Antenatal glucocorticoids reset the level of baseline and hypoxemia-induced pituitary-adrenal activity in the sheep fetus during late gestation

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Fletcher, Andrew J. W., Xiao Hong Ma, Wen X. Wu, Peter W. Nathanielsz, Hugh H. G. McGarrigle, Abigail L. Powden, and Dino A. Giussani. Antenatal glucocorticoids reset the level of baseline and hypoxemia-induced pituitary-adrenal activity in the sheep fetus during late gestation. Am J Physiol Endocrinol Metab 286: E311–E319, 2004.—This study examined the effects of dexamethasone treatment on fetal hypothalamo-pituitary-adrenal (HPA) axis function and HPA responses to subsequent acute hypoxemia in the ovine fetus during late gestation. Between 117 and 120 days (term: ~145 days), 12 fetal sheep and their mothers were catheterized under halothane anesthesia. From 124 days, 6 fetuses were continuously infused intravenously with dexamethasone (1.80 ± 0.15 μg kg⁻¹ h⁻¹ in 0.9% saline at 0.5 ml/h) for 48 h, while the remaining 6 fetuses received saline at the same rate. Two days after infusion, when dexamethasone had cleared from the fetal circulation, acute hypoxemia was induced in both groups for 1 h by reducing the maternal fraction of inspired O₂. Fetal dexamethasone treatment transiently lowered fetal basal plasma cortisol, but not ACTH, concentrations. However, 2 days after treatment, fetal basal plasma cortisol concentration was elevated without changes in basal ACTH concentration. Despite elevated basal plasma cortisol concentration, the ACTH response to acute hypoxemia was enhanced, and the increment in plasma cortisol levels was maintained, in dexamethasone-treated fetuses. Correlation of fetal plasma ACTH and cortisol concentrations indicated enhanced cortisol output without a change in adrenocortical sensitivity. The enhancements in basal cortisol concentration and the HPA axis responses to acute hypoxemia after dexamethasone treatment were associated with reductions in pituitary and adrenal glucocorticoid receptor mRNA contents, which persisted at 3–4 days after the end of treatment. These data show that prenatal glucocorticoids alter the basal set point of the HPA axis and enhance HPA axis responses to acute stress in the ovine fetus during late gestation.

dexamethasone; hypoxemia

IN THE SHEEP FETUS DURING LATE GESTATION, episodes of acute stress evoke integrated endocrine responses that facilitate fetal survival during the period of adversity. The fetal ovine hypothalamo-pituitary-adrenal (HPA) axis responds to acute hypoxemia (1, 5, 22), hemorrhage (54), acidemia (53), and hypotension (38, 52) with increases in arterial plasma concentrations of ACTH (1, 5, 22) and adrenocortical output of cortisol (26). The maturational effects of synthetic or natural glucocorticoids on a number of fetal systems are well established (21, 29). However, few studies have investigated the maturational effects of glucocorticoids on the responses of the fetal HPA axis to an episode of stress, such as acute hypoxemia. This is important, because fetal acute hypoxemia may accompany labor and delivery (25), both of which are likely to occur during, or shortly after, the administration of synthetic glucocorticoids in clinical practice. It is probable that any maturational effects of glucocorticoids on fetal pituitary-adrenal responses to acute hypoxemia, and the mechanisms mediating them, will be influenced by glucocorticoid negative feedback if the episode of hypoxemia occurs during the period of elevated glucocorticoid concentration. Indeed, previous studies have shown that treatment of fetal sheep with either cortisol (1, 33) or dexamethasone (18, 35) abolished 1) the hypoxemia-induced increases in plasma ACTH and cortisol, 2) the elevations in corticotropin-releasing hormone (CRH) mRNA content in the paraventricular nuclei of the hypothalamus, and 3) proopiomelanocortin mRNA levels in the pars distalis of the pituitary gland during the period of treatment. However, the effects of fetal treatment with synthetic glucocorticoids on the responses of the fetal HPA axis to acute hypoxemia, when the episode of oxygen deprivation occurs after the clearance of the steroid from the fetal circulation, remain unknown.

Hence, the current study determined the effects of fetal intravenous infusion with dexamethasone for 48 h on ovine fetal ACTH and cortisol responses to a 1-h episode of acute hypoxemia occurring at 48 h after the end of the infusion period. To address possible mechanisms of changes in basal and stimulated fetal HPA axis function produced by dexamethasone treatment, fetal plasma ACTH and cortisol concentrations were correlated during normoxic and hypoxic conditions. In addition, after the end of the experimental protocol, 1) fetal pituitary and adrenal glucocorticoid receptor (GR) mRNA levels were measured by Northern analysis to address possible contributions of glucocorticoid-induced changes in feedback regulation of the fetal HPA axis, and 2) gross histological examination of the adrenals was performed by light microscopy.

MATERIALS AND METHODS

Use of Animals and Surgical Preparation

All surgical and experimental procedures were performed under the UK Animals (Scientific Procedures) Act 1986. Between 117 and 120 days of gestation (dGA; term is ~145 days), 12 Welsh Mountain
sheep fetuses and their mothers were surgically prepared for long-term recording under general anesthesia, as described previously in detail (18). Briefly, food, but not water, was withheld from the ewes for 24 h before surgery. After induction with 20 mg/kg intravenous sodium thiopentone (Intraval Sodium; Rhone Mérieux, Dublin, Ireland), general anesthesia (1.5–2.0% halothane in 50:50 O2:N2O) was maintained using positive pressure ventilation. A Teflon catheter was inserted into a maternal femoral artery and its tip advanced into the descending aorta. The gravid uterus was exposed via a midline abdominal incision. After exteriorization of the fetal head, translucent PVC catheters (0.58 or 0.86 mm ID; 0.96 or 1.52 mm OD; Crittily Electrical Products, NSW, Australia) were inserted into a fetal carotid artery, jugular vein, and the amniotic cavity. At the end of surgery, the vascular catheters were filled with heparinized saline (80 IU heparin/ml in 0.9% NaCl) and sealed with brass pins. All catheters were exteriorized via a small incision in the maternal flank and housed in a pouch sutured to the skin.

Postoperative Care
Ewes were housed in individual pens, had free access to water and hay, were fed concentrates twice daily (100 g; Sheep Nuts no. 6; H&C Beart, Kings Lynn, UK), and generally resumed normal feeding patterns within 24 h of surgery. The ewes received 2 days of postoperative analgesia (3 g daily of oral phenylbutazone; Equipalzem Paste E-pp, Arnolds Veterinary Products, Shropshire, UK) if required. Antibiotics were administered daily to the ewe (0.20–0.25 mg/kg im Depocillin; Mycofarm, Cambridge, UK), to the fetus (150 mg/kg iv ampicillin, Penbritin; SmithKline Beecham Animal Health, Surrey, UK), and into the amniotic cavity (300 mg Penbritin). Vascular catheters were maintained patent by a slow continuous infusion of heparinized saline at 0.1 ml/h.

Experimental Procedure
At least 4 days after surgery, the fetuses were randomly assigned to one of two experimental groups. From 124 days, 6 fetuses were continuously infused intravenously with dexamethasone (dexamethasone sodium phosphate; Merck, Sharp, Dohme, Herts., UK) in heparinized saline for 48 h at a rate of 4.23 ± 0.28 μg/h delivered at 0.5 ml/h (1.80 ± 0.15 μg·kg⁻¹·h⁻¹, corrected retrospectively for fetal weight measured at the end of the experimental protocol). The remaining 6 fetuses were infused intravenously with heparinized saline at the same rate (0.5 ml/h) to act as age-matched controls. Maternal caudal aortic and fetal carotid arterial blood samples were taken daily for analysis of arterial blood gases and acid/base status and for measurement of plasma hormone concentrations. Samples were collected daily at 10:00 AM on the 2 days before the infusion began (days 1 and 0), on the 2 days of infusion (days 1 and 2), and on the 2 days after infusion (days 3 and 4). At 128 days, 2 days after the end of infusion, all fetuses were subjected to an acute episode of hypoxemia, induced by reducing the maternal fraction of inspired O2 (FEO2). The acute hypoxemia protocol was based on a 3-h protocol: 1 h of normoxia; 1 h of hypoxemia; 1 h of recovery, as described previously (23). At the start of the protocol, a large, transparent, polythene respiratory hood was placed over the ewe’s head, and air was passed through at a rate of 40 l/min. After a 1-h control period of normoxia, fetal hypoxemia was induced for 1 h by switching the gas mixture breathed by the ewe to 9% O2 in N2 (18 l/min air; 22 l/min N2), with small amounts of CO2 (1.2 l/min) added to the inspirate. This mixture was designed to reduce fetal arterial pressure of O2 (PaO2) to 11–13 Torr while minimizing changes in fetal arterial pressure of CO2 (PaCO2). After the 1-h period of hypoxemia, the hood was removed from the ewe’s head, and the ewe was allowed to breathe room air for the 1-h recovery period.

Paired maternal aortic and fetal carotid arterial blood samples (0.3 ml) were collected at 15-min intervals throughout the hypoxemia protocol for the measurement of blood gases and acid-base status. In addition, a fetal carotid arterial blood sample was taken 5 min after the onset of hypoxemia to confirm that fetal PaO2 had fallen to the desired level. Paired maternal and fetal arterial blood samples (5 ml) were collected for hormone measurement at 15 min (early) and 45 min (late) of normoxia, at 15 min (early) and 45 min (late) of hypoxemia, and at 45 min of recovery. Blood samples for hormone analyses were collected into K²/EDTA-treated tubes kept on ice and were centrifuged at 4,000 rpm for 4 min at 4°C. Plasma samples were stored at −70°C until analyses. All hormone measurements were performed within 2 mo of sample collection.

Biochemical Analyses
Maternal aortic and fetal carotid arterial blood gas status, acid/base status, and hemoglobin concentrations were determined using an ABL5 blood gas analyzer and OSM2 hemoximeter (Radiometer, Copenhagen, Denmark). Measurements in maternal and fetal blood were corrected to 38 and 39.5°C, respectively. Plasma immunoreactive ACTH (IR-ACTH), cortisol, and dexamethasone concentrations were determined by RIA and validated for use in ovine plasma.

Dexamethasone. Plasma dexamethasone levels were measured after ether extraction by use of tritium-labeled dexamethasone as tracer, as described in detail previously (18). All values were corrected for recovery (86%). The interassay coefficients of variation (CVs) for three control plasma pools (1.8, 5.4, and 26.7 nmol/l) were 14.6, 9.3, and 8.2%, respectively. The lower detection limit of the assay was 0.2 nmol/l. The anti-dexamethasone antiserum showed a 1.6% cross-reactivity against cortisol and cross-reactivities of <0.5% against 11-deoxycortisol, corticosterone, testosterone, progesterone, and estradiol.

IR-ACTH. Maternal and fetal plasma IR-ACTH concentrations were measured using a commercially available double-antibody 125I RIA kit (Incatar, Wokingham, UK) (18). The lower limit of detection of the assay was 10–25 pg/ml. The interassay CV was 8.4%. The intra-assay CVs for two plasma pools (37 and 150 pg/ml) were 3.6 and 4.1%, respectively. The assay had negligible (<0.01%) cross-reactivities with α-melanocortin-stimulating hormone, β-endorphin, β-lipotropin, leucine enkephalin, methionine enkephalin, bombesin, calcitonin, parathyroid hormone, follicle-stimulating hormone, arginine vasopressin (AVP), oxytocin, and substance P.

Cortisol. Maternal and fetal plasma cortisol concentrations were measured as described previously (41). After ethanol extraction, samples were analyzed by RIA with [3H]cortisol as tracer. The lower limit of detection of the assay was 1.0–1.5 ng/ml. The intra- and interassay CVs were 5.3 and 13.0%, respectively. The antiserum showed no detectable cross-reactivity with dexamethasone. The cross-reactivities of the antiserum at 50% binding with other cortisol-related compounds were 0.5% for cortisone, 2.3% for corticosterone, 0.3% for progesterone, and 4.6% for deoxycorticosterone.

Postmortem Procedures
After the end of the experimental protocol, at 130 ± 1 days, ewes were humanely killed with a lethal dose of sodium pentobarbitone (40 mg/kg iv Pentoject; Animalcare, York, UK) and the fetuses by exsanguination under the materno-fetal pentobarbitone anesthesia. In all of the fetuses, the right adrenal gland was removed, fixed in 10% formal saline, and stored at 5°C for subsequent biometric measurements with light microscopy. The pituitary glands were removed from the hypophysial fossa of the sphenoid bone and, together with the left adrenal glands, were frozen in liquid N2 and stored at −80°C for subsequent measurement of GR mRNA.

Fetal Adrenal Histology
Within 4 days of collection, the fetal right adrenal glands (saline infused, n = 6; dexamethasone treated, n = 6) were removed from the fixative and prepared for histological examination. The adrenal glands...
were bisected along their longitudinal axis, and sections were prepared from the cut surfaces. The sections were stained using the ferric ferricyanide technique, with new fuchsin as counterstain. Sections were examined under light microscopy, and the boundary between adrenal cortex and medulla was determined by visual inspection. For each section, measurements of the thickness of the cortex and medulla were made in triplicate with a calibrated eyepiece graticule.

Measurement of Pituitary and Adrenal GR mRNA Content

GR mRNA content was determined in the whole homogenized pituitary and the left adrenal glands using Northern blot analysis, and values were expressed relative to β-actin mRNA. Polyadenylated RNA was extracted from frozen fetal pituitaries and adrenals by oligo(dT) cellulose affinity chromatography with a commercial kit (Micro-Fast Track 2.0, Invitrogen, San Diego, CA). Purity of RNA compared with DNA and protein contamination was determined by the ratio of absorbance at 260 to 280 nm (pure RNA 260/280 absorbance ratio ≈ 2.0).

Electrophoretic separation of RNA and capillary transfer. Each sample (2 μg of mRNA) (denatured in 50% deionized formamide; 9% formaldehyde) was subjected to denaturing agarose gel electrophoresis (6% formaldehyde, 1.0% agarose) in MOPS [20 mM 3-(N-morpholino)-propanesulfonic acid, 5 mM sodium acetate, 1 mM EDTA, pH 7.0]. Electrophoresis on all of the experimental samples was performed on the same gel series, and lanes were randomly assigned. After electrophoresis, the RNA was capillary-transferred onto nylon membranes (Gene Screen Plus; DuPont-NEN) and air-dried. RNA quality was assessed by ethidium bromide gel staining.

Prehybridization and hybridization. Membranes containing fetal pituitary and adrenal mRNA were prehybridized at 42°C in a commercial 50% formamide-based buffer (Northern Max, Ambion, TX). Specific mRNA was quantified and analyzed by hybridization in the same buffer as prehybridization to a radiolabeled 32P-labeled human GRα cDNA probe (no. 67200; American Type Cell Culture) (24). The 32P-labeled GRα cDNA probe (1 × 106 counts/min·μg·ml−1 prehybridization volume) was denatured at 100°C for 5 min and then rapidly chilled before addition to the hybridization mixture. The mixture was then left for 16 h at 42°C to allow hybridization to reach equilibrium. The membranes were washed initially at room temperature with 2 × SSC for 10 min. The membranes were then washed twice under more stringent conditions at 65°C for 15 min in 0.5 × SSC and 0.1% SDS. Autoradiography (XRP-5) with one Cronex intensifying screen was performed at −80°C for varying lengths of time, on the basis of abundance of message, to obtain signals within the linear range of densitometric measurements.

For quantification of RNA, scanning densitometry was utilized on autoradiographs of the hybridized mRNA. Quantity of RNA was expressed as arbitrary absorbance units. The membranes were then rehybridized for β-actin, allowing each sample to be corrected for RNA loading relative to β-actin mRNA content.

Data and Statistical Analyses

Values for all variables are expressed as means ± SE unless otherwise indicated. Statistical significance for comparisons between daily blood gases, acid/base status, and hormone concentrations was assessed using two-way repeated-measures ANOVA and the Tukey post hoc test. Statistical significance for differences between pituitary and adrenal GR mRNA expression between groups was assessed by Student’s t-test for unpaired data. Statistical significance of the linear fit of the log[IR-ACTH] vs. [cortisol] correlations within each group was assessed using the Pearson product-moment correlation coefficient test. In this study, adrenal sensitivity to ACTH is taken to be the gradient of the linear fit of the log[IR-ACTH] vs. [cortisol] correlation; i.e., a measure of the increment in [cortisol] per unit increment in log[IR-ACTH]. To compare correlations between treatment groups, comparisons between the slopes and intercepts of the linear regression lines derived for each treatment group were made according to Armitage and Berry (3). In all cases, significance was accepted when P < 0.05.

RESULTS

Daily Changes in Plasma Hormone Concentrations

In both groups of animals, maternal plasma dexamethasone concentrations remained unaltered from preinfusion values throughout the experimental protocol (Fig. 1). Although dexamethasone was not detected in saline-infused fetuses throughout the experimental protocol, fetal dexamethasone treatment produced a significant elevation in fetal plasma dexamethasone concentration, averaging 1.91 ± 0.18 nmol/l over the 48 h of infusion (Fig. 1). In the dexamethasone-treated fetuses, plasma dexamethasone was again undetectable within 48 h after the cessation of infusions (Fig. 1). In both groups of mothers and in the saline-infused fetuses, plasma IR-ACTH and cortisol concentrations remained unaltered from preinfusion values throughout the experimental protocol (Fig. 1). Although there was a significant reduction in basal plasma cortisol concentration by 24 h of infusion in dexamethasone-treated fetuses compared with saline-infused controls, plasma cortisol concentration was significantly elevated compared with control fetuses by 48 h after the end of the infusion period in the presence of unchanged fetal plasma IR-ACTH concentrations (Fig. 1).

Daily Changes in Blood Gases and Acid-Base Status

Values for maternal blood gases and arterial pH (pHₐ) remained unaltered from preinfusion baseline throughout the infusion protocol and were similar between the two groups of animals (pHₐ 7.47 ± 0.01 vs. 7.49 ± 0.01; PaCO₂ 35.2 ± 0.4 vs. 34.0 ± 0.2 Torr; PaO₂ 100.4 ± 1.6 vs. 101.3 ± 2.1 Torr; acid/base excess 3.0 ± 0.4 vs. 3.0 ± 0.2 meq/l; hematocrit 26.2 ± 0.7 vs. 28.0 ± 0.6%; saline vs. dexamethasone; P > 0.05). Preinfusion values for fetal blood gases and pH were similar between saline-infused and dexamethasone-treated fetuses (pHₐ 7.36 ± 0.01 vs. 7.35 ± 0.01; PaCO₂ 51.2 ± 0.8 vs. 51.2 ± 1.1 Torr; PaO₂ 22.2 ± 0.8 vs. 23.2 ± 0.6 Torr; O₂ saturation of hemoglobin 65.0 ± 1.5 vs. 69.7 ± 2.3%; hemoglobin concentration 8.4 ± 0.4 vs. 8.5 ± 0.3 g/dl; and hematocrit 27.2 ± 1.3 vs. 27.7 ± 0.9%; saline vs. dexamethasone; P > 0.05) and remained unaltered from baseline in both groups throughout the experimental protocol.

Blood Gases and Acid-Base During the Acute Hypoxemia Protocol

Maternal levels. During normoxia, values for maternal pHₐ (7.50 ± 0.01 vs. 7.50 ± 0.01), PaCO₂ (36.0 ± 1.3 vs. 35.6 ± 1.5 Torr), PaO₂ (95.6 ± 1.7 vs. 95.8 ± 2.6 Torr), acid/base excess (4.2 ± 0.7 vs. 4.8 ± 1.0 meq/l), and hematocrit (24.8 ± 1.6 vs. 27.8 ± 1.3%) were not significantly different between saline-infused and dexamethasone-treated animals. During hypoxemia, maternal PaO₂ levels achieved (38.8 ± 3.5 vs. 32.0 ± 2.1 Torr) were similar in both groups. These PaO₂ changes occurred without alterations from baseline in any other maternal variable in either group. During recovery, PaO₂ reverted to normoxic levels in mothers of both saline-infused and dexamethasone-treated fetuses.
**Fetal levels.** Changes in fetal carotid blood gases and acid-base status during the acute hypoxemia protocol are shown in Table 1. In both treatment groups, the reduction in fetal PaO₂ occurred rapidly after the onset of the hypoxic challenge, and values of PaO₂ were similar between treatment groups at 5 min of hypoxemia (saline: 13.4 ± 0.5 Torr; dexamethasone: 14.0 ± 0.7 Torr; P > 0.05) and throughout the hypoxic challenge (Table 1). Although fetal PaO₂ returned to baseline levels by 15 min of recovery in the saline-infused fetuses, fetal PaO₂ was transiently elevated above normoxic baseline levels at 15 min of recovery in the dexamethasone-treated fetuses (Table 1). There were no changes in fetal PaCO₂ in either saline-infused or dexamethasone-treated fetuses (Table 1). In both groups, fetal acidemia developed during late hypoxemia and recovery and was associated with a reduction in base excess (Table 1). The magnitude of the acidemia was greater in fetuses exposed to hypoxemia after dexamethasone treatment than in control fetuses (Table 1).

**Plasma IR-ACTH and Cortisol During the Acute Hypoxemia Protocol**

During normoxia, maternal plasma IR-ACTH (58.5 ± 19.8 vs. 56.5 ± 10.9 pg/ml) and cortisol (29.4 ± 17.2 vs. 34.2 ± 8.4

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**Table 1. Fetal carotid blood gas and acid/base status during acute hypoxic challenge**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hypoxemia</th>
<th>Recovery</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N15</td>
<td>N45</td>
<td>H15</td>
</tr>
<tr>
<td>pHₐ</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>7.34±0.02</td>
<td>7.34±0.02</td>
<td>7.32±0.01</td>
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<tr>
<td>After dexamethasone</td>
<td>7.33±0.02</td>
<td>7.32±0.02</td>
<td>7.28±0.02</td>
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<tr>
<td>PₐCO₂, Torr</td>
<td></td>
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<tr>
<td>Saline</td>
<td>52.6±1.2</td>
<td>53.2±1.5</td>
<td>51.8±1.5</td>
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<tr>
<td>After dexamethasone</td>
<td>51.0±1.7</td>
<td>51.4±1.6</td>
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<tr>
<td>PₐO₂, Torr</td>
<td></td>
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<tr>
<td>Saline</td>
<td>22.2±0.5</td>
<td>22.8±0.9</td>
<td>12.0±0.1</td>
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<tr>
<td>After dexamethasone</td>
<td>24.0±0.8</td>
<td>24.4±1.1</td>
<td>12.6±0.4</td>
</tr>
<tr>
<td>ABE, meq/l</td>
<td></td>
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<tr>
<td>Saline</td>
<td>1.6±1.2</td>
<td>1±1.2</td>
<td>-0.4±1.3</td>
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<tr>
<td>After dexamethasone</td>
<td>-0.3±1.4</td>
<td>-0.6±1.2</td>
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</tr>
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</table>

Values are means ± SE at 15 (N15) and 45 (N45) min of normoxia, 15 (H15) and 45 (H45) min of hypoxemia, and 15 (R15) and 45 (R45) min of recovery for fetuses exposed to 1 h of hypoxemia after saline infusion (n = 6) or after dexamethasone treatment (n = 6). Fetal blood gas values were corrected to 39.5°C. pHₐ, arterial pH; PₐCO₂, arterial CO₂ partial pressure; PₐO₂, arterial O₂ partial pressure; ABE, base excess. Significant differences (P < 0.05); differences by post hoc analysis indicating a *significant main effect of time vs. normoxia; a **significant main effect of treatment vs. saline (two-way repeated-measures ANOVA + Tukey test).
ng/ml) concentrations were similar in the saline-infused and dexamethasone-treated groups. In both groups, maternal plasma IR-ACTH and cortisol concentrations remained unchanged from baseline values throughout the hypoxic protocol.

Changes in absolute values for fetal plasma IR-ACTH and cortisol concentrations during the acute hypoxic protocol and the correlation between fetal plasma IR-ACTH and cortisol concentrations during normoxia and hypoxemia for both treatment groups are shown in Fig. 2. In saline-infused fetuses, significant increases in plasma IR-ACTH and cortisol occurred during acute hypoxemia (Fig. 2A). In these fetuses, plasma IR-ACTH concentrations returned toward baseline during recovery, but plasma cortisol concentrations remained elevated at 45 min after the cessation of the hypoxic challenge. In dexamethasone-treated fetuses, normoxic baseline values for plasma cortisol, but not IR-ACTH, concentrations were significantly elevated at 48 h after cessation of the dexamethasone infusion compared with values measured in saline-infused fetuses (Fig. 2A). In these fetuses, fetal plasma concentrations of IR-ACTH were significantly elevated at 45 min of hypoxemia and during the recovery period compared with values measured in saline-infused controls (Fig. 2A). Despite increased fetal basal plasma cortisol concentrations after dexamethasone treatment, the magnitude of the increments during acute hypoxemia in circulating IR-ACTH concentration was greater, and that of cortisol concentration was at least as large, in fetuses preexposed to dexamethasone treatment as in the saline-infused fetuses (Fig. 2B). In both saline-infused and dexamethasone-treated fetuses, significant linear relationships were found between the logarithm of fetal plasma IR-ACTH concentrations and the corresponding plasma cortisol concentrations (saline: \( r = 0.543, P < 0.01 \); dexamethasone: \( r = 0.669, P < 0.01 \); Fig. 2C). Comparison of these linear relationships by the method of Armitage and Berry (3) confirmed that the intercepts (\( P < 0.05 \)), but not the gradients (\( P > 0.05 \)), of the linearized plots of the logarithm of the plasma IR-ACTH vs. cortisol concentrations were different between saline-infused fetuses and fetuses exposed to hypoxemia after dexamethasone treatment (Fig. 2C), suggesting enhanced cortisol output without a change in adrenal sensitivity to ACTH.

Ex Vivo Adrenal and Pituitary Measurements

Biometric and histological measurements for the adrenal glands in saline-infused and dexamethasone-treated fetuses are shown in Table 2. Dexamethasone treatment had no effect on any of the morphometric variables measured (Table 2).

Northern blot analyses for GR mRNA content in the pituitary and left adrenal glands of saline-infused and dexamethasone-treated fetuses are shown in Fig. 3. In saline-infused fetuses, the GR-to-β-actin (GR/β-actin) mRNA ratio in the
adrenal glands was approximately one-eighth of the values measured in the pituitary glands (Fig. 3C). Preexposure to dexamethasone for 48 h produced a significant reduction in both fetal pituitary and adrenal GR/β-actin mRNA ratios at 130 ± 1 days (Fig. 3, A and C).

**DISCUSSION**

The data reported in the present study show that 1) fetal treatment with dexamethasone transiently lowers fetal basal plasma cortisol, but not IR-ACTH, concentrations; 2) 2 days after the end of fetal treatment with dexamethasone, basal plasma cortisol is substantially elevated without any effect on basal plasma IR-ACTH concentrations; 3) 2 days after the end of fetal treatment with dexamethasone, the plasma IR-ACTH response to acute hypoxemia is enhanced, whereas the increment in plasma concentrations of cortisol is maintained; 4) correlation of fetal plasma IR-ACTH and cortisol concentrations during normoxia and hypoxemia after dexamethasone treatment indicates an upward parallel shift in the relationship, suggesting enhanced cortisol output without a change in adrenocortical ACTH sensitivity; and 5) the enhancements in basal plasma cortisol concentration and in the HPA axis responses to acute hypoxemia after the period of dexamethasone treatment are associated with reductions in pituitary and adrenal GR mRNA contents, which persist at 3–4 days after the end of treatment.

Clinical dosing regimens expose the fetus to initially high, but then rapidly decreasing, concentrations of synthetic steroid (4). In the current study, the fetuses were exposed to a continuous intravenous infusion of dexamethasone during the 48-h treatment period by use of a dosing regimen that is well characterized for studies in the ovine fetus (14, 18, 19). Fetal, as opposed to maternal, treatment with dexamethasone has been used in these studies to assess the direct effects of the synthetic glucocorticoids on fetal cardiovascular and endocrine variables, both under basal conditions and during acute hypoxemia. This approach minimizes the confounding influences of glucocorticoid-induced changes in maternal metabolic, endocrine, and cardiovascular variables and possible differences in transplacental passage of glucocorticoids between animals.

The purpose of the present study was to investigate possible maturational effects of synthetic glucocorticoids on basal HPA axis function as well as the response of the axis to a period of acute stress in the ovine fetus during late gestation. Previous studies, including our own, that have addressed aspects of these questions investigated the effects of fetal exposure to steroids during normoxia and hypoxemia after the period of dexamethasone treatment (1–3). Preexposure to dexamethasone transiently lowers fetal basal plasma cortisol concentrations, thereby allowing any maturational effects of the steroid on the capacity of the fetal HPA axis to respond to stress to be assessed without confounding effects of simultaneous negative feedback from the synthetic glucocorticoid. It is possible that the enhanced fetal basal plasma cortisol concentrations in the 48-h period after infusion are attributable, at least in part, to a “rebound” effect of the fetal HPA axis in response to removal of dexamethasone, rather than dexamethasone-mediated mater-
uation of the fetal HPA axis per se. However, a maturational effect of dexamethasone on the HPA axis is supported both by the findings of this study, in which the fetal plasma cortisol response to the superimposed acute hypoxemic challenge in the postinfusion period was enhanced (an effect not attributable to rebound), and the results of other reported studies. For example, Sloboda and colleagues (46, 47) found that prenatal synthetic glucocorticoid exposure produced modifications to the ovine HPA axis that were present even at 1 yr of postnatal life, suggesting persisting modification of HPA axis function (46).

The data reported in the present study on basal plasma IR-ACTH and cortisol concentrations are consistent with those of clinical studies in humans in which fetal or neonatal suppression of HPA axis function by synthetic glucocorticoids reversed after the cessation of treatment (20, 48, 49). Similarly, studies in sheep have demonstrated that cord plasma ACTH was higher, and plasma cortisol tended to be elevated, in fetuses near term whose mothers had previously been repeatedly injected with betamethasone at 104, 111, and 118 days of gestation (47). Other studies in sheep report that a single maternal intramuscular injection of dexamethasone (12 mg) suppresses both fetal plasma ACTH and cortisol concentrations by 24 h after treatment (4), whereas at 48 h of fetal intravenous betamethasone infusion, fetal plasma cortisol concentration is unaltered from baseline despite a reduction in fetal plasma ACTH concentration (2).

The few studies that have investigated the effect of fetal glucocorticoid exposure on stimulated HPA axis function in either the fetal or postnatal periods show conflicting results. Although maternal antenatal dexamethasone treatment in rhesus monkeys at 132–133 dGA (term is ~165 dGA) elevated postisolation-stress plasma cortisol concentrations compared with controls at 9 mo of postnatal age (50), Dodic et al. (15) demonstrated that treatment of pregnant ewes with intramuscular injections of dexamethasone at 1 or 2 mo of gestation had no effect on basal or ACTH-stimulated increases in plasma cortisol concentration in offspring at 4, 10, and 19 mo of postnatal age. Similarly, an intramuscular injection of dexamethasone (100 μg·kg⁻¹·day⁻¹) in pregnant dams during the last one-third of gestation resulted in offspring that had elevated basal plasma corticosterone concentrations but similar additional increments in plasma corticosterone concentrations in response to restraint, compared with controls at 16 wk of postnatal age (28). Investigations by Sloboda et al. (46) reported that prenatal exposure to glucocorticoids could have differential effects on postnatal basal and stimulated HPA axis function according to the time of gestation when the steroid was administered and whether it was given directly into the fetal or the maternal compartment. At 6 mo postnatal age, neither maternal nor fetal prenatal betamethasone administration altered significantly the ACTH and cortisol responses to exogenous CRH + AVP. However, in animals at 1 yr of postnatal age, a previous single maternal injection of betamethasone resulted in significantly elevated basal and stimulated cortisol levels, without significant change in the ACTH response. In contrast, betamethasone administration to the fetus resulted in significantly attenuated ACTH responses to CRH + AVP at 1 yr compared with control animals, but these were not associated with any significant changes in basal or stimulated cortisol levels. The effects of differences in species, glucocorticoid dose, and route of administration may account, at least in part, for the varying observations reported in these studies.

In addition to suppressing HPA axis activity via glucocorticoid-mediated negative feedback, dexamethasone and other glucocorticoids, such as cortisol, may exert maturational or modifying effects, particularly if administered for prolonged periods, on components of the axis via several mechanisms. These effects include changes at the level of the fetal adrenal cortex (12), including maturation of adrenal steroidogenic pathways (10); changes in responsiveness to ACTH (6, 7, 17, 30, 36, 40); changes in the gain of neural influences on the adrenal cortex (16, 22, 38); and a shift in the ratio of bioactive to immunoreactive ACTH produced by the pituitary under basal and/or stressful conditions (8, 9, 57). Alternatively, glucocorticoids may affect mechanisms that regulate local tissue availability of biologically active cortisol in the HPA axis, thereby modulating the gain of glucocorticoid-mediated negative feedback, such as the activities of placental and target-tissue 11β-hydroxysteroid dehydrogenase (11β-HSD), corticosteroid-binding globulin in plasma, and the population of GR in the HPA axis (31, 34, 44, 47, 55). To address some of the mechanisms mediating changes in basal and stimulated fetal HPA axis function produced by dexamethasone treatment, the present study 1) correlated fetal plasma IR-ACTH and cortisol concentrations during normoxic and hypoxemic conditions; 2) determined fetal pituitary and adrenal GR mRNA levels as markers of possible changes in glucocorticoid-mediated negative feedback of the HPA axis at the level of either the pituitary or the adrenal glands; and 3) examined the gross histology of the adrenal gland by light microscopy.

Correlation of fetal plasma IR-ACTH and cortisol concentrations during normoxia and hypoxemia after dexamethasone treatment produced parallel upward shifts in the plasma IR-ACTH vs. cortisol plots, suggesting enhanced cortisol output without a change in adrenocortical sensitivity to ACTH. Other results of the present study show no effect of dexamethasone on adrenocortical mass. Therefore, any maturational effects on steroidogenic capacity of dexamethasone could be mediated via effects on the activities of adrenal steroidogenic enzymes (10, 11, 39, 45). Furthermore, Ross et al. (42) showed that premature elevations in circulating cortisol, before the normal prepartum surge in fetal cortisol, suppressed the expression of 11β-HSD type 2 mRNA in the adrenal of the sheep fetus. Similar effects of dexamethasone on cortisol bioactivity within the fetal adrenal gland could enhance adrenocortical steroidogenic capacity.

In the current study, the rebound in basal fetal cortisol concentrations and in HPA axis responses to acute stress after the period of dexamethasone infusion were associated with reductions in pituitary and adrenal gland GR mRNA contents that persisted 3–4 days after the end of dexamethasone treatment. Assuming corresponding reductions in the levels of biologically active GR proteins, this provides a mechanism to account for enhanced fetal HPA activity after the end of the dexamethasone infusion period. These findings are consistent with those of previous investigators who have demonstrated both ontogenic and glucocorticoid-induced changes in neonatal and adult HPA axis GR mRNA expression (32, 34, 43, 44, 56). Indeed, the studies of Levitt et al. (28) reported that intramuscular injection of dexamethasone in pregnant dams during the last one-third of gestation resulted in offspring that had ele-
vated basal plasma corticosterone concentrations, similar additional increments in plasma corticosterone concentrations in response to restraint, and suppressed hippocampal GR mRNA and mineralocorticoid mRNA levels. These investigators also proposed that resulting reductions in glucocorticoid-mediated negative feedback could have contributed to the elevated basal corticosterone concentrations and to the maintained magnitude of the stress response (28). Furthermore, when fetal rats were exposed to increased levels of maternally derived corticosterone by inhibiting 11B-HSD activity with carbonoxolone administered daily throughout gestation, the adult offspring had elevated basal corticosterone concentrations and reduced GR mRNA content in the paraventricular nucleus, but no alteration in hippocampal GR or mineralocorticoid receptor mRNA, compared with control animals (51). In contrast, Sloboda et al. (47) reported that treatment of pregnant ewes with repeated betamethasone injections at 104, 111, and 118 days of gestation resulted in significant elevations in fetal plasma ACTH concentrations and increased pars distalis GR mRNA levels in pituitaries of fetuses near term. Finally, Dean and Matthews (13) demonstrated that alteration of fetal guinea pig hippocampal GR mRNA 24 h after maternal antenatal dexamethasone treatment was sex specific, as female fetuses had elevated plasma cortisol concentrations and elevated hippocampal GR mRNA levels compared with males and vehicle-treated controls. In the present study, there were equal proportions of male and female fetuses in control and dexamethasone-treated fetuses, but sample sizes were too small to distinguish any effects of the sex of the fetus on GR mRNA expression resulting from the dexamethasone treatment.

The implications of the observed changes in basal and stimulated HPA axis function during (18) and following (present study) the period of dexamethasone treatment remain unclear. Activation of the fetal HPA axis, with associated increases in plasma ACTH and cortisol concentrations, is a well-characterized and integral part of the fetal defense response to episodes of acute hypoxemia (1, 5, 22). In the present study, fetal plasma dexamethasone concentrations during infusion (1.91 ± 0.18 nmol/l; means ± SE during the 48-h infusion regimen for all treated fetuses) were approximately one-fifth of the mean value measured in umbilical arterial blood samples taken from human infants at cesarean section 12 h after the completion of a course of maternal antenatal glucocorticoid treatment (5 mg dexamethasone im every 12 h for 48 h) (27). Therefore, it could be argued that these doses of dexamethasone are of clinical relevance and that, although antenatal glucocorticoid therapy may mature the fetal endocrine responses to the acute hypoxemia associated with labor and delivery, only fetuses delivered after the course of glucocorticoid exposure will experience the full benefit of the maturational effect of the steroid.

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