arterial P02, Pco2, and pH were determined with a blood gas analyzer (ABL4, Radiometer, Copenhagen, Denmark) with measurements corrected to a fetal temperature of 39.5°C. Arterial oxygen saturation and hemoglobin were measured in duplicate with an OSM3 Hemoximeter device (Radiometer). Oxygen content was calculated with a capacity of 1.34 ml oxygen/g hemoglobin.

irACTH, cortisol, and PGE2 were determined using radioimmunoassay that we have described and validated previously (25, 26). The combined inter- and intra-assay coefficients of variation were 7–14%. Hormone estimates of less than the assay sensitivity for each assay were ascribed that value for the purpose of analysis.

In situ hybridization. The method for in situ hybridization has been described previously (23). Briefly, coronal sections (15 μm) of fetal pituitaries were cut on a cryostat (Tissue-Tek, Miles Canada, Etobicoke, Ontario, Canada), mounted onto poly-L-lysine (Sigma Chemical, St. Louis, MO)-coated slides, dried, postfixed in 4% paraformaldehyde for 5 min, rinsed in phosphate-buffered saline (2 times for 1 min), and dehydrated in an alcohol series. The slides were removed from alcohol, allowed to air dry at room temperature, and then incubated overnight in a moist chamber at 42°C with the radiolabeled oligonucleotide probe in hybridization buffer. The hybridization buffer used for these experiments contained 4X standard sodium citrate (SSC; 1× SSC = 150 mM sodium chloride, 15 mM sodium citrate), 50% deionized formamide, 50 mM sodium phosphate (pH 7.0), 1 mM sodium pyrophosphate (pH 7.0), 0.02% bovine serum albumin, 200 μg/ml hydroxylized salmon sperm DNA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 10% dextran sulfate, and 40 mM dithiothreitol. The oligonucleotide probes were labeled using terminal deoxynucleotidyl transferase (GIBCO, Burlington, Ontario, Canada) and [35S]deoxyadenosine 5’-(α-thio)triphosphate (1,300 Ci/mmol; New England Nuclear Du Pont Canada, Mississauga, Ontario, Canada) to a sp act of 1.0 × 106 cpm/μg. The labeled probe was used at a concentration of 500 cpm/μl. Labeled probe in hybridization buffer (200 μl) was applied to each slide and incubated overnight. After a wash for 30 min in 1× SSC at room temperature and for 30 min in 0.1× SSC (2× each), dehydrated in ethanol, dried, and exposed in X-ray film (XAR 5, Kodak). The X-ray films were developed using standard procedures.

The antisense POMC deoxyribonucleotide probes were 45 bases long and were made by solid-phase synthesis by use of an Applied BioSystems DNA synthesizer and purified on an 8% polyacrylamide/8 M urea preparative sequencing gel. The POMC probe was complementary to bases 711–756 of the porcine POMC gene (9). Control 45-mer sense oligonucleotides were also synthesized, and no signal was observed when these were hybridized with sections adjacent to those desribed for the antisense probes.

**Data analysis.** Changes in arterial blood gases and fetal endocrine measurements were analyzed using a two-way unbalanced correlation matrix analysis of variance with repeated measures (BMDP 5V, BMDP Statistical Software, Los Angeles, CA) to compare the effect of time, group (embolized vs. control), and interaction between group and time. If a significant effect of group or time was found (P < 0.05), within animal comparisons were conducted with multiple-comparison Bonferroni’s t-test, and between-group comparisons were made using BMDP 5V. All results are presented as means ± SE for the number of fetuses studied.

To compare levels of POMC mRNA, sections from control and experimental animals were processed simultaneously. The sections were exposed together with 14C-labeled standards (American Radiochemical, St Louis, MO). The optical density of the autoradiographic film was quantified using a computerized image analysis system ( Imaging Research, St. Catharine’s, Ontario, Canada), and the results were expressed as absorbance after subtraction of background values. Comparison between groups was performed using the values from ≥10 sections per animal. The level of the pituitary at which analysis was undertaken was consistent throughout. Because POMC mRNA is regionally distributed in the pituitary of fetal sheep (22), analyses of the supr

[22]
at the level of analysis was also determined. Grouped data were statistically analyzed using one-way analysis of variance followed by unpaired $t$-test (BMDP). Significant differences were set at $P < 0.05$.

**RESULTS**

Fetal morphometry, fetal blood gases, and hemoglobin. Table 1 summarizes fetal morphometric measurements at postmortem examination. The mean fetal body weight was reduced by 28% in embolized fetuses. The mean ponderal index, as a standard index to estimate the presence of intrauterine growth restriction, was lower in embolized fetuses ($P = 0.048$). In addition, the brain-to-liver weight ratio was higher ($P = 0.001$) in the embolized fetuses because of a small liver and a normal brain weight typical of that seen with redistribution of blood flow toward the brain during fetal hypoxemia and referred to as "asymmetrical" intrauterine growth restriction and placental insufficiency. In contrast, "symmetrical" intrauterine growth restriction refers to fetuses with a smaller brain as well as a smaller liver, which is usually the result of genetically small fetuses with normal placental function.

Fetal placental embolization caused progressive fetal hypoxemia (analysis of variance, effect of group and time for $Cao_2$, both $P < 0.0001$, Fig. 1, top). The mean fetal $Cao_2$ decreased progressively from a preembolization value of $2.92 \pm 0.12$ mmol/l to $1.49 \pm 0.30$ mmol/l on day 21 postembolization ($P < 0.0001$). There was partial recovery in fetal $Cao_2$, of the preembolization sample on each day until day 9, after which fetal $Cao_2$ remained significantly lower than control values ($P < 0.05$, Fig. 1, top). Changes in fetal arterial $PO_2$ paralleled changes observed in $Cao_2$ (analysis of variance effect of group and time, both $P < 0.0001$). Although fetal arterial $PCO_2$ tended to be higher in the embolized group (analysis of variance, $P = 0.036$), it decreased at the end of each daily embolization (Fig. 1, middle). A similar pattern of decreasing fetal arterial $PCO_2$ during the day was observed in the control group. Although fetal arterial pH in embolized fetuses decreased slightly postembolization, it recovered to control levels every day (Fig. 1, bottom) at the time fetal blood samples were taken for hormone measurements. Two-way analysis of variance indicated a significant effect of time but not of treatment on fetal arterial pH (analysis of variance, time effect, $P < 0.0001$). The fall in fetal pH was due to a small decrease in fetal arterial base excess (analysis of variance, time effect, $P < 0.0001$). Hemoglobin concentrations increased progressively in both embolized and control groups (analysis of variance, time effect, $P < 0.0001$). Maternal $Cao_2$, $PO_2$, and pH remained unchanged over 21 days, and there were no significant differences between groups (Table 2).

Fetal and maternal $trACTH$ and cortisol. Two-way analysis of variance demonstrated a significant effect of group ($P < 0.01$) and time ($P < 0.0001$) and a significant interaction between group and time ($P < 0.01$) on both variance, $P < 0.0001$). The fall in fetal pH was due to a small decrease in fetal arterial base excess (analysis of variance, time effect, $P < 0.0001$). Hemoglobin concentrations increased progressively in both embolized and control groups (analysis of variance, time effect, $P < 0.0001$). Maternal $Cao_2$, $PO_2$, and pH remained unchanged over 21 days, and there were no significant differences between groups (Table 2).

**Table 1. Morphometric data at postmortem examination**

<table>
<thead>
<tr>
<th></th>
<th>Embolized ($n = 6$)</th>
<th>Control ($n = 6$)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, days</td>
<td>129.5 ± 0.2</td>
<td>129.2 ± 0.4</td>
<td>0.925</td>
</tr>
<tr>
<td>Body weights, kg</td>
<td>2.60 ± 0.29</td>
<td>3.61 ± 0.43</td>
<td>0.070</td>
</tr>
<tr>
<td>Ponderal index, 100 g/cm³</td>
<td>2.69 ± 0.13</td>
<td>3.03 ± 0.06</td>
<td>0.048</td>
</tr>
<tr>
<td>Brain, g/kg body wt</td>
<td>16.3 ± 0.6</td>
<td>13.9 ± 1.0</td>
<td>0.073</td>
</tr>
<tr>
<td>Liver, g/kg body wt</td>
<td>28.3 ± 1.5</td>
<td>34.6 ± 0.88</td>
<td>0.004</td>
</tr>
<tr>
<td>Adrenal, g/kg body wt</td>
<td>0.19 ± 0.03</td>
<td>0.11 ± 0.01</td>
<td>0.030</td>
</tr>
<tr>
<td>Brain-to-liver weight ratio</td>
<td>0.58 ± 0.02</td>
<td>0.40 ± 0.03</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of pregnant sheep/group. Ponderal index was fetal body wt/(crown-to-rump length)$^9$.

Fig. 1. Fetal femoral artery oxygen content ($Cao_2$), $PCO_2$, and pH (means ± SE) before and at the end of each of the daily fetal placental embolization periods (horizontal bar). O, control ($n = 6$); o, embolized ($n = 6$). There was partial recovery in fetal oxygenation until day 9, after which fetal $Cao_2$ remained significantly lower than control values. *Significantly lower ($Cao_2$ and pH) or higher ($PCO_2$) than control values ($P < 0.05$).

Fig. 2. Fetal hemoglobin concentration (means ± SE) before each of the daily fetal placental embolization periods (horizontal bar). Fetal hemoglobin increased with advancing gestation in both groups and was not altered by chronic embolization.
FETAL PITUITARY-ADRENAL AXIS DURING LONG-TERM HYPOXEMIA

Table 2. Maternal arterial blood gases, oxygen content, and hormonal data on days 1, 10, and 21 of fetal placental embolization

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 10</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.449 ± 0.028</td>
<td>7.448 ± 0.008</td>
<td>7.448 ± 0.014</td>
</tr>
<tr>
<td>PaO2</td>
<td>103.0 ± 1.5</td>
<td>108.9 ± 3.6</td>
<td>110.4 ± 3.7</td>
</tr>
<tr>
<td>CaO2, mmol/l</td>
<td>5.67 ± 0.30</td>
<td>5.57 ± 0.37</td>
<td>5.74 ± 0.36</td>
</tr>
<tr>
<td>ACTH, pg/ml</td>
<td>63.3 ± 3.6</td>
<td>58.9 ± 3.0</td>
<td>60.5 ± 2.8</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>8.8 ± 3.5</td>
<td>9.0 ± 2.2</td>
<td>4.2 ± 1.1</td>
</tr>
<tr>
<td>PGE2, pg/ml</td>
<td>264 ± 26</td>
<td>283 ± 49</td>
<td>320 ± 73</td>
</tr>
<tr>
<td><strong>Embolized (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.482 ± 0.011</td>
<td>7.471 ± 0.013</td>
<td>7.444 ± 0.015</td>
</tr>
<tr>
<td>PaO2</td>
<td>100.4 ± 3.1</td>
<td>110.7 ± 5.0</td>
<td>110.3 ± 3.8</td>
</tr>
<tr>
<td>CaO2, mmol/l</td>
<td>5.36 ± 0.15</td>
<td>5.52 ± 0.26</td>
<td>5.84 ± 0.30</td>
</tr>
<tr>
<td>ACTH, pg/ml</td>
<td>58.5 ± 7.9</td>
<td>54.1 ± 5.2</td>
<td>51.6 ± 6.0</td>
</tr>
<tr>
<td>Cortisol, ng/ml</td>
<td>9.1 ± 3.7</td>
<td>6.2 ± 1.3</td>
<td>4.4 ± 2.4</td>
</tr>
<tr>
<td>PGE2, pg/ml</td>
<td>272 ± 31</td>
<td>287 ± 51</td>
<td>368 ± 57</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of pregnant sheep/group. CaO2, oxygen content; ACTH, adrenocorticotropic hormone; PGE2, prostaglandin E2.

fetal irACTH and cortisol values (Fig. 3). The mean baseline fetal plasma irACTH concentration in the embolized group on day 1 was 17 ± 3 pg/ml, which was not significantly different from the value of 19 ± 2 pg/ml in controls. On day 4 of embolization, irACTH increased significantly (P < 0.05) above controls but then returned to control levels on days 6–8. However, on day 10, fetal plasma irACTH levels increased again (P < 0.05) and remained elevated above control values up to day 21 (Fig. 3, top). The mean fetal plasma cortisol concentration in the embolized group was not significantly different from that of the control group from day 1 to day 18, and then it surged up on days 20 and 21 (P < 0.0001; Fig. 3, bottom). In the control group, mean fetal plasma irACTH and cortisol did not change significantly from day 1 to day 21. There was no significant effect of time or embolization on maternal plasma irACTH and cortisol concentrations, which remained unaltered throughout the study (Table 2).

Fetal and maternal PGE2. The mean fetal plasma PGE2 concentrations in the embolized and control fetuses are shown in Fig. 4. Analysis of variance revealed a significant effect of group (P < 0.05) and a significant interaction between group and time (P < 0.05). In embolized fetuses, the mean plasma PGE2 concentration on day 1 preembolization was 436 ± 49 pg/ml, which was not significantly different from values of 453 ± 65 pg/ml in controls. The fetal plasma PGE2 levels increased gradually and remained significantly elevated above control values from day 16 to day 21. In the control group, fetal plasma PGE2 did not change significantly throughout the study. Maternal plasma PGE2 concentrations in both groups remained unaltered throughout the study (Table 2).

POMC mRNA in fetal pituitaries. POMC mRNA was present in both the pars distalis and the pars intermedia, but not in the pars nervosa, of the pituitaries in the embolized and control fetuses (Fig. 5, A–D). The levels of pituitary POMC mRNA were more than 10-fold greater in the pars intermedia than in the pars distalis. The regional distribution for POMC mRNA within the pars distalis was not significantly different between...
Fig. 5. Localization of proopiomelanocortin (POMC) mRNA in the fetal pituitary after in situ hybridization of coronal sections with 35S-labeled 45-mer antisense oligonucleotide probe specific for POMC mRNA in a control fetus (a and b) and an embolized fetus (c and d). In the pars distalis, POMC mRNA was present at highest levels in the inferior region (base of gland), with lower levels in the superior region (area around the pars intermedia). PD, pars distalis; PI, pars intermedia. Arrow, pars intermedia in embolized fetus. Exposure time: (a and c), 4 days; (b and d), 4 h. Color code indicates relative optical density.

groups; the level in the inferior region was 1.7-fold greater than in the superior region in controls and 2.1-fold greater in embolized fetuses (Fig. 6A). Therefore, chronic hypoxemia did not alter the normal distribution of POMC mRNA within the pars distalis. After 21 days of progressive hypoxemia, POMC mRNA levels in the pars distalis were not significantly different between groups (Figs. 5 and 6A). However, POMC mRNA levels decreased in the pars intermedia of embolized fetuses to values that were 40% less than in controls ($P < 0.05$, Figs. 5 and 6B).

DISCUSSION

This study has shown that, during fetal placental embolization for 21 days between 0.74 and 0.88 of gestation of fetal sheep, there were progressive increases in fetal plasma irACTH and PGE$_2$ after day 10 and day 16, respectively, and that these were not associated with a significant increase in cortisol until days 20 and 21. We also demonstrated that the levels of POMC mRNA in the pars distalis of the fetal pituitary, the site of synthesis and release of ACTH, were not significantly different from those of controls, whereas POMC mRNA levels decreased in the pars intermedia after 21-day hypoxemia. The changes in POMC mRNA expression in the fetal pituitary do not support the hypothesis that long-term fetal hypoxemia causes premature activation of the pituitary-adrenal function under the current experimental conditions.

During development, the fetus may be exposed to a variety of stresses, including hypoxemia, that may influence growth and the timing of birth. It is likely that the activities of the fetal endocrine system, particularly the HPA axis, contribute to the ability of the fetus to adjust and adapt to chronic stress in utero. Some studies have demonstrated the effect of short-term hypoxemia (4, 15) and long-term hypoxemia (12, 17) on fetal pituitary-adrenal function in fetal sheep. In these
ACTH concentrations return to control values, whereas although a reduction in fetal Cao, was always ob-
known about the effect of chronic hypoxemia on adrenal cortisol concentrations remain elevated (4, 15). Little is
known about the effect of chronic hypoxemia on adrenal cortisol. However, after 12-16 h of hypoxemia, plasma
24 h initially causes an increase in both ACTH and embolization.
lower than control values from day 10 to day 21 of studies, oxygen transfer to the fetus was reduced by lowering the fraction of oxygen inspired by the mother (12, 17) or by decreasing uteroplacental blood flow with a vascular clamp around the common internal iliac artery (4, 15). However, these methods were not particularly appropriate as models of long-term fetal hypoxemia, because fetal hemoglobin concentration increased as compensatory adaptation within 2-3 days during the course of the studies, and fetal Cao, was no longer different from control levels. The methodology used in the present study to induce fetal hypoxemia allowed a predetermined reduction of the fetal Cao,.
Although a reduction in fetal Cao, was always ob-
tained, the fetal oxygenation partially recovered before the next embolization day until day 9. These findings are similar to those we reported previously (6). Long-
term hypoxemia induced by daily fetal placental embo-
lization in our model was characterized by a progressive decline in fetal Cao, that remained significantly lower than control values from day 10 to day 21 of embolization.

During late gestation, prolonged hypoxemia lasting 24 h initially causes an increase in both ACTH and cortisol. However, after 12-16 h of hypoxemia, plasma ACTH concentrations return to control values, whereas cortisol concentrations remain elevated (4, 15). Little is known about the effect of chronic hypoxemia on adrenal sensitivity, but we hypothesized that chronic stress would activate fetal pituitary-adrenal function at a time when the adrenals are still relatively unrespon-
sive to ACTH. ACTH administration in vivo leads to an increase in fetal adrenal weight and cortical hypertrophy (18). We observed almost a doubling in fetal adrenal weight in the embolized fetuses in the present study, which might have been the result of chronic hypoxemia and a progressive increase in fetal plasma ACTH. However, our findings that long-term fetal hypoxemia caused an increase in plasma ACTH but little increase in plasma cortisol concentrations until 126 days of gestation suggest that the sensitivity of the fetal adrenal cortex to ACTH remained relatively unal-
tered. It is possible that secretion of steroids other than cortisol might have changed earlier during the emboli-
ization period. However, cortisol is the main product of the ovine fetal adrenal gland. Androstenediione is pro-
duced, but ongoing studies show that its plasma concentra-
tions are unchanged or even reduced during hypox-

Our results are in part consistent with those of a recent study that examined the changes in adrenal responsiveness in fetal sheep after long-term hypoxia caused by maternal exposure to high altitude (3,820 m) from 30 days of gestation (12). It was reported that cortisol secretion in response to an ACTH challenge was blunted in the hypoxic fetus on 126 and 136 days of gestation, suggesting that long-term hypoxemia did not increase the adrenal sensitivity to ACTH. However, in that report, long-term hypoxemia did not alter basal ACTH and cortisol concentrations between 126 and 140 days of gestation (12). The difference between their results and ours may be that Harvey et al. (12) induced fetal hypoxemia at an early gestational age, such that there was no difference in Cao, between “hypoxic” fetuses and controls because of a higher hemoglobin concentration in fetuses exposed to high altitude.

In confirmation of our previous study of 10-day embolization in late gestation (25), we found that fetal plasma PGE, concentrations increased and remained elevated between days 16 and 21 of hypoxemia. PGE, is known to stimulate rises in plasma cortisol even at a time when the fetal adrenals are relatively unrespon-
sive to ACTH (20). Because PGE, is a potent stimulator of both cortisol (20) and ACTII (28) release, it is possible that elevated fetal PGE, and ACTH synergized in their action on adrenal cortex and increased cortisol concentrations on days 20 and 21 of embolization. However, we cannot discount the possibility that the increase in fetal adrenal cortisol output on days 20 and 21 is due to the effect of sustained elevation of plasma ACTH over the preceding days (26). PGE, induced cortisol might have changed earlier during the emboli-
ization period. However, cortisol is the main product of the ovine fetal adrenal gland. Androstenediione is pro-
duced, but ongoing studies show that its plasma concentra-
tions are unchanged or even reduced during hypox-
nal blood volume (29). We suggest that increased production of PGE\textsubscript{2} and cortisol plays an important role to maintain fetal homeostasis under conditions of chronic hypoxemia.

After 21 days of embolization, we found that POMC mRNA levels in the pars intermedia of the fetal pituitary had decreased by ~40%, whereas levels in the pars distalis were not significantly different from controls, even though plasma ACTH concentrations were elevated. Previously, we reported that POMC mRNA levels in the pars distalis were elevated after 6 and 48 h of hypoxemia (2, 22). It is therefore possible that POMC mRNA levels in the pars distalis had been elevated at earlier times during the embolization protocol, but that these levels were later inhibited by the rise in plasma cortisol seen on days 20 and 21. Our previous studies have shown that increased endopeptidase (corticotropin-releasing hormone (CRH)), CRH, and AVP stimulate activation of the pituitary POMC mRNA are more susceptible to the negative feedback effects of glucocorticoids than are basal values (22). However, we cannot rule out the possibility that transient increases in POMC mRNA, occurring immediately after or during the period of stress on day 21 of the experimental period, would not have been detected in tissue collected 4 h after that episode.

In contrast to the lack of change in levels of POMC mRNA in the pars distalis, levels of POMC mRNA in the pars intermedia were significantly decreased compared with controls. This is similar to the response seen after 48 h of hypoxemia (2). These results clearly show that there is differential regulation of POMC expression in the two zones of the pituitary in response to the stress of hypoxemia.

The major factors regulating POMC expression in the pars distalis are likely corticotropin-releasing hormone (CRH) and AVP (30). CRH and AVP stimulate the corticotropes to secrete ACTH in the fetal sheep (3), and CRH and AVP stimulate increased accumulation of POMC mRNA in corticotroph cells prepared from term fetal sheep and maintained in vitro (Matthews and Challis, unpublished observations). Previously, we showed that the levels of CRH in the parvocellular region of the paraventricular nucleus were elevated in response to short-term hypoxemia (21), but, as with POMC mRNA in the pituitary, these levels were attenuated after concomitant glucocorticoid treatment. Thus, it seems likely the CRH mRNA levels at 21 days may have been similar in the two groups of animals, as we found for POMC mRNA. This would not have precluded changes in CRH mRNA at earlier times in the experimental protocol.

Regulation of the pars intermedia is multifactorial. Its differential control may be related to effects of one or all of dopamine, \textgamma-aminobutyric acid (GABA), and CRH. Dopamine, acting through the D\textsubscript{2} receptor (24), inhibits levels of POMC mRNA in the rat pars intermedia (5). Systemic infusion of a dopamine agonist to fetal sheep in late gestation leads to a fall in POMC mRNA levels in the pars intermedia without change in levels of POMC mRNA in the pars distalis, showing the differential effects on these two zones of the gland (S. G. Matthews, M. Fraser, and J. R. G. Challis, unpublished observations). Others have reported that carunclectomy, which causes long-term fetal hypoxemia in sheep, is associated with a significant reduction in circulating prolactin concentrations (11). Plasma prolactin is decreased by dopamine treatment, suggesting that dopamine synthesis in the hypothalamus may be increased after hypoxic stress. This could provide an explanation for the present results. In addition, GABA inhibits POMC mRNA levels in melanotroph cells in the pars intermedia through GABAergic neurones (30), and, in the rat, CRH has been reported to decrease levels of POMC in the pars intermedia (14). Further studies are required to evaluate the relative importance of these different agents in the context of the present results.

The two regions of the fetal pituitary are thought to secrete different posttranslational products of POMC into the fetal circulation. POMC is processed in the pars intermedia to larger molecular weight products that are endogenous inhibitors of ACTH action (27). A fall in POMC expression in the pars intermedia after hypoxemia could result in a decrease in the output of these large molecular weight peptides and a concomitant increase in the proportion of biologically active ACTH in peripheral plasma. In contrast, the pars distalis is thought to process POMC predominately to ACTH (23). An alternative explanation for the rise in plasma ACTH is the absence of changed levels of POMC in the pars distalis after 21 days of embolization may be that the levels of endopeptidase(s) activity required for POMC processing have changed, without alteration, in POMC mRNA expression. However, there is no information on the prohormone convertase enzymes in the fetal sheep pituitary at the present time.

In conclusion, 21-day repetitive fetal placental embolization, resulting in fetal hypoxemia, caused a progressive increase in irACTH from days 10–21 and in PGE\textsubscript{2} from days 16–21, without a significant increase in cortisol until days 20 and 21. POMC mRNA levels in the pars distalis were not different from those of controls, possibly because of negative feedback effects of cortisol on the pituitary gland, preventing premature activation of the pituitary-adrenal axis during chronic fetal hypoxemia. In contrast, POMC mRNA in the pars intermedia decreased after 21-day hypoxemia, indicating a differential regulation of POMC mRNA expression in the pars distalis and pars intermedia. The altered responses in HPA function may be important as adaptive mechanisms for the fetus to prevent preterm delivery; they may, however, contribute to growth restriction in utero.

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