

Pituitary-Adrenal Responses to Acute Hypoxemia During and After Maternal Dexamethasone Treatment in Sheep

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ABSTRACT

The effects of maternal dexamethasone treatment on hypothalamic-pituitary-adrenal axis function were determined during basal and hypoxemic conditions in maternal and fetal sheep. Under halothane, ewes and their fetuses were catheterized at 117 d gestation (term = 145 d). Starting at 124 d, the ewes received i.m. injections of two doses of either dexamethasone (12 mg) or saline at 24-h intervals. All animals experienced one episode of hypoxemia when the dexamethasone was present in the maternal and fetal circulations [125 ± 1 d (H1)] and a second episode of hypoxemia when the steroid was no longer detectable in either the maternal or fetal circulations [128 ± 1 d (H2)]. The fall in partial pressure of oxygen in arterial blood in response to hypoxia was similar in the two episodes in both the fetal and the maternal blood. Maternal dexamethasone treatment diminished maternal and fetal basal plasma cortisol but not ACTH during the normoxic period of H1 but not H2. In control animals, hypoxemia induced increases in fetal but not maternal ACTH and cortisol concentrations. In dexamethasone-treated animals, ma-

ternal ACTH and cortisol concentrations also remained unchanged from baseline in both H1 and H2. In contrast, fetal plasma ACTH and cortisol responses to hypoxemia were significantly suppressed during H1 but not H2. Correlation of fetal plasma ACTH and cortisol concentrations suggested diminished cortisol output without a change in adrenocortical responsiveness in dexamethasone-treated fetuses during H1 but not H2. Maternal treatment with dexamethasone transiently suppressed maternal and fetal basal hypothalamic-pituitary-adrenal axis function and the fetal plasma ACTH and cortisol responses to acute hypoxemia in sheep. (*Pediatr Res* 56: 864–872, 2004)

Abbreviations

CBG, corticosteroid-binding globulin
dGA, days of gestational age
HPA, hypothalamo-pituitary-adrenal
POMC, pro-opiomelanocortin

Since the pioneering discovery of Liggins (1) that glucocorticoids are essential for the maturation of fetal systems in preparation for extrauterine life, synthetic glucocorticoids, such as dexamethasone, have been used routinely in human clinical practice to treat pregnant women who are at risk of preterm delivery (2–4). Such prophylactic treatment mimics the normal parturition increase in endogenous cortisol output by the fetal adrenal that switches tissue accretion to differentiation (5). Antenatal glucocorticoid treatment has resulted in significant decreases in the incidence of respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, and neonatal mortality associated with preterm birth (3,6).

Despite the clear benefits of antenatal glucocorticoid therapy, concerns have been expressed about the safety of the current dosing regimen, in particular with regards to maternal and fetal adrenal suppression resulting from negative feedback by the synthetic steroid (4). In addition, the effects of synthetic glucocorticoids on the fetal defense responses to hypoxemic insults have been identified by the National Institutes of Health in the United States as an area of research needed to guide intrapartum clinical care (4). This is important for two reasons. First, the ovine hypothalamic-pituitary-adrenal (HPA) axis is activated in response to acute hypoxemia in the mother and the fetus and plays an integral role in the fetal defense response to acute hypoxemia (7). Second, episodes of acute hypoxemia are common during pregnancy, in particular during labor and delivery (8). Because antenatal glucocorticoids are given to pregnant women who are most at risk of preterm delivery, it is likely that delivery and therefore episodes of acute hypoxemia will occur either during or shortly after maternal steroid treatment.

Received October 30, 2003; accepted April 1, 2004.

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This work was supported by Tommy's—the Baby Charity, UK.

J.K.J. is currently a fellow of the New Zealand Foundation for Research and Technology, and D.A.G. is currently a Fellow of the Lister Institute for Preventive Medicine, UK.

DOI: 10.1203/01.PDR.0000145253.92052.60

Previous studies in the ovine fetus during late gestation suggested that the timing of an episode of acute hypoxemia relative to antenatal treatment with glucocorticoids might determine the balance between the maturational and the suppressive effects of these steroids on the fetal HPA axis (9). Direct infusion of the ovine fetus with low doses of dexamethasone during late gestation has been shown to suppress pituitary-adrenal responses to an episode of acute hypoxemia that occurred during treatment (10). However, a similar hypoxemic challenge that occurred 2 d after treatment, in the absence of glucocorticoid negative feedback, resulted in enhanced adrenal cortisol output without alterations in adrenal cortical sensitivity to ACTH (9). However, in clinical practice, synthetic glucocorticoids are administered to the mother and not to the fetus. In addition, in contrast to the effect of antenatal glucocorticoid therapy on the fetal HPA axis, little attention has been paid to the effects of this treatment on maternal pituitary-adrenal responses. Hence, this study determined the effects of maternal treatment with dexamethasone in doses and dose intervals used in human clinical practice on both maternal and fetal pituitary-adrenal functions during basal conditions and in response to acute hypoxemia in sheep.

METHODS

Surgical Preparation

Seventeen Welsh Mountain ewes that were carrying fetuses of known gestational age were used in the study. All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. All food but not water was withdrawn from the animals for 24 h before surgery.

Surgery was performed under aseptic conditions at 117 ± 1 d of gestation (dGA; term is ~ 145 dGA). Anesthesia was induced with sodium thiopentone (20 mg/kg i.v. Intraval Sodium; Rhone Mérieux, Dublin, Ireland) and maintained with 1 to 2% halothane in 50:50 O₂/N₂O. In brief, after abdominal and uterine incisions, the fetal head was exteriorized for insertion of a catheter (i.d. 0.86 mm, o.d. 1.52 mm; Critchly Electrical Products, NSW, Australia) into a carotid artery with the tip of the catheter extended to the ascending aorta. The catheter was filled with heparinized saline (100 i.u. heparin/ml in 0.9% NaCl) and plugged with a brass pin, and the uterine incision was closed in layers. A Teflon catheter was inserted into the maternal femoral artery and advanced into the descending aorta. A third catheter was placed in the amniotic cavity. Antibiotics were administered to the fetus through the carotid artery (300 mg of ampicillin; Penbritin, SmithKline-Beecham Animal Health, Surrey, UK) and into the amniotic cavity (300 mg of ampicillin). All catheters were exteriorized through a keyhole incision in the maternal flank and housed in a pouch sutured to the mother's skin.

Postoperative Care

Animals were housed in individual pens with access to hay and water *ad libitum*. Concentrates were fed twice daily (100 g; Sheep Nuts #6; H&C Beart Ltd., Kings Lynn, UK). All ewes received antibiotics (0.20–0.25 mg/kg i.m. Depocillin; Myco-

farm, Cambridge, UK) immediately after surgery and daily for 3 d. Patency of fetal vascular catheters was maintained by a slow continuous infusion of heparinized saline (100 i.u. heparin/ml at 0.1 mL/h in 0.9% NaCl) containing antibiotic (10 mg/ml of benzylpenicillin; Crystapen, Schering-Plough, Animal Health Division, Welwyn Garden City, UK).

Experimental Procedures

At least 5 d after surgery, at 122 ± 1 dGA, the animals were divided randomly into two experimental groups. Ten ewes received 2×12 -mg injections of dexamethasone (dexamethasone sodium phosphate; Merck Sharpe, Dohme Ltd., Herts, UK) in 2 mL of saline (0.9% NaCl) 24 h apart. Seven ewes received two injections of 2 mL of saline 24 h apart to serve as age-matched sham controls.

All ewes and their fetuses were subjected to two separate episodes of acute hypoxemia, induced by reducing the maternal fraction of inspired oxygen, at 125 ± 1 dGA (8 h after the second maternal injection; hypoxemia 1 (H1)) and again at 128 ± 1 dGA [3 d after the second dexamethasone injection; hypoxemia 2 (H2)]. The rationale for the first episode of acute hypoxemia was to determine whether HPA axis responses to acute stress were affected when dexamethasone was present in the maternal and fetal circulations. The acute hypoxemic challenge was performed 8 h after the second injection at a time when fetal plasma concentrations of dexamethasone were similar to those measured during direct fetal treatment (10). The rationale for the second episode of acute hypoxemia was to determine the long-term effects of glucocorticoid exposure after the dexamethasone had cleared from both the maternal and the fetal circulations. The protocol for acute hypoxemia involved a 3-h experiment consisting of 1 h of normoxia, 1 h of hypoxemia, and 1 h of recovery. In brief, for 1 h, air was passed at a rate of ~ 40 L/min into a large, transparent, polythene bag placed over the ewes' head. After this control period, maternal and fetal hypoxemia was induced by changing the concentrations of gases breathed by the ewe to 9% O₂ in N₂ with 2 to 3% CO₂. This mixture was designed to reduce maternal and fetal arterial partial pressure of oxygen in arterial blood to ~ 35 and 12 mm Hg, respectively, while maintaining arterial isocapnia. After the 1-h period of hypoxemia, the ewe was returned to breathing air for the 1-h recovery period.

Measurements and Biochemical Analyses

During the hypoxemia protocols, arterial blood samples (4 mL) were collected at 15 and 45 min of normoxia, after 15 and 45 min of hypoxemia, and after 45 min of recovery. The descending aortic blood samples (0.4 mL) were drawn into sterile syringes and analyzed for measurement of arterial blood gases, percent saturation of O₂ in Hb, Hb concentration, and acid/base status 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. All blood samples for hormone analysis were collected into K⁺/EDTA-treated tubes, kept on ice, and centrifuged at 4000 rpm for 4 min at 4°C. Plasma samples were stored at -70°C until analyses.

ACTH

Maternal and fetal plasma ACTH concentrations were measured using a commercially available double antibody ^{125}I RIA kit (Incstar Ltd, Wokingham, UK). The lower limit of detection for the assay was between 10 and 25 pg/mL. The intra-assay coefficients of variation for two plasma pools (37 and 150 pg/ml) were 3.6 and 4.1%, respectively. The interassay coefficient of variation was 8.4%. The cross-reactivities for the assay were <0.01% for α -MSH, β -endorphin, β -lipotrophin, leucine enkephalin, methionine enkephalin, bombesin, calcitonin, PTH, FSH, AVP, oxytocin, and substance P.

CORTISOL

Maternal and fetal plasma cortisol concentrations were measured by RIA validated for use in ovine plasma, as described previously (11). The lower limit of detection for the assay was 1.0–1.5 ng/ml. The intra- and interassay coefficients of variations were 5.3 and 7.8%, respectively. The cross-reactivities of the antiserum at 50% binding with other cortisol-related compounds were 0.5% cortisone, 2.3% corticosterone, 0.3% progesterone, and 4.6% deoxycortisol.

DEXAMETHASONE

Plasma dexamethasone levels were measured after ether extraction using tritium-labeled dexamethasone as tracer, as described previously (10). All values were corrected for recovery (86%). The interassay coefficients of variation 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 antidexamethasone antiserum showed a 1.6% cross-reactivity against cortisol and cross-reactivities of <0.5% against 11-deoxycortisol, corticosterone, testosterone, progesterone, and estriol.

STATISTICAL ANALYSES

Values for all variables are expressed as means \pm SEM. All measured variables were first analyzed for normality of distribution. All data obtained were parametric and were analyzed using two-way ANOVA with repeated measures (Sigma Chemical Co.-Stat; SPSS Inc, Chicago, IL) followed by an appropriate *post hoc* test. A comparison between the slopes and intercepts of regression curves was conducted according to Armitage and Berry (12). For all comparisons, statistical significance was accepted at $p < 0.05$.

RESULTS

Dexamethasone concentrations. In saline-injected ewes and their fetuses, dexamethasone immunoreactivity was undetectable in the circulation throughout the experiment. In contrast, plasma concentrations of dexamethasone were significantly elevated in dexamethasone-injected ewes (6.83 ± 0.20 nmol/L; $n = 6$) and their fetuses (3.83 ± 0.32 nmol/L; $n = 6$) during H1. No dexamethasone could be detected in either the maternal or the fetal circulations at the time of H2.

Blood gas and metabolic status. Values for maternal basal arterial pH, Pco_2 , Po_2 , percentage saturation of Hb, percentage hematocrit, and concentrations of Hb were similar in saline- and dexamethasone-treated ewes during H1 and H2 (Table 1). Values for fetal arterial pH, Pco_2 , Po_2 , and percentage saturation of Hb were similar in saline- and dexamethasone-treated fetuses during H1 and H2 (Table 2). Whereas fetal basal concentrations of Hb and percentage hematocrit were similar in saline- and dexamethasone-treated fetuses during H1, both of these were significantly decreased in dexamethasone-treated fetuses during the basal period of H2 (Table 2).

In the mother, acute hypoxemia induced significant reductions in maternal arterial Po_2 and percentage saturation of Hb and significant increases in plasma concentrations of Hb during both H1 and H2 (Table 1). These alterations occurred without significant changes in maternal arterial pH, Pco_2 , or percentage hematocrit. During recovery, a reduction from baseline occurred in maternal arterial Pco_2 , and maternal arterial Po_2 tended to shoot above prehypoxemic levels. All other variables returned toward baseline values (Table 1).

In the fetus, acute hypoxemia induced significant reductions in arterial Po_2 and percentage saturation of Hb, with significant increases in plasma concentrations of Hb, but without alterations in arterial Pco_2 or hematocrit during H1 and H2 (Tables 1 and 2). Hypoxemia elicited mild progressive acidemia in all fetuses. The magnitude of the fetal acidemic response to acute hypoxemia was significantly greater in dexamethasone compared with saline groups during H1 but not H2. Whereas fetal arterial pH remained lower than baseline, all other variables returned toward baseline during recovery (Table 2).

ACTH and cortisol. In dexamethasone animals, basal plasma concentrations of ACTH tended to be lower in the maternal and fetal circulations during H1; however, these differences did not reach statistical significance. During H2, basal plasma concentrations of ACTH were not significantly different in saline or dexamethasone groups in either the maternal or the fetal circulations. Conversely, both maternal and fetal plasma concentrations of cortisol were significantly lower during the basal period of H1 but not of H2 in dexamethasone-compared with saline-treated animals (Figs. 1 and 2).

During the acute hypoxemic periods of H1 and H2, there were no significant alterations from baseline in the maternal plasma concentrations of ACTH or cortisol in any animal (Fig. 1). In contrast, in the saline fetuses, fetal plasma concentrations of ACTH and cortisol were significantly increased for the duration of both hypoxemic challenges and remained elevated during the recovery periods. In dexamethasone-treated fetuses, there was no hypoxemia-induced increase in plasma ACTH or cortisol during H1. However, during H2, the ACTH and cortisol responses had returned and were similar in magnitude to those seen in saline-treated fetuses (Figs. 1 and 2).

When the relationship between the fetal plasma ACTH and cortisol concentrations was analyzed for each of the two episodes of acute hypoxemia, the intercept but not the slope of the relationship was significantly lower in dexamethasone than saline fetuses during H1 (Fig. 3A). No differences in the relationship between fetal plasma ACTH and cortisol concentrations were observed during H2 (Fig. 3B). The relationship

Table 1. Arterial blood gas and metabolic status during H1 (A) and H2 (B) in saline- and dexamethasone-treated ewes

	Baseline		Hypoxemia		Recovery	
	N15	N45	H15	H45	R15	R45
H1						
pH _a						
Saline	7.50 ± 0.01	7.50 ± 0.01	7.49 ± 0.02	7.49 ± 0.01	7.50 ± 0.02	7.48 ± 0.01
Dexamethasone	7.53 ± 0.02	7.52 ± 0.01	7.52 ± 0.02	7.53 ± 0.02	7.53 ± 0.02	7.51 ± 0.02
Paco ₂ (mm Hg)						
Saline	34 ± 1	34 ± 1	35 ± 2	35 ± 2	32 ± 1*	35 ± 1
Dexamethasone	36 ± 2	37 ± 1	36 ± 1	36 ± 1	33 ± 1*	36 ± 1
Pao ₂ (mm Hg)						
Saline	97 ± 2	101 ± 2	37 ± 2*	37 ± 2*	104 ± 2	100 ± 2
Dexamethasone	96 ± 1	96 ± 2	35 ± 1*	35 ± 1*	100 ± 2	97 ± 2
SatHb (%)						
Saline	98.8 ± 0.5	99.0 ± 0.5	60.3 ± 4.9*	59.4 ± 3.9*	99.3 ± 1.6	100.0 ± 0.9
Dexamethasone	96.0 ± 1.2	95.3 ± 1.2	54.5 ± 4.1*	53.5 ± 3.4*	93.6 ± 2.7	95.0 ± 1.8
[Hb] (g/dL)						
Saline	8.7 ± 0.3	8.8 ± 0.4	10.3 ± 0.6*	10.2 ± 0.4*	9.3 ± 0.4	9.2 ± 0.5
Dexamethasone	9.4 ± 0.4	9.1 ± 0.4	11.0 ± 0.4*	10.7 ± 0.5*	9.8 ± 0.5	9.4 ± 0.5
Hct (%)						
Saline	27 ± 1	27 ± 1	28 ± 1	29 ± 1	28 ± 1	28 ± 1
Dexamethasone	29 ± 1	28 ± 1	30 ± 1	30 ± 1	29 ± 1	28 ± 1
H2						
pH _a						
Saline	7.49 ± 0.01	7.49 ± 0.01	7.49 ± 0.01	7.50 ± 0.01	7.52 ± 0.01	7.48 ± 0.01
Dexamethasone	7.51 ± 0.01	7.50 ± 0.01	7.51 ± 0.01	7.49 ± 0.01	7.53 ± 0.01	7.48 ± 0.01
Paco ₂ (mm Hg)						
Saline	37 ± 1	35 ± 1	36 ± 2	36 ± 1	31 ± 1*	35 ± 1
Dexamethasone	35 ± 1	32 ± 1	36 ± 1	37 ± 1	33 ± 2	36 ± 1
Pao ₂ (mm Hg)						
Saline	97 ± 2	99 ± 1	38 ± 2*	37 ± 3*	105 ± 1*	100 ± 1
Dexamethasone	98 ± 2	98 ± 3	35 ± 1*	35 ± 1*	104 ± 2*	97 ± 2
SatHb (%)						
Saline	96.5 ± 1.6	96.4 ± 1.7	57.4 ± 3.8*	57.2 ± 5.6*	98.2 ± 1.8	96.7 ± 1.9
Dexamethasone	95.5 ± 1.2	95.4 ± 1.4	54.2 ± 3.3*	52.5 ± 3.0*	96.3 ± 1.4	94.7 ± 1.3
[Hb] (g/dL)						
Saline	8.7 ± 0.3	8.5 ± 0.2	10.1 ± 0.4*	10.3 ± 0.4*	8.8 ± 0.1	8.8 ± 0.2
Dexamethasone	8.7 ± 0.5	8.1 ± 0.5	10.1 ± 0.8*	10.0 ± 0.6*	8.4 ± 0.5	8.5 ± 0.5
Hct (%)						
Saline	26 ± 1	26 ± 1	28 ± 1	29 ± 1	26 ± 1	26 ± 1
Dexamethasone	26 ± 1	26 ± 1	28 ± 2	29 ± 2	26 ± 2	26 ± 1

Values are means ± S.E.M. for 7 saline and 10 dexamethasone treated ewes. Arterial blood samples were taken at 15 and 45 minutes of normoxia (N), hypoxemia (H) and recovery (R). Statistical differences (P < 0.05) are: *versus normoxia (Two Way repeated measures (RM) ANOVA + Tukey test). Blood gas values were corrected to 38°C. pH_a, arterial pH; SatHb, saturation of hemoglobin; [Hb], concentration of hemoglobin; Hct, hematocrit.

between maternal ACTH and cortisol concentrations during acute hypoxemia could not be analyzed because of the narrow range of maternal ACTH values.

DISCUSSION

This study showed that maternal administration of dexamethasone in doses and dose intervals used in human clinical practice transiently depressed basal cortisol concentrations in both the mother and the fetus without an apparent effect on basal ACTH concentrations. In control animals, acute hypoxemia induced significant increases in fetal but not maternal ACTH and cortisol concentrations. In contrast, fetal plasma ACTH and cortisol responses to acute hypoxemia were significantly suppressed when the dexamethasone was present in the fetal circulation but not 3 d later, when the dexamethasone had cleared from the maternal and the fetal circulations. The relationship between fetal plasma ACTH and cortisol concentrations showed that cortisol output was diminished without an

apparent change in adrenocortical ACTH sensitivity when dexamethasone was present in the fetal circulation.

Dexamethasone concentrations. The plasma concentrations of dexamethasone measured in fetal sheep were similar to values measured in human umbilical cord blood within 24 h of a course of antenatal glucocorticoid therapy (13,14). Although maternal injection with dexamethasone would have exposed the ewe and the fetus to initially high then rapidly decreasing concentrations of synthetic glucocorticoid, the plasma concentrations in maternal and fetal blood at the onset of H1 were similar to those reported previously 8 h after injection in the pregnant ewe and the fetus (15) and to those reported in the fetus after 24 h of direct i.v. infusion of the fetus with low doses of dexamethasone (10).

Basal pituitary-adrenal function. Maternal dexamethasone transiently depressed both maternal and fetal plasma concentrations of cortisol in the present study without any apparent alteration in plasma ACTH concentrations, consistent with

Table 2. Arterial blood gas and metabolic status during H1 and H2 in saline- and dexamethasone-treated fetuses

	Baseline		Hypoxemia		Recovery	
	N15	N45	H15	H45	R15	R45
H1						
pH _a						
Saline	7.36 ± 0.01	7.35 ± 0.01	7.32 ± 0.02	7.28 ± 0.03*	7.24 ± 0.02*	7.28 ± 0.02*
Dexamethasone	7.32 ± 0.01	7.31 ± 0.01	7.28 ± 0.02	7.18 ± 0.02*†	7.13 ± 0.02*†	7.20 ± 0.02*†
Paco ₂ (mm Hg)						
Saline	51 ± 1	51 ± 1	51 ± 2	49 ± 2	48 ± 1	49 ± 1
Dexamethasone	50 ± 2	51 ± 1	50 ± 1	50 ± 2	47 ± 2	47 ± 1
Pao ₂ (mm Hg)						
Saline	23 ± 1	23 ± 1	13 ± 1*	12 ± 1*	26 ± 1	24 ± 1
Dexamethasone	24 ± 2	24 ± 2	12 ± 1*	12 ± 1*	29 ± 2	25 ± 2
SatHb (%)						
Saline	61.8 ± 3.8	59.4 ± 3.7	24.6 ± 2.3*	22.8 ± 2.0*	60.5 ± 3.4	59.0 ± 3.5
Dexamethasone	62.3 ± 3.3	63.1 ± 3.3	27.7 ± 2.8*	25.8 ± 2.7*	64.1 ± 3.6	61.8 ± 3.5
[Hb] (g/dL)						
Saline	10.0 ± 0.4	9.8 ± 0.3	10.8 ± 0.4*	10.6 ± 0.3*	9.5 ± 0.4	9.7 ± 0.4
Dexamethasone	9.5 ± 0.3	9.6 ± 0.3	10.5 ± 0.3*	10.2 ± 0.3*	9.5 ± 0.4	9.0 ± 0.3
Hct (%)						
Saline	32 ± 2	32 ± 2	33 ± 2	34 ± 2	32 ± 2	31 ± 1
Dexamethasone	31 ± 1	31 ± 1	33 ± 1	33 ± 1	30 ± 1	30 ± 1
H2						
pH _a						
Saline	7.36 ± 0.01	7.36 ± 0.01	7.31 ± 0.01	7.25 ± 0.02*	7.21 ± 0.03*	7.27 ± 0.04*
Dexamethasone	7.33 ± 0.02	7.33 ± 0.02	7.28 ± 0.03	7.20 ± 0.04*	7.15 ± 0.04*	7.21 ± 0.03*
Paco ₂ (mm Hg)						
Saline	52 ± 1	52 ± 1	52 ± 2	51 ± 1	50 ± 1	50 ± 1
Dexamethasone	52 ± 1	52 ± 1	53 ± 1	52 ± 1	48 ± 2	50 ± 1
Pao ₂ (mm Hg)						
Saline	22 ± 1	23 ± 1	12 ± 1*	12 ± 1*	26 ± 2	24 ± 2
Dexamethasone	23 ± 2	23 ± 1	12 ± 1*	13 ± 1*	26 ± 2	24 ± 2
SatHb (%)						
Saline	61.33 ± 2.4	63.0 ± 3.2	26.9 ± 1.9*	24.2 ± 2.2*	61.7 ± 3.0	56.2 ± 4.1
Dexamethasone	62.8 ± 2.7	57.9 ± 1.7	26.2 ± 2.3*	24.6 ± 2.4*	57.9 ± 2.4	55.4 ± 4.3
[Hb] (g/dL)						
Saline	9.9 ± 0.5	9.9 ± 0.6	10.8 ± 0.6*	10.7 ± 0.5*	9.4 ± 0.5	9.4 ± 0.5
Dexamethasone	7.4 ± 0.6†	7.3 ± 0.5†	7.9 ± 0.6†	8.0 ± 0.6*†	7.1 ± 0.5†	7.0 ± 0.4†
Hct (%)						
Saline	31 ± 2	31 ± 2	33 ± 2	33 ± 2	31 ± 2	31 ± 2
Dexamethasone	26 ± 1†	26 ± 1†	27 ± 2†	28 ± 2†	26 ± 1†	25 ± 2†

Values are means ± S.E.M. for 7 saline and 10 dexamethasone treated ewes. Arterial blood samples were taken at 15 and 45 minutes of normoxia (N), hypoxemia (H) and recovery (R). Statistical differences ($P < 0.05$) are: *versus normoxia and †versus saline (Two Way repeated measures (RM) ANOVA + Tukey test). Blood gas values were corrected to 39.5°C. pH_a, arterial pH; SatHb, saturation of hemoglobin; [Hb], concentration of hemoglobin; Hct, hematocrit.

previous studies of maternal (15) and fetal (10) treatment with synthetic glucocorticoids. The anterior pituitary and hypothalamus contain glucocorticoid receptors (16) and are responsive to cortisol negative feedback during late gestation under basal (17,18) and stimulated conditions (1,19). Because dexamethasone has 7-fold greater affinity for glucocorticoid receptors than cortisol (2), its actions on hypothalamic and pituitary glucocorticoid receptors may account for potent feedback inhibition of maternal and fetal basal plasma concentrations of cortisol in this study.

At least two mechanisms may explain the lack of significant differences in basal plasma concentrations of ACTH. First, the tendency of ACTH to be lower, despite falling outside statistical significance, may be sufficient to induce significant suppression of endogenous adrenocortical glucocorticoid output in the mother and the fetus. Second, in the fetus, dexamethasone may alter pro-opiomelanocortin (POMC) processing and the ratio of biologic:immunologic ACTH released from the ante-

rior pituitary. Both precursors and ACTH are present in (20,21) and secreted from (22,23) the ovine fetal pituitary during late gestation. Changes in the ratio of bioactive to immunoreactive ACTH secreted by pituitary cells have been observed in response to cortisol both *in vivo* (24) and *in vitro* (25,26). In addition, the higher molecular weight precursors POMC and pro-ACTH have been shown to inhibit the cortisol response to ACTH in ovine fetal adrenal cells *in vitro* (22,27). Because the ACTH antibody used in this study binds to the 1- to 24-amino acid sequence common to POMC and pro-ACTH and its processed, bioactive form ACTH, the values in this study represent both bioactive ACTH and its higher molecular weight precursors.

Other aspects of HPA axis feedback regulation that may be altered by dexamethasone include an effect on corticosteroid-binding globulin (CBG). The presence of CBG maintains relatively low free cortisol concentrations close to term, and this has been suggested to reduce the degree of cortisol-

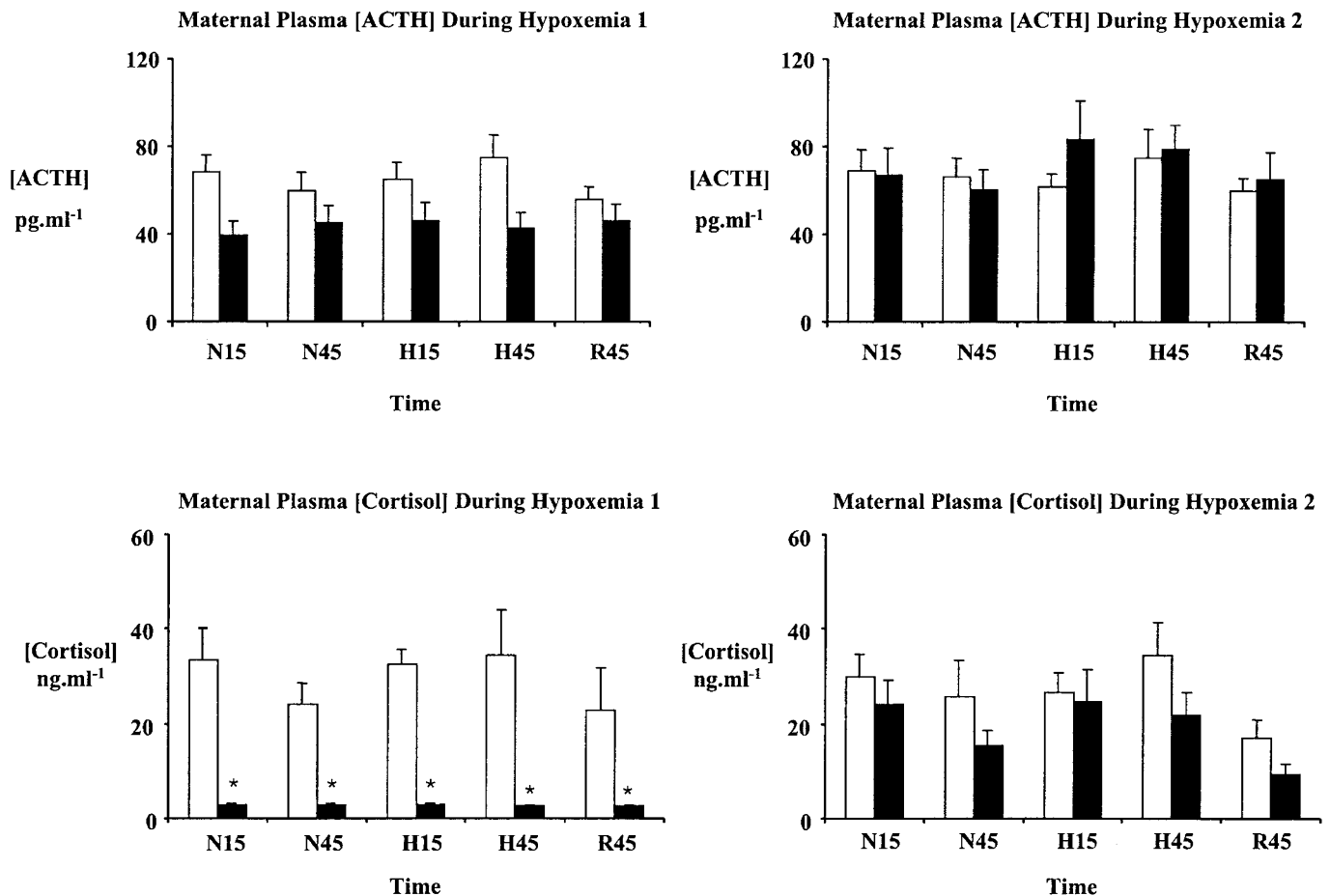


Figure 1. Plasma concentrations of ACTH and cortisol during H1 (A) and H2 (B) in saline- and dexamethasone-treated ewes. Values are means \pm SEM for seven saline-treated (\square) and 10 dexamethasone-treated (\blacksquare) ewes. Arterial blood samples were taken at 15 and 45 min of normoxia (N), hypoxemia (H), and recovery (R). * $p < 0.05$ vs saline (two-way repeated measures ANOVA + Tukey test).

mediated negative feedback on pituitary gland ACTH output (28). Cortisol stimulates an increase in CBG in the fetal circulation by altering expression in the fetal liver and modifying the extent of CBG glycosylation in a way that would reduce metabolic clearance (28). Sloboda *et al.* (29) demonstrated increased levels of plasma CBG in the umbilical cord of sheep at 7 d after the end of a course of repeated maternal betamethasone injections. Similar persistence of increased levels of CBG may contribute to the increase in fetal HPA axis in the period after dexamethasone treatment in the current study.

Pituitary-adrenal responses during acute hypoxemia. Hypoxemia-induced increases in fetal plasma concentrations of ACTH and cortisol, appropriate for the degree of hypoxemia and the gestational age of the fetus (10,30–33), were prevented by maternal dexamethasone, consistent with previous studies in fetal sheep (10,34,35). Both direct fetal i.v. infusion with dexamethasone (10) and dexamethasone implants near the paraventricular nucleus (36) abolished the ACTH and cortisol responses to acute hypoxemia in the ovine fetus during late gestation. Likewise, dexamethasone significantly suppressed systemic corticotrophin-releasing factor and AVP stimulated ACTH and cortisol responses in fetal sheep (35).

In dexamethasone animals in the present study, basal and stimulated plasma ACTH and cortisol concentrations were

similar to saline controls after the dexamethasone had cleared from the maternal and the fetal circulations, consistent with previous studies in lambs (37), adult sheep (38), and human neonates (39–41). Conversely, Fletcher *et al.* (9) reported not only restored but also enhanced ACTH responses to acute hypoxemia 2 d after cessation of direct fetal treatment with low doses of dexamethasone. Further studies of the HPA axis have shown a tendency toward hypoactivity at 1 y of age and hyperactivity of the axis at 3 y of age (42) in sheep. In the present study, a lack of significant differences in HPA function between dexamethasone and control fetuses 3 d after the second maternal injection therefore may represent simple withdrawal of glucocorticoid negative feedback or an insufficient period for manifestation of dexamethasone-induced maturation and/or resetting of the HPA axis.

Because significant reductions in arterial P_{O_2} occurred in both ewes and their fetuses, the lack of maternal HPA axis response is unlikely to reflect differences in the severity of the challenge but rather differences in maternal and fetal sensitivity to acute hypoxemia. In addition, although it is known that the ovine placenta is not permeable to ACTH (43) and that maternal cortisol is metabolized in the placenta by the enzyme 11 β -hydroxysteroid dehydrogenase (44), the absence of any significant alterations in maternal plasma ACTH and cortisol

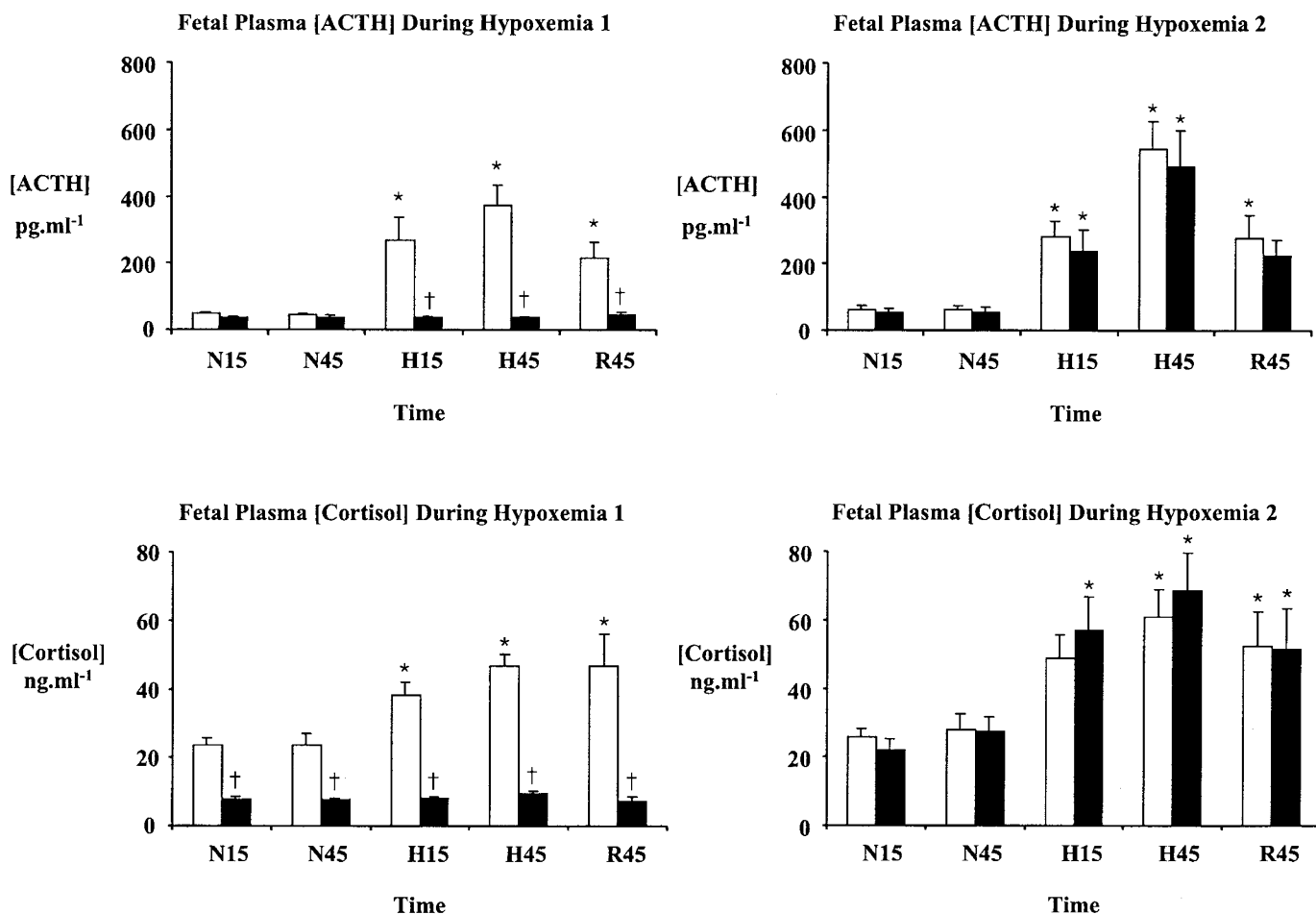


Figure 2. Plasma concentrations of ACTH and cortisol during H1 (A) and H2 (B) in saline- and dexamethasone-treated fetuses. Values are means \pm SEM for seven saline-treated (\square) and 10 dexamethasone-treated (\blacksquare) fetuses. Arterial blood samples were taken at 15 and 45 min of normoxia (N), hypoxemia (H), and recovery (R). * $p < 0.05$ vs normoxia and † $p < 0.05$ vs saline (two-way repeated measures ANOVA + Tukey test).

concentrations confirms the fetal origin of the alterations in ACTH and cortisol in response to acute hypoxemia in fetal plasma.

Previous studies have suggested a role for the splanchnic sympathetic nerves innervating the adrenal gland in the steroidogenic response to stress, because stimulation of the splanchnic nerves doubled and section of these nerves halved the increase in plasma concentrations of cortisol in response to exogenous ACTH in conscious, hypophysectomized calves (45,46) and lambs (47). Similarly, the increase in plasma concentrations of cortisol in response to acute hypoxemia were attenuated or completely abolished in carotid chemodenervated sheep (33) and llama (48) fetuses, respectively. Hence, Giusani *et al.* (33) suggested that in the fetus during late gestation, a reflex arc triggered by the carotid chemoreceptors and mediated by splanchnic nerve efferents may increase cortisol secretion either during acute hypoxemic stress directly and/or by enhancing the sensitivity of the adrenal cortex to circulating ACTH. In the present study, the lack of an increase in plasma cortisol in the absence of an increase in plasma ACTH during H1 does not support a direct role for the splanchnic nerves in inducing adrenocortical cortisol secretion. Rather, these data support the hypothesis that splanchnic nerve efferents may

increase cortisol secretion during acute stress by enhancing the sensitivity of the adrenal cortex to the prevailing plasma concentration of ACTH. Alternatively, dexamethasone-induced negative feedback at the level of the adrenal gland itself may suppress both neural and endocrine regulation of adrenal cortisol secretion.

Activation of the HPA axis is integral to the fetal defense response to acute hypoxemic stress, a physiologic challenge that is common during labor and delivery (8). In the present study, maternal administration of dexamethasone in doses and dose intervals used in human clinical practice significantly suppressed fetal HPA axis activation in response to an acute hypoxemic challenge that occurred during but not 3 d after treatment. The results of the present study therefore suggest that the timing of an episode of acute hypoxemia in relation to maternal administration of synthetic glucocorticoids may determine the capacity of the fetal pituitary-adrenal axis to respond to the challenge. Furthermore, an increase in plasma concentrations of cortisol is essential for mobilization of fuel reserves at birth to maintain a supply of oxidative substrates during the transition from parenteral to enteral nutrition (5). Suppression of the HPA axis at the time of delivery therefore may have adverse consequences for the immediate adaptation

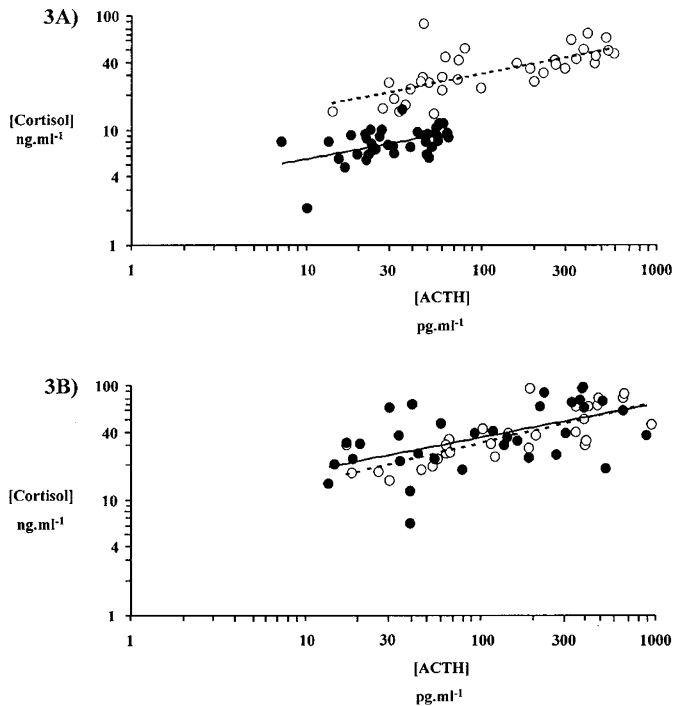


Figure 3. Linear regression of fetal plasma ACTH and cortisol concentrations during H1 (A) and H2 (B) in saline (○) and dexamethasone (●) treated fetuses. Data points are for all paired ACTH and cortisol samples in individual fetuses obtained during H1 (A) or H2 (B). There was a significant change in the intercept ($p < 0.05$) but not the slope of the relationship between plasma ACTH and cortisol in dexamethasone-treated fetuses during H1 but not H2. Saline H1: $y = 0.301x + 0.889$ ($n = 7$, $r = 0.689$, $p < 0.05$); dexamethasone H1: $y = 0.283x + 0.467$ ($n = 10$, $r = 0.479$, $p < 0.05$); saline H2: $y = 0.361x + 0.783$ ($n = 7$, $r = 0.782$, $p < 0.05$); dexamethasone H2: $y = 0.299x + 0.957$ ($n = 10$, $r = 0.560$, $p < 0.05$).

of the newborn to extrauterine life. This work, of course, does not suggest that antenatal glucocorticoid therapy should be abandoned, but it reemphasizes that repeated treatment should be discouraged and that new clinical regimens should concentrate on achieving an optimal dose that maintains the beneficial maturational effects of the steroid while minimizing the unwanted adverse side effects.

Acknowledgments. We acknowledge Paul Hughes for help during surgery and Sue Nicholls for the routine care of the animals in this study.

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