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Activation of the Baboon Fetal Pituitary-Adrenocortical Axis at Midgestation by Estrogen: Responsivity of the Fetal Adrenal Gland to Adrenocorticotropic Hormone In Vitro[1]

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ABSTRACT

We have previously demonstrated that increased expression of fetal pituitary proopiomelanocortin mRNA and the induction of enzymes catalyzing fetal adrenal cortisol formation at term are regulated by estrogen-induced changes in placental oxidation of maternal cortisol to cortisone. To test the hypothesis that induction of fetal pituitary adrenocorticotropic hormone (ACTH) production by estrogen-induced changes in placental cortisol oxidation results in increased responsivity of the fetal adrenal gland to ACTH, in the present study we compared fetal adrenal sensitivity to ACTH in vitro at midgestation in untreated controls and in animals treated at this time in gestation with estrogen. Fetal adrenals were obtained on Day 100 (n = 7) and Day 165 (n = 5; term = Day 184) from untreated baboons and on Day 100 following maternal treatment with estradiol (s.c.; Days 70–100; n = 10) or androgen precursor (n = 3). Adrenal slices (15–25 mg) were perifused (100 μl/min; 37°C) with Medium 199 (no phenol red); media were collected at 10-min intervals and assayed for cortisol and dehydroepiandrosterone. Secretion of cortisol and dehydroepiandrosterone reached equilibrium after 140 min of perifusion; therefore, basal release was calculated as the mean steroid concentrations during 190–240 min. Adrenal slices were then perifused for 20 min with saline or ACTH at 240 (0.001 nmol), 370 (0.01 nmol), and 490 (0.1 nmol) min, and an overall average cortisol/dehydroepiandrosterone secretion rate (pg/min/mg) between 240–600 min was calculated. Basal cortisol production at Day 165 (85 ± 11) exceeded ($p < 0.05$) that at Day 100 in adrenals of control (9 ± 3) and estrogen-treated (13 ± 3) animals. In contrast, dehydroepiandrosterone production by control adrenals of Day 165 (13 ± 4) was lower than that at Day 100 in control (30 ± 6) and estrogen-treated (20 ± 3) animals. Thus, the ratio of dehydroepiandrosterone:cortisol secretion was significantly ($p < 0.05$) higher at midgestation (4.93 ± 2.15) than at term (0.16 ± 0.05) and was reduced ($p < 0.05$) in adrenals of estrogen-treated baboons (1.87 ± 0.38). In all groups, basal secretion of cortisol and dehydroepiandrosterone was not altered after infusion of saline. In contrast, ACTH increased ($p < 0.05$) cortisol secretion by ≥ 50% in adrenals of Day 165 (116 ± 20) and in those of estrogen-treated (18 ± 3) but not untreated (10 ± 4) baboons of Day 100. ACTH increased ($p < 0.05$) androgen production by estrogen-treated adrenals of Day 100 to a greater ($p < 0.05$) extent (515% ± 161%) than that of controls at Day 100 (154% ± 81%). These data indicate that estrogen treatment in vivo at midgestation induced a ratio of dehydroepiandrosterone:cortisol secretion in vitro in fetal adrenals that mimicked the ratio near term and was associated with increased steroid hormone production in response to ACTH. Therefore, we suggest that activation of the fetal hypothalamic-pituitary-adrenal axis at term and at midgestation following maternal estrogen administration is associated with increased responsivity of the fetal adrenal to ACTH.

INTRODUCTION

We have proposed that estrogen via regulation of placental cortisol-cortisone metabolism regulates the fetal hypothalamic-pituitary-adrenal axis (HPAA) and the timing of the onset of de novo cortisol production by the fetus [1, 2]. Thus, using isotope dilution methods in vivo we have demonstrated that de novo production of cortisol by the fetus is negligible at midgestation in controls and that it increased to 22% after maternal treatment with estrogen [3]. Moreover, the specific activities of fetal adrenal steroidogenic enzymes and the cell signaling enzyme protein kinase A at midgestation were increased following treatment with estradiol to values comparable to those at term [4, 5]. In a recent study [6] we also confirmed that expression of the mRNA for the adrenocorticotropic hormone (ACTH) precursor molecule proopiomelanocortin (POMC), as well as the number of cells expressing ACTH peptide in the fetal baboon anterior pituitary, was greater in late gestation when estrogen levels are elevated than at midgestation and was increased at midgestation by prematurely elevating estrogen to levels typically observed late in pregnancy. Collectively, these studies support our hypothesis that the induction of fetal adrenal steroidogenic maturation by the estrogen-regulated increase in placental oxidation of maternal cortisol (normally at term and experimentally at midgestation) is the result of activation of fetal pituitary function. However, although the number of cells expressing and presumably secreting ACTH peptide is increased at term and after estrogen treatment at midgestation, it remains to be demonstrated that the fetal adrenal has indeed been exposed to increased concentrations of ACTH as hypothesized.

With regard to adrenal function, it is well established that pulsatile as well as chronic administration of ACTH to the sheep fetus increases the sensitivity (e.g., steroid production) of the fetal adrenal to ACTH as measured in vitro [7, 8].

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Moreover, it has been suggested that the increase in responsiveness to ACTH probably involves changes in ACTH receptor-guanosine triphosphate (GTP) coupling and/or an increase in the enzymes necessary for cortisol biosynthesis [8, 9]. Therefore, it would appear that the sensitivity of the ovine fetal adrenal to ACTH in vitro is dependent upon the extent to which the gland was exposed to ACTH in vivo. Assuming that the latter also occurs in the baboon fetus, in the present study we compared fetal adrenal sensitivity to ACTH in vitro at midgestation in untreated controls and in animals treated at this time in gestation with estrogen. The aim was to test the hypothesis that the induction of fetal pituitary ACTH secretion by estrogen-induced changes in placental cortisol oxidation results in increased responsivity of the fetal adrenal gland to ACTH.

MATERIALS AND METHODS

Animals

Female baboons (*Papio anubis*), weighing 10–15 kg, were housed individually in metabolic cages in air-conditioned quarters under standardized conditions as described previously [10]. Females were paired with males for 5 days at the anticipated time of ovulation as estimated by menstrual cycle history and turgescence of the external sex skin. Baboons were used strictly in accordance with USDA regulations and the NIH Guide for the Care and Use of Laboratory Animals (Publication 85–23, 1985). The experimental protocol employed was approved by the Institutional Animal Care and Use Committee of Eastern Virginia Medical School.

Fetal adrenal glands were obtained on Day 100 (n = 7) and Day 165 (n = 5) of gestation (term = Day 184) from untreated baboons and on Day 100 from baboons treated daily with a maximal dosage of 0.5 (n = 2), 1.0 (n = 3), or 2.0 (n = 5) mg estradiol benzoate (Sigma Chemical Company, St. Louis, MO) suspended in 0.5 ml sesame oil and injected s.c. in increasing concentrations (0.25–2.0 mg/day, doubling the dosage every 5 days between Days 70 and 100 of gestation; i.e., 0.25 mg on Days 70–74, 0.5 mg on Days 75–79, etc.). In three additional baboons studied on Day 100, pellets of the aromatizable androgen androstenedione (50 mg; Innovative Research Products, Toledo, OH) were implanted s.c. in the abdominal region of the mother in increasing numbers between Days 70 and 94 of gestation (i.e., 2 on Day 70, 4 on Day 78, 6 on Day 86, and 8 on Day 94) as described previously [1]. At 2-day intervals between Days 80 and 100 or Days 145 and 165 of gestation, baboons were sedated with ketamine-HCl, and a maternal saphenous blood sample (4–7 ml) was collected. Serum was stored at −20°C until assayed for estradiol by a solid-phase ^{125}I RIA (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA) as described previously [1]. On Day 100 or 165, animals were sedated with ketamine-HCl (10 mg/kg BW; Parke Davis, Detroit, MI) and then anesthetized with halothane:nitrous oxide [1], and the fetus and placenta were delivered. After exsanguination, the fetal adrenals were quickly removed and placed in ice-cold 0.9% saline.

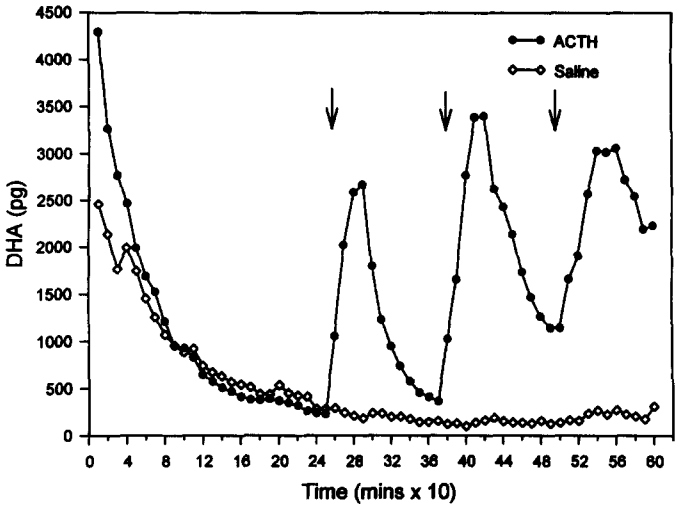

FIG. 1. Concentrations of dehydroepiandrosterone (DHA) released into the medium by representative sections of baboon fetal adrenal gland perifused for 600 min with Medium 199 (100 μl/min). Basal release was calculated as the mean of the steroid concentrations in all chambers during experimental time 190–240 min. Saline without or with ACTH was infused over a 20-min period at 240 min (0.001 nmol ACTH/min/100 μl), 370 min (0.01 nmol), or 490 min (0.1 nmol). The total amount of steroid secreted into the medium following infusion of saline/ACTH was determined, and mean secretion rate (pg/min/mg tissue) was calculated and compared with respective pretreatment secretion in each chamber.

Perifusion Protocol

Fetal adrenals were trimmed of fat, weighed, and then sliced into pieces weighing 15–25 mg each. Three random pieces were placed into prefabricated 3-ml syringes fitted with glass wool; they were maintained upright in a 37°C circulating water bath and then perifused with Medium 199 (Gibco Corp., Bethesda, MD) without phenol red (pH 7.4) delivered via a peristaltic pump at a rate of 100 μl/min. Medium was collected at 10-min intervals into glass tubes maintained on ice and was stored at −20°C until assayed by RIA for cortisol and dehydroepiandrosterone content as described previously [1, 11].

Preliminary studies confirmed that the concentration of cortisol and dehydroepiandrosterone secreted into the medium was relatively (± 10%) similar between the chambers. Moreover, after an extensive and relatively rapid release of steroid into the medium, hormone secretion rates stabilized and reached equilibrium between 140 and 160 min after onset of perifusion (Fig. 1). Therefore, basal hormone release (pg/min/mg tissue) was calculated as the mean of the steroid concentrations in all three chambers during experimental time 190–240 min. To determine the responsivity of fetal adrenal tissues to ACTH, ACTH (Cortrosyn, ACTH$^{1-24}$; Organon, Orange, NJ; 25 IU/250 μg) was infused into two

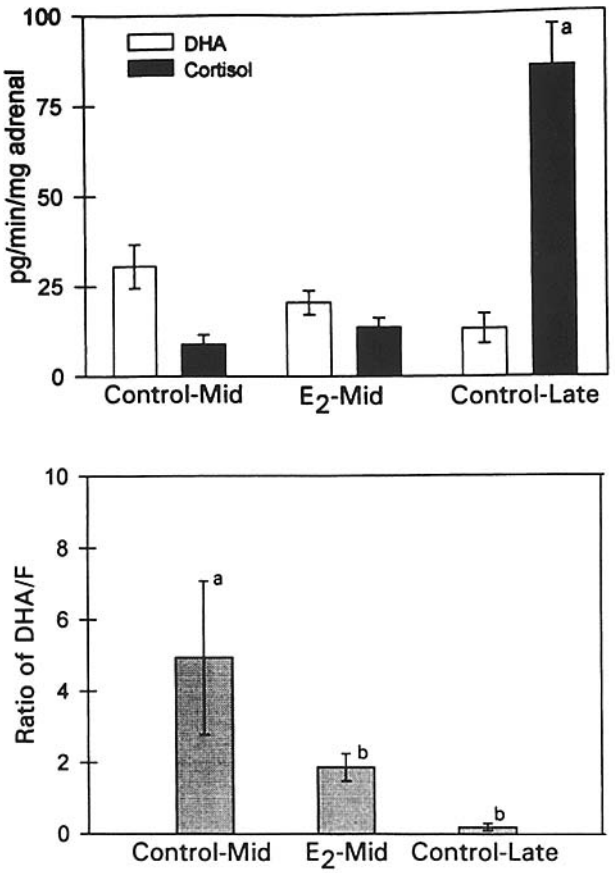

FIG. 2. Top panel: Basal secretion rates of dehydroepiandrosterone (DHA) and cortisol by perifused fetal adrenal glands obtained from untreated baboons at mid (Day 100; n = 7) and late (Day 165; n = 5) gestation (term = 184 days) and at midgestation following maternal treatment with androstenedione or estradiol (E_2; n = 13; see Table 1 notes and Fig. 1 legend for details). Letter superscript indicates that mean (± SE) value exceeds ($p = 0.05$) that in adrenals of midgestation with/without estrogen treatment (ANOVA; Duncan's Multiple Range test). Bottom panel: Ratio of basal secretion rate of dehydroepiandrosterone:cortisol (F) at mid- and late gestation. Mean values with different letter superscripts differ from each other at $p < 0.05$ (ANOVA; Duncan's Multiple Range test).

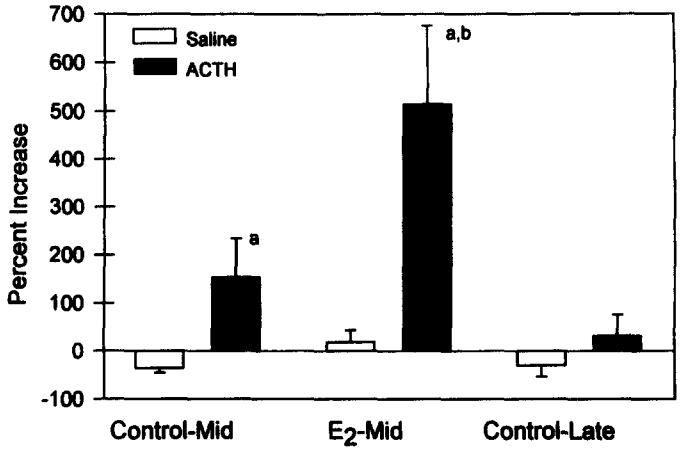

FIG. 3. Percentage change (mean ± SE) in basal secretion rate of dehydroepiandrosterone following infusion in vitro of ACTH or saline to baboon fetal adrenal glands obtained at late gestation and at midgestation with/without maternal treatment with androstenedione or estradiol (E_2; see Table 2 notes and Fig. 1 legend for experimental details). Superscript a indicates that mean value exceeds that with saline infusion ($p < 0.05$; t-test); superscript b indicates that mean value exceeds respective mean values in adrenals of untreated controls at mid and late gestation ($p < 0.05$; ANOVA; Duncan's Multiple Range test).

of the three chambers over a 20-min period at experimental times 240 min (0.001 nmol ACTH/100 µl Medium 199/min), 370 min (0.01 nmol/min), and 490 min (0.1 nmol/min). The remaining chamber received vehicle alone at the designated times. The amounts of steroid secreted into the medium after infusion of ACTH or vehicle alone were combined, and the overall average secretion rate for tissue in each chamber was calculated and compared with the respective pretreatment (e.g., basal) secretion rate. In addition, because the variance in basal steroid secretion rates between animals was relatively large, the percentage change in steroid secretion per chamber was also calculated and expressed as (pg steroid/min after ACTH [or vehicle] − pg steroid before ACTH [or vehicle])/pg steroid before ACTH (or vehicle).

The concentration of total (free and esterified) cholesterol remaining in fetal adrenals perifused with or without ACTH was determined essentially as described by Carr and Simpson [12]. Briefly, adrenals were homogenized in 2.0 ml 0.9% NaCl; after addition of 2000 cpm [1,2,6,7^{3}H]cholesteryl linoleate (SA 60 Ci/mmol; Dupont-NEN, Boston, MA) in 10 µl ethanol to estimate procedural losses, samples were extracted twice with 6 ml ice-cold chloroform:methanol (2:1 [v/v]). After evaporation of solvent, residues were dissolved in 250 µl isopropanol, and 50-µl aliquots were utilized for cholesterol assay using a commercially available enzymatic assay kit (Sigma).

Serum estradiol, fetal adrenal weight, and the basal secretion rates of cortisol and dehydroepiandrosterone were compared between the groups by ANOVA [13] with multiple comparison of the means according to Duncan [14]. Comparison of the effects of ACTH treatment and saline vehicle alone was performed by Student's t-tests for paired observations, while comparison of the effects of vehicle and ACTH between the groups was carried out by ANOVA and multiple comparison of the means according to Duncan [14].

RESULTS

Maternal serum estradiol concentrations (Table 1) were similar between animals treated at midgestation with androstenedione (1.4 ng/ml) and those treated with estradiol benzoate at daily dosages of 0.5 mg (1.3 ng/ml) or 1.0 mg (1.5 ng/ml). The overall mean (± SE) serum estradiol concentration in these baboons (1.5 ± 0.2 ng/ml) was 2-fold greater ($p < 0.05$) than that in untreated baboons on Day 100 but was similar to that of late gestation (Table 1). Estradiol concentrations were further increased ($p < 0.05$) by administration of 2.0 mg estradiol benzoate to a mean value

TABLE 1. Fetal adrenal and body weight and maternal serum estradiol (E_2) concentrations in baboons at mid- and late gestation and at midgestation following treatment with androstenedione or estradiol.[a]

Treatment in vivo	n	Body weight (grams)	Wet weight (mg/2 adrenals)	E_2 (ng/ml)[b]
Midgestation				
Control	7	176 ± 9[c]	112 ± 14[c]	0.7 ± 0.1[c]
Androstenedione	3	159 ± 10[c]	105 ± 6[c]	1.4 ± 0.3[d]
E_2 (0.5 and 1.0 mg)	5	160 ± 19[c]	98 ± 12[c]	1.5 ± 0.1[d]
E_2 (2.0 mg)	5	209 ± 21[c]	102 ± 11[c]	3.6 ± 0.5[e]
Late gestation				
Control	5	726 ± 23[d]	347 ± 19[d]	1.8 ± 0.4[d]

[a]Baboons delivered on Day 100 (mid) and Day 165 (late) of gestation (term = Day 184) to mothers untreated or treated s.c. with increasing numbers of 50 mg implants of androstenedione or with various maximal dosages of estradiol benzoate injected s.c. daily in increasing concentrations between Days 70 and 100 of gestation.
[b]Serum estradiol (mean ± SE) determined in maternal saphenous venous samples obtained at 1- to 2-day intervals on Days 80–100 or Days 145–165 of gestation.
[c,d,e]Values (mean ± SE) with different superscripts differ from each other at $p < 0.05$ (ANOVA and Duncan's Multiple Range test).

that was also greater ($p < 0.05$) than the value on Day 165 (Table 1).

Although fetal adrenal weight was 3-fold greater ($p < 0.05$) at term than at midgestation, adrenal weight was not changed significantly by any of the treatment regimens (Table 1). Similar changes were noted for fetal body weight; thus the ratio of adrenal weight to body weight was lower near term than at midgestation and was not altered at midgestation by an experimental increase in estrogen.

As indicated in Figure 2, in adrenals from untreated controls obtained at midgestation the mean (± SE) basal secretion rate of dehydroepiandrosterone (30.4 ± 6.1 pg/min/mg) exceeded that of cortisol (8.9 ± 2.7 pg/min/mg), whereas the reverse was true in adrenals from untreated controls near term. Thus, the ratio of dehydroepiandrosterone:cortisol secretion by perifused adrenals of midgestation baboons (4.93 ± 2.15) was greater ($p < 0.05$) than that of adrenals obtained near term (0.16 ± 0.05; Fig. 2). Moreover, the ratio of steroid secretion at midgestation was decreased ($p < 0.05$) 2-fold in fetal adrenals from baboons in which estrogen production was experimentally increased (1.87 ± 0.38) at this time in pregnancy.

The effects of ACTH (and saline vehicle) on the mean basal secretion rates of dehydroepiandrosterone by perifused adrenals at mid- and late gestation, expressed as a percentage increase (or decrease) in average hormone secretion rate during experimental time 250–600 min, are depicted in Figure 3. In all three treatment groups, basal secretion of dehydroepiandrosterone was not altered by 20-min bolus infusions of saline. In contrast, as compared to the effects of saline, ACTH infusion increased ($p < 0.05$) secretion of dehydroepiandrosterone in adrenals of control (154 ± 81%) and estrogen-treated animals (515 ± 161%) of midgestation but not in adrenals of near-term baboons (32 ± 44%). Similar observations were made when data were expressed as the difference in the mass (pg/min/mg tissue) of dehydroepiandrosterone secreted before and after infusion of saline or ACTH (Table 2). Thus ACTH infusion increased ($p < 0.05$) mean basal dehydroepiandrosterone secretion by 53.3 ± 33.9 pg/min/mg and 69.3 ± 18.9 pg/min/mg by adrenals of midgestation controls and estradiol-treated baboons, respectively, compared with the respective values (−15.3 ± 9.7 and 2.4 ± 4.6 pg/min/mg) following infusion of saline. ANOVA also confirmed that the percentage increase in dehydroepiandrosterone secretion elicited by ACTH in adrenals of estrogen-treated animals was greater ($p < 0.05$) than that elicited in adrenals of controls at midgestation and at term (Fig. 3). Although this difference was not confirmed statistically when data were analyzed as the difference in mass of dehydroepiandrosterone secreted, the latter was attributable primarily to the large variation in dehydroepiandrosterone secretion observed in general and in one adrenal in particular (Table 2).

Cortisol secretion by control midgestation adrenals was not significantly increased by ACTH, whether data were expressed as percentage change (Fig. 4) or as the difference in mass of steroid secreted (Table 3). In contrast, cortisol secre-

TABLE 2. Effect of ACTH in vitro on basal secretion rates (pg/min/mg tissue) of dehydroepiandrosterone by baboon fetal adrenals obtained at mid- and late gestation.[a]

	Saline			ACTH		
Treatment in vivo	Before	After	Difference[b]	Before	After	Difference[b]
Midgestation						
Control	35.3 ± 14.6	20.0 ± 6.0	−15.3 ± 9.7	27.9 ± 5.6	81.1 ± 38.5[c]	53.3 ± 33.9[d]
E_2	18.2 ± 3.7	20.6 ± 5.4	2.4 ± 4.6	21.8 ± 4.1	91.1 ± 19.8	69.3 ± 18.9[d]
Late gestation						
Control	15.0 ± 6.6	14.9 ± 9.9	−0.1 ± 5.5	12.3 ± 3.5	16.9 ± 6.2	4.5 ± 5.7

[a]Baboons delivered on Day 100 (mid) and Day 165 (late) of gestation to mothers untreated or treated with androstenedione or estradiol between Days 70 and 100 of gestation (See Table 1 for details). Adrenals were perifused for 600 min with Medium 199 and concomitantly for 20 min with ACTH or saline starting at experimental times 240, 370, and 490 min.
[b]Mean ± SE difference in the average dehydroepiandrosterone secretion rate (pg/min/mg) between 250–600 min and 190–240 min (see Fig. 2). Individual values for each adrenal preparation were utilized to calculate the overall mean (± SE) percent increase in steroid secretion following saline or ACTH depicted in Figure 3.
[c]Value in one animal (271 pg/min/mg) exceeded the overall mean value (49 ± 26 pg/min/mg) in adrenals of the remaining 6 baboons.
[d]Different ($p < 0.05$) from respective mean value following saline treatment (*t*-test).

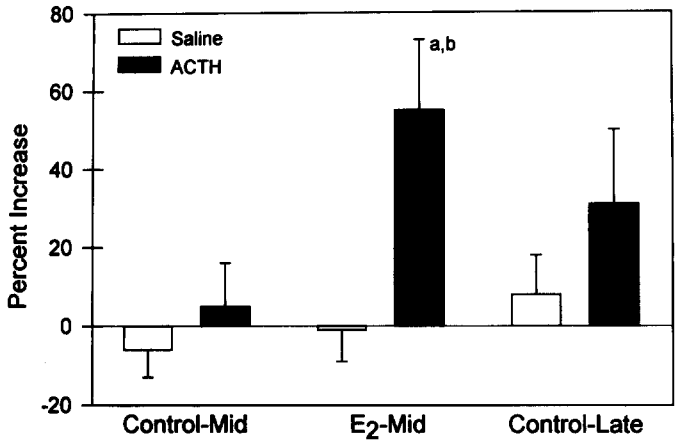

FIG. 4. Percentage change (mean ± SE) in basal secretion rate of cortisol following infusion in vitro of ACTH or saline to baboon fetal adrenal glands obtained at late gestation and at midgestation with/without maternal treatment with androstenedione or estradiol (E_2; see Table 2 notes and Fig. 1 legend for experimental details). Superscript a indicates that mean value exceeds that with saline infusion ($p < 0.05$; *t*-test); superscript b indicates that mean value exceeds respective mean values in adrenals of untreated controls at midgestation ($p < 0.05$; ANOVA; Duncan's Multiple Range test).

tion was increased by 55 ± 18% ($p < 0.05$), or 5.4 ± 2.2 pg/min/mg tissue ($p < 0.05$; Table 3), by ACTH in adrenals of midgestation baboons treated with estradiol. In adrenals of term animals, ACTH increased ($p < 0.05$) cortisol secretion by 30.7 ± 13.9 pg/min/mg (Table 3), although this increase was not confirmed statistically when data were expressed as percentage (34 ± 17%) change (Fig. 4). ANOVA confirmed that the ACTH-induced increase in the mass of cortisol secreted by adrenals of near-term animals exceeded that of tissue from midgestational baboons (Table 3).

The data in Table 4 indicate that the amount of total cholesterol (e.g., free and esterified) remaining in fetal adrenals after perifusion for 10 h was similar in tissues receiving saline and those receiving ACTH during the study period. Regardless of in vitro treatment, cholesterol concentrations in the adrenals of term baboons were greater ($p < 0.05$) than in tissue of animals at midgestation and were not altered at midgestation by treatment in vivo with estrogen.

TABLE 4. Cholesterol concentrations (μg/mg protein) in baboon fetal adrenals of mid- and late gestation after perifusion in vitro with saline or ACTH.[a]

Treatment in vivo	n	Saline	ACTH
Midgestation			
Control	5	84 ± 8[b]	90 ± 11
Estradiol	6	76 ± 11	83 ± 5
Late gestation			
Control	4	213 ± 65[c]	210 ± 67[c]

[a] See notes in Tables 1 and 2 for details of animal treatment and adrenal incubation.
[b] Mean ± SE total cholesterol (free and esterified) remaining after perifusion and treatment in vitro with or without ACTH.
[c] Different ($p < 0.05$) from respective value at midgestation (ANOVA; Duncan's Multiple Range test).

DISCUSSION

The results of the present study indicate that the secretion of dehydroepiandrosterone by the baboon fetal adrenal in response to ACTH in vitro at midgestation is enhanced in tissues obtained from animals treated in vivo with estradiol, an experimental condition that we [6] have previously shown enhances POMC mRNA expression and the number of cells expressing ACTH peptide within the fetal anterior pituitary. Moreover, in contrast to the inability of the fetal adrenal of untreated baboons at midgestation to secrete cortisol in response to ACTH, fetal adrenals of estrogen-treated baboons displayed a consistent increase in cortisol production after infusion of ACTH in vitro. Previous studies in the sheep demonstrated that treatment of the fetus with ACTH increased serum cortisol concentrations [8] and cortisol production by isolated adrenal cells subsequently treated in vitro with ACTH [15]. Moreover, infusion of the sheep fetus with ACTH at 75% of gestation induced development of the ACTH-sensitive adenylate cyclase system [16], as well as the specific activity of rate-limiting enzymes in the steroidogenic pathway leading to cortisol production [17] within the adrenal. Collectively, these findings in the fetal sheep support the suggestion that the degree of fetal adrenal responsivity to ACTH in vitro is correlated with the amount of ACTH to which this fetal tissue is exposed in vivo and that this response is due in part to increased activity of steroidogenic enzymes, as well as receptor mediated cell-signaling pathways. In the baboon, we

TABLE 3. Effect of ACTH in vitro on basal secretion rates (pg/min/mg tissue) of cortisol by baboon fetal adrenals obtained at mid- and late gestation.[a]

	Saline			ACTH		
Treatment in vivo	Before	After	Difference[b]	Before	After	Difference[b]
Midgestation						
Control	8.3 ± 3.2	7.7 ± 3.1	−0.6 ± 0.4	9.9 ± 2.9	10.8 ± 3.8	0.9 ± 1.5
E_2	13.0 ± 2.4	12.7 ± 2.7	−0.3 ± 0.9	12.9 ± 2.1	18.2 ± 2.7	5.4 ± 2.2[c]
Late gestation						
Control	86.6 ± 18.7	89.2 ± 14.8	0.4 ± 10.1	84.8 ± 8.2	115.6 ± 19.9	30.7 ± 13.9[c,d]

[a] See notes of Tables 1 and 2 for details of animal treatment.
[b] See notes of Table 2 and legend of Figure 2. Individual values for each adrenal preparation were utilized to calculate the overall mean (± SE) percentage increase in steroid secretion following saline or ACTH depicted in Figure 4.
[c] Different ($p < 0.05$) from respective mean value following saline treatment (*t*-test).
[d] Different ($p < 0.05$) from respective values at mid-gestation (ANOVA; Duncan's Multiple Range test).

have previously demonstrated that the specific activities of protein kinase A [5], 17-hydroxylase-C17,20-lyase, and Δ^5-3β-hydroxysteroid dehydrogenase-isomerase [4] in the fetal adrenal were increased at midgestation concomitant with an increase in fetal pituitary POMC mRNA expression subsequent to estrogen-induced alterations in placental cortisol oxidation [4]. The results of the present study, therefore, support the hypothesis that increased responsivity of fetal adrenal dehydroepiandrosterone secretion to ACTH in vitro, and the ontogenesis of ACTH-dependent cortisol secretion at midgestation following maternal estrogen treatment in the baboon, are the result of increased exposure of the fetal adrenal in utero to ACTH. Moreover, on the basis of our previous studies, we suggest that these changes in adrenal response to increased ACTH production are dictated by estrogen and its ability to regulate placental 11β-hydroxysteroid dehydrogenase activity [18, 19]. The increase in 11β-hydroxysteroid dehydrogenase catalyzes the oxidation of cortisol to cortisone, leading to decreased transfer of maternal cortisol to the fetus [3] and consequently enhanced expression of fetal pituitary POMC mRNA.

As previously demonstrated in humans and nonhuman primates (see [2] for review), there is a developmental change in the pattern of fetal adrenal steroidogenesis during the course of gestation from exclusive production of the Δ^5-C19 androgen dehydroepiandrosterone at midgestation to dominant synthesis of the Δ^4-C21 steroid cortisol near term. Indeed, results of the present study confirmed that the relatively high ratio of dehydroepiandrosterone to cortisol secretion by midgestational adrenals was reduced in adrenals of near-term animals. Significantly, in adrenals of baboons in which estrogen production was experimentally increased at midgestation, this ratio of steroid secretion was reduced more than 2-fold. Collectively, these findings support the suggestion that the ontogenetic change in the qualitative pattern of adrenal steroidogenesis is also regulated by increased exposure of the fetal adrenal to ACTH in vivo, presumably via induction of enzymes important to cortisol production, most notably the Δ^5-3β-hydroxysteroid dehydrogenase-isomerase enzyme catalyzing synthesis of Δ^4 steroid products.

It is well established that the Δ^5-3β-hydroxysteroid dehydrogenase enzyme catalyzing cortisol synthesis is localized exclusively within the definitive zone cells of the primate fetal adrenal gland [20, 21]. Production of dehydroepiandrosterone, in contrast, occurs primarily in the fetal cortical cells, which constitute more than 85% of the total fetal adrenal gland throughout most of intrauterine development (see [2] for review). Therefore, the results of the present study, as well as those previously published demonstrating a 4-fold increase in Δ^5-3β-hydroxysteroid dehydrogenase activity [4] at midgestation following maternal estrogen treatment, presumably reflect a corresponding increase in ACTH-dependent proliferation of the definitive zone cells of the fetal adrenal gland. This 4-fold increase, however, was still substantially less than the level of Δ^5-3β-hydroxysteroid dehydrogenase activity that is observed normally near term and reflects an increase in the number of definitive zone cells as determined histologically [2, 4]. Moreover, despite an increase in the responsivity of the fetal cortical cells to ACTH in estrogen-treated baboons of midgestation, this change was not associated with an increase in fetal adrenal weight as occurs normally late in gestation [4, 5]. Whether the latter reflected differences in the quantity of ACTH secreted and/or the length of time the gland was exposed to ACTH remains to be determined. However, it is well known that the regulation of fetal adrenal growth is multifactorial in nature and thus involves factors in addition to ACTH (see [2] for review). For example, specific growth factors of fetal adrenal and/or placental origin clearly have the capacity to stimulate fetal adrenal growth in vitro, and their effects appear to depend upon or be modulated by ACTH as originally suggested by Roos [22] and by Jaffe et al. [23]. Moreover, ACTH-induced expression of growth factors by cultured human fetal adrenal glands can be attenuated by secretogogues that activate protein kinase C. Because of the complex cellular organization of the fetal adrenal gland and its requirement to produce C19-steroid estrogen precursors and cortisol with advancing pregnancy, it appears that fetal adrenal growth, cellular differentiation, and steroidogenesis, while collectively dependent upon ACTH, may each require a select complex of auxiliary factors/second messengers that make their appearance at different times in gestation as maturation of the fetus takes place.

On a quantitative basis, basal cortisol production by the adrenal of near-term baboon fetuses and glandular responsivity to ACTH in vitro clearly exceeded respective values measured in untreated as well as estrogen-treated animals of midgestation. However, although the difference in the amount of cortisol secreted in response to ACTH at term (30 pg/min/mg) was significantly greater than that measured at midgestation (0.9 pg/min/mg), this was not confirmed when data were expressed as a percentage change over basal levels at mid (5%)- and late (34%) gestation. Although the latter could reflect the marked variation in responsivity observed between the groups, it is difficult to directly compare the percentage change in the secretion of cortisol at mid- and late gestation since the amount of cortisol secreted at these two times in pregnancy differs by about 10-fold. Thus, a 5% increase in cortisol production at midgestation would not be comparable to a 5% increase at term. This was not the case when the cortisol or the dehydroepiandrosterone response to ACTH in untreated control and estrogen-treated animals was compared at midgestation, because the absolute amount of these steroids secreted by fetal adrenals derived from these two animal treatment groups was relatively comparable.

In the present study, steroid substrate cholesterol was not added to the perifusion medium of the fetal adrenal system.

It is well known that low density lipoprotein (LDL)-cholesterol esters in addition to de novo cholesterol production are the major sources of substrate for hormonogenesis in the human [24] and baboon [2, 25] fetal adrenal. In the present study, total (i.e., free and esterified) cholesterol concentrations in the fetal adrenal after perifusion for 10 h were significant and were not depleted or reduced in tissues treated in vitro with ACTH. Similar observations were made by Mason and Rainey [26], who demonstrated in human fetal adrenal cell cultures that steroidogenesis in the absence of LDL-cholesterol could be maintained almost entirely by the de novo pathway, which exhibited a turnover rate of approximately 3 h. Thus, the results of the present study do not appear to be influenced or compromised by substrate availability.

Although fetal serum estrogen concentrations were 4-fold greater at term than at midgestation and increased by 35–50% at midgestation after maternal treatment with estradiol [6, 27], it is unlikely that the increased responsivity of the baboon fetal adrenal at midgestation in estrogen-treated animals and at term in controls reflected a direct stimulatory effect of estrogen on the fetal adrenal gland. Thus, in both fetal and neonatal adrenal glands, estrogen has been shown to act as a competitive inhibitor of the enzymes 17-hydroxylase [28] and Δ^5-3β-hydroxysteroid dehydrogenase-isomerase [29]. Moreover, we have proposed, using both in vitro [25, 30] and in vivo [31] experimental paradigms, that during primate pregnancy a feedback system is operative in which placental estrogen secreted into the fetus suppresses the responsivity of the fetal adrenal to ACTH with respect to dehydroepiandrosterone production, a mechanism that may be important in maintaining estrogen production within a physiologically normal level.

In summary, on the basis of our previous [3, 6] and present studies, we propose that activation of the baboon fetal pituitary-adrenocortical axis normally at term, and at midgestation by estrogen-induced alterations in placental cortisol metabolism, results in increased POMC mRNA expression/ACTH production by fetal pituitary corticotrophs and increased responsivity of the fetal adrenal to ACTH.

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