Role of Hypothalamic-Pituitary Axis in EGF Action on Maturation of Adrenal Gland in Fetal Rhesus Monkey *In Vivo*

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ABSTRACT

We determined the route of action of epidermal growth factor (EGF) [intraperitoneal (IP) *versus* intraamniotic administration] on adrenal development and whether its effects are mediated via the fetal hypothalamic-pituitary axis in the fetal rhesus monkey *in vivo*. EGF (40 μ g) was administered IP ($n = 9$) or intraamniotic ($n = 6$) at 121, 123, 125, and 127 d gestation (term, approximately 165 ± 10 d gestation). In addition, a competitive corticotropin-releasing factor antagonist ([D-phenylalanine¹², Norleucine^{21,38}] corticotropin-releasing factor¹²⁻⁴¹ to block fetal pituitary ACTH secretion; 400 μ g IP) and metyrapone (11 β -hydroxylase inhibitor to block adrenal cortisol synthesis; 15 mg IP and 15 mg intraamniotic) were administered, in combination with EGF (EGF+BLOCK; 40 μ g IP; $n = 4$ fetuses). Control fetuses ($n = 6$) received saline injections in an equivalent volume. On gestational d 128, a hysterotomy was performed, and fetal adrenals were collected for morphometric analyses and immunocytochemical localization of 3 β -hydroxysteroid dehydrogenase (3 β HSD) and cytochrome P-450 11 β -hydroxylase/aldosynthase. Definitive zone (DZ) width and cortical width of 3 β HSD staining were significantly greater ($p < 0.05$) in the EGF IP-treated fetuses compared with controls and EGF+BLOCK. With EGF IP, 3 β HSD was increased in the DZ and induced extensively in the transitional zone of the fetal adrenal cortex, and cytochrome P-450 11 β -hydroxylase/aldosynthase immunoreactivity was induced to detectable levels in the DZ. The administration of EGF+BLOCK inhibited the expression of 3 β HSD in the transitional zone, but 3 β HSD expression was still increased in the DZ and cytochrome P-450 11 β -hydroxylase/

aldosynthase immunoreactivity was induced in the DZ. EGF intraamniotic administration had no significant effect on the width of the DZ or cortical width of 3 β HSD staining compared with controls. These data suggest that EGF acts via the hypothalamic-pituitary axis to modulate adrenal cortical growth and functional maturation of the transitional zone (the putative zona fasciculata), whereas EGF can act independently of the hypothalamic-pituitary axis to stimulate functional maturation of the DZ (the putative zona glomerulosa). (*Pediatr Res* 50: 210–216, 2001)

Abbreviations

ACTH, adrenocorticotrophic hormone
CYP11B2, aldosynthase (EC 1.14.99)
3 β HSD, 3 β -hydroxysteroid dehydrogenase (EC 1.1.)
CRF, corticotropin-releasing factor
CYP11B1, cytochrome P-450 11 β -hydroxylase (EC 1.14.15.4)
CYP11B, cytochrome P-450 11 β -hydroxylase/aldosynthase
CYP11B-ir, cytochrome P-450 11 β -hydroxylase/aldosynthase immunoreactivity
DZ, definitive zone
BLOCK, [D-phenylalanine¹², Norleucine^{21,38}] CRF^{12,41} plus metyrapone
EGF, epidermal growth factor
FZ, fetal zone
H&E, hematoxylin and eosin
IA, intraamniotic
TGF α , transforming growth factor- α
TZ, transitional zone

The development of the steroidogenic capacity of the fetal adrenal gland is necessary for the maintenance of intrauterine

homeostasis, development of enzyme systems necessary for extrauterine life, and, at least in some species, the timing of

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parturition. Secretion of cortisol from the fetal adrenal is necessary for the maturation of essential organ systems and plays a key role in the fetal response to intrauterine stress (1–5). After birth, the secretion of aldosterone from the newborn adrenal is a key component of the renin-angiotensin system and is essential for the maintenance of fluid balance and blood pressure during postnatal life. Previous studies in the human and nonhuman (rhesus monkey) primate fetus have indicated that the adrenal gland comprises three functional zones: 1) the large, inner FZ, which has the enzymes necessary for dehydroepiandrosterone sulfate production beginning early in gestation; 2) the TZ between the inner FZ and outer DZ, which possesses enzymes necessary for cortisol production beginning in late gestation; and 3) the outer, DZ, which appears to function as a reservoir of progenitor cells, which may populate the remainder of the gland, and does not acquire a steroidogenic phenotype with the capacity to produce mineralocorticoids until near term (1, 2, 6–13). Recently, we have shown that timing of the induction of 3β HSD in the TZ and CYP11B-ir in the DZ are coincident with increased circulating concentrations of cortisol and aldosterone in late gestation (12). These data suggest that functional maturation of the fetal adrenal cortex is dependent on the specific zonal expression of 3β HSD and CYP11B, which are pivotal enzymes in adrenal steroidogenesis (1, 2). Thus, to understand the functional maturation of the primate fetal adrenal, it is important to determine the factors that regulate induction of 3β HSD and CYP11B expression.

Recently, we have demonstrated that administration of EGF into the fetal peritoneal and amniotic fluid cavities stimulated adrenal cortical growth and the expression of 3β HSD in the adrenal cortex of the fetal rhesus monkey *in vivo* (14). Studies have shown that EGF can stimulate the secretion of CRF from the hypothalamus and ACTH from the pituitary (15). The presence of specific receptors for EGF on both DZ and FZ cells has been demonstrated, and EGF stimulates proliferation of human fetal adrenal cells *in vitro* (16). It has also been shown that EGF stimulates secretion of cortisol and aldosterone as well as angiotensin II-induced aldosterone secretion from the adrenal gland (17). Thus, EGF may act both directly and indirectly via the fetal hypothalamic-pituitary axis to stimulate adrenal growth and steroidogenesis. In the rhesus monkey, amniotic fluid contains substantial amounts of a factor that binds to the EGF receptor in radioreceptor assays. This could reflect EGF or TGF α , the latter having been identified in amniotic fluid in other studies. TGF α is a member of the EGF family and acts specifically via the EGF receptor (15). In these studies, we have used EGF, rather than TGF α , because it was made available to us as a recombinant protein in sufficient amounts for *in vivo* studies. To determine the route of action of EGF on adrenal development, in one group of fetuses we administered EGF IA and in a second group of fetuses we administered EGF directly into the fetus by the IP route. In addition, to determine whether the effects of EGF were directly on the adrenal gland or mediated via the hypothalamic-pituitary axis, we administered EGF IP in combination with a competitive CRF antagonist (to block fetal pituitary ACTH secretion). In the present study, we examined the effects of

EGF administration and the mode of action of EGF on adrenal growth variables using morphometric techniques and determined the pattern of localization of 3β HSD and CYP11B in the fetal rhesus adrenal using immunocytochemical techniques.

METHODS

Animals and EGF administration. Twenty-five pregnant rhesus monkeys (*Macaca mulatta*) were obtained from the time-mated breeding colony at the California Regional Primate Research Center, University of California, Davis, CA, U.S.A. Animals were maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Animals*, and the protocols used in this study were approved by the Institutional Animal Use and Care Committee at the University of California, Davis. Before IA or IP injections, the dams were administered ketamine hydrochloride (10 mg/kg), and EGF administration was performed with ultrasound guidance as described previously (18). Recombinant human EGF was provided by Chiron (Emeryville, CA, U.S.A.). EGF (40 μ g in 1 mL of saline) was administered via the IP route ($n = 9$) or IA ($n = 6$) at 121, 123, 125, and 127 d gestation (term, 165 ± 10 d). In four fetuses, a competitive CRF antagonist ([D-phenylalanine¹², Norleucine^{21,38}] CRF^{12–41} to block fetal pituitary ACTH secretion; 400 μ g IP) and metyrapone (CYP11B1 inhibitor to block adrenal cortisol synthesis; 15 mg IP and 15 mg IA) were also administered, in combination with EGF IP (EGF+BLOCK; 40 μ g) at the same gestational times. The CRF antagonist was provided by Dr. Jean Rivier [The Salk Institute, San Diego, CA, U.S.A. (19)], and the dose chosen was previously shown to be effective in the fetal rhesus monkey at modulating pituitary ACTH secretion by blocking the fetal hemorrhage-induced increase in fetal plasma ACTH concentrations (1, 20). Metyrapone treatment was included in the experimental paradigm, to determine whether the stimulatory effects of EGF treatment observed on fetal lung maturation (18, 21–23) were direct or mediated via the fetal adrenals. The data on the effects of EGF+BLOCK, EGF IP, or EGF IA on lung development are the subject of a separate publication (in preparation). In six control fetuses, saline was administered (1 mL of saline IP and 1 mL of saline IA). At 128 d gestation (78% of gestation), hysterotomies were performed, and fetal tissues were collected for analysis (see below), as described previously (18). Body and adrenal weights were obtained, and fetal adrenals were fixed in formalin (10%) for morphologic and immunocytochemical analyses as described (14).

Immunocytochemistry. To localize 3β HSD and CYP11B protein in adrenals from fetal rhesus monkeys, we used a modification of the peroxidase/antiperoxidase method as described previously (14). A human placenta type-1 3β HSD (1:200) polyclonal antibody was used and characterized previously (14) (generously provided by Dr. J. Ian Mason, Department of Clinical Biochemistry, University of Edinburgh, Scotland, U.K.). The CYP11B1/CYP11B2 (1:4000) polyclonal antibody was raised in rabbits against immunopurified ovine CYP11B enzyme and has been characterized previously (22, 24) and validated for use in primate adrenal tissue (12) (gen-

erously provided by Professor Pieter Swart, Department of Biochemistry, University of Stellenbosch, South Africa).

The ovine CYP11B antibody does not distinguish between 11β -hydroxylase and aldosterone synthase (24), which are encoded by separate but highly homologous genes, CYP11B1 and CYP11B2, respectively (25, 26), and therefore we have referred to this immunoreactive product as CYP11B-ir. Negative control sections were used in this study for all adrenals, in which the primary antisera was replaced with normal rabbit serum at dilutions equivalent to those for each antibody, *i.e.* 1:200 and 1:4000 for 3β HSD and CYP11B, respectively. The immunocytochemical signal was visualized using 3,5 diaminobenzidine tetrahydrochloride, and the appearance of the light brown product was observed under a light microscope. To compare among the treatment groups, the same reaction time with 3,5 diaminobenzidine tetrahydrochloride was used for adrenal sections from control, EGF-treated, and EGF+BLOCK-treated fetuses.

Adrenal morphology and morphometry. Adrenal morphometric measurements and quantification of 3β HSD staining were made using a video-generated image analysis (Videometric 150, Oncor Inc, Gaithersburg, MD, U.S.A.) as described previously (13, 14), and assessments were conducted by an individual blinded to the treatment groups. In brief, the image-analysis system permits the investigator to select the spectral composition of any desired color and allows one to establish limits of its true brilliance and intensity. These spectral values can be saved for equivalent measurements in successive sessions. The light microscope video-image is scanned automatically for the presence of the defined color, and the quantification (intensity) of 3β HSD staining (number of pixels) is presented as the number of grains per unit area of the image. In this study, the color limits were set manually to detect the brown immunocytochemical reaction product and to eliminate unstained background tissue. Triplicate measurements were made of the amount of 3β HSD staining in a defined area ($20 \times 10^3 \mu\text{m}^2$) in three histologic sections from each adrenal gland from all fetuses and expressed as arbitrary units per square micrometer. Six measurements were made of the width of the DZ in H&E-stained sections and of the width of cortical 3β HSD staining in sections of adrenals from control, EGF-treated, and EGF+BLOCK-treated animals.

Statistical analysis. The effect of EGF or EGF+BLOCK treatment on the adrenal to body weight ratio was compared using a one-way ANOVA. Comparisons of the effect of EGF or EGF+BLOCK treatment on width of the DZ, cortical width of 3β HSD staining, and amount of 3β HSD staining were made using a two-way ANOVA with treatment (*i.e.* EGF IP, EGF IA, EGF+BLOCK, control) and replication. The two-way ANOVA tested for variation among morphometric measurements of the adrenal sections from one animal, among animals within a treatment group, and among control and treated groups. The Fisher's protected least square difference test was used after ANOVA to compare significant differences among mean values, and $p < 0.05$ was considered to be significant. All data are expressed as mean $\pm$ SEM. Data were analyzed using SuperANOVA (Abacus Concepts, Berkeley, CA,

U.S.A.) on a Macintosh personal computer (Apple Computer Inc, Cupertino, CA, U.S.A.).

RESULTS

Fetal body weight, adrenal weight, and adrenal growth.

Fetal body weights did not significantly differ among the control (273.3 ± 19.6 g), EGF IP (288.1 ± 26.2 g), EGF IA (296.5 ± 35.9 g), and EGF+BLOCK groups (307.3 ± 27.5 g). Fetal adrenal weights were greater in the EGF IP group (278.5 ± 57.1 mg) when compared with the control group (188.1 ± 33.5 mg). In contrast, the fetal adrenal weights were not different among the EGF IA (240.8 ± 82.2 mg), EGF+BLOCK (224.3 ± 53.0 mg), and control fetuses. Similar data were found when examining the adrenal weight to body weight ratio. Adrenal to body weight ratios were significantly greater ($p < 0.05$) in the EGF IP group (1.01 ± 0.05 g/kg) compared with control fetuses (0.69 ± 0.05 g/kg; Fig. 1). In contrast, the adrenal to body weight ratios were not different among the EGF IA, EGF+BLOCK, and control fetuses (Fig. 1). The width of the DZ from EGF IP-treated fetuses was approximately twice the width of the DZ from control fetuses (Figs. 2 and 3, A and C). In contrast, the width of the DZ was not significantly different among the EGF IA, EGF+BLOCK, and control fetuses (Figs. 2 and 3, A, B, and D).

Immunocytochemistry. The width of the DZ and cortical width of 3β HSD staining were significantly greater ($p < 0.05$) in the EGF IP group ($132 \pm 18 \mu\text{m}$, $329 \pm 25 \mu\text{m}$) compared with the control group ($73 \pm 8 \mu\text{m}$, $141 \pm 9 \mu\text{m}$) and EGF+BLOCK group ($69 \pm 3 \mu\text{m}$, $189 \pm 19 \mu\text{m}$; Figs. 2 and 4). EGF IA administration had no significant effect ($p > 0.05$) on the width of the DZ or cortical width of 3β HSD staining ($106 \pm 10 \mu\text{m}$, $152 \pm 12 \mu\text{m}$) compared with controls (Figs. 2 and 4).

Figure 1. Adrenal to body weight ratio measurements from control ($n = 6$; open bars), EGF IP ($n = 9$; forward-angled hatched bars), EGF IA ($n = 6$; reverse-angled hatched bars), and EGF+BLOCK ($n = 4$; solid bars) rhesus monkey fetuses at 128 d gestation. Data are expressed as mean $\pm$ SEM. A one-way ANOVA was used to compare the control and treated animals, and $p < 0.05$ was considered significant. Groups designated with the same letter are not significantly different from each other, whereas groups designated with different letters are significantly different from each other.

Figure 2. An image-analysis system was used to measure the DZ width on H&E-stained adrenal glands from control ($n = 6$; open bars), EGF IP ($n = 9$; forward-angled hatched bars), EGF IA ($n = 6$; reverse-angled hatched bars), and EGF+BLOCK ($n = 4$; solid bars) rhesus monkey fetuses at 128 d gestation. For each animal, a mean of six measurements of the width of the DZ was made, and the data are expressed as mean $\pm$ SEM for control and treated fetuses. A two-way ANOVA with treatment and replication as factors was used to compare the control and treated animals, and $p < 0.05$ was considered significant. Groups designated with the same letter are not significantly different from each other, whereas groups designated with different letters are significantly different from each other.

In the control animals, 3β HSD immunostaining was present in the DZ and did not extend greatly into the TZ. In fetuses that received EGF IA alone or EGF+BLOCK, 3β HSD immunostaining was also present in the DZ and a small TZ was present (Figs. 4 and 5, A, B, and D). However, the intensity of staining for 3β HSD protein in the DZ was increased in the EGF+BLOCK group when compared with the control group (Figs. 5D and 6). In contrast to control animals, there was abundant 3β HSD immunostaining in the DZ, and 3β HSD was induced extensively in the TZ of the fetal adrenal after administration of EGF IP alone (Figs. 4, 5B, and 6). In parallel with these observations on 3β HSD localization, the mean width of cortical 3β HSD staining was significantly greater ($p < 0.05$) in

Figure 4. The cortical width of 3β HSD staining of adrenal glands from control ($n = 6$; open bars), EGF IP ($n = 9$; forward-angled hatched bars), EGF IA ($n = 6$; reverse-angled hatched bars), and EGF+BLOCK ($n = 4$; solid bars) rhesus monkey fetuses at 128 d gestation. For each animal, six measurements of the cortical width of 3β HSD staining were made using an image-analysis system. A two-way ANOVA with treatment and replication as factors was used to compare the control and treated animals, and $p < 0.05$ was considered significant. Groups designated with the same letter are not significantly different from each other, whereas groups designated with different letters are significantly different from each other. Data are expressed as mean $\pm$ SEM.

the EGF IP-treated fetuses compared with control, EGF IA, and EGF+BLOCK fetuses, as determined by image analysis (Fig. 4).

It should be noted in the EGF IA fetuses that although there was a trend for an increase in the adrenal to body weight ratio (Fig. 1) and width of the DZ (Fig. 2), this was not paralleled by any significant change in cortical width (Fig. 4), the amount (Fig. 6) of 3β HSD staining, or the pattern of localization of 3β HSD (Fig. 5B).

In control and EGF IA animals, CYP11B-ir was present in the TZ and FZ, but was not detectable in the DZ (Fig. 5, E and G). EGF administration into the IP cavity induced CYP11B-ir in the DZ (Fig. 5I). However, the effect of EGF IP on staining for CYP11B in the DZ was not blocked by coadministration with the CRF antagonist and metyrapone (Fig. 5K). Although CYP11B-ir was detected in the FZ cells, it was not detectable in adrenomedullary cells in any of the treatment groups (Fig. 5, F, H, J, and L). No staining was detected when the primary antisera were replaced with normal rabbit serum (data not shown).

DISCUSSION

In the fetal rhesus monkey *in vivo*, adrenal cortical growth and functional maturation of the adrenal gland are stimulated by EGF administration into the peritoneal and amniotic fluid cavities (14). In the present study, to extend these observations, we have determined the route of action of EGF on the fetal adrenal. We have found that EGF administered via the IP route, but not the IA route, significantly stimulated adrenal cortical

Figure 3. Photomicrographs of H&E-stained sections (8 μ m) of adrenal glands from control (A), EGF IA (B), EGF IP (C), and EGF+BLOCK (D) rhesus monkey fetuses at 128 d gestation. The open bar across the top of each panel identifies the region directly underneath, namely, the DZ, TZ, FZ, and adrenal capsule (C). The scale bar represents 150 μ m.

Figure 5. Photomicrographs of 8- μ m sections of 128 d gestation rhesus monkey fetal adrenal glands from control (A), EGF IA (B), EGF IP (C), and EGF+BLOCK (D) fetuses, stained for 3 β HSD, and control (E, F), EGF IA (G, H), EGF IP (I, J), and EGF+BLOCK (K, L) fetuses, stained for CYP11B-ir, in which brown staining identifies adrenocortical cells that are immunopositive for 3 β HSD or CYP11B-ir, respectively. The sections stained for CYP11B-ir are counterstained with hematoxylin to identify cell nuclei (blue). For panels A–E, G, I, and K, the open bar across the top of each panel identifies the region immediately underneath, namely, the Dz, Tz, Fz, and adrenal capsule (C). Panels F, H, J, and L are photomicrographs of the central corticomedullary region, in which the adrenomedullary cells interdigitate with FZ cells of the adrenal cortex. The arrows identify CYP11B-ir FZ cells adjacent to or within the unstained adrenomedullary (AM) area. The scale bar represents 150 μ m.

Figure 6. The amount of 3βHSD staining (arbitrary units per square micrometer) from adrenal glands from control ($n = 6$; open bars), EGF IP ($n = 9$; forward-angled hatched bars), EGF IA ($n = 6$; reverse-angled hatched bars), and EGF+BLOCK ($n = 4$; solid bars) rhesus monkey fetuses at 128 d gestation. For each animal, triplicate measurements of the 3βHSD staining were made using an image-analysis system. A two-way ANOVA with treatment and replication as factors was used to compare the control and treated animals, and $p < 0.05$ was considered significant. Groups designated with the same letter are not significantly different from each other, whereas groups designated with different letters are significantly different from each other. Data are expressed as mean $\pm$ SEM.

growth. EGF receptors are present on human fetal adrenal cells, and EGF stimulates the proliferation of these cells *in vitro* (16); therefore, we postulated that this may be a direct action of EGF on adrenal cells (14). Alternatively, EGF has been shown to increase both CRF and ACTH release (15) and potentiate ACTH-mediated steroidogenesis in the human fetal adrenal (27); therefore, EGF may mediate its effects on the adrenal gland by activation or modulation of the hypothalamic-pituitary axis.

To provide further insight into the mechanism of action of EGF on adrenal development and to assess the role of the fetal hypothalamic-pituitary axis in mediating the effects of EGF on adrenal growth, we administered the competitive CRF antagonist [D-phenylalanine¹², Norleucine^{21,38}] CRF¹²⁻⁴¹ in combination with EGF. We found that the effects of EGF on increasing adrenal weight and stimulating the growth of the DZ were blocked by the administration of the CRF antagonist. These data suggest that EGF mediates its effects on adrenal growth not by a direct action on the fetal adrenal but indirectly via the fetal hypothalamic-pituitary axis. It is clear that from previous studies that this CRF antagonist is effective in the fetal rhesus monkey at modulating pituitary ACTH secretion as it has been shown to block the fetal hemorrhage-induced increase in fetal plasma ACTH concentrations (1, 20). We also note that the CRF antagonist was able to block the secondary effects of metyrapone. Recently, we have shown that metyrapone treatment alone dramatically stimulated adrenal cortical growth (28). Metyrapone blocks cortisol synthesis by inhibiting CYP11B1 activity and thus removes the negative feedback effect of cortisol to activate the fetal hypothalamic-pituitary axis and stimulate adrenal growth. These findings provide further evidence to demonstrate the effectiveness of the CRF

antagonist in blocking fetal pituitary ACTH secretion and also suggest that EGF acts via the fetal hypothalamic-pituitary axis to stimulate adrenal cortical growth. However, in the present study we did not measure fetal plasma ACTH concentrations.

The effects of EGF administration on stimulating the premature morphologic maturation of the primate fetal adrenal are paralleled by the stimulation of the functional maturation of the adrenal gland. The induction of CYP11B protein in the DZ and 3βHSD protein in the TZ of the fetal adrenal are markers for the functional differentiation of the fetal into the adult cortex (9, 12, 13). Between 110 and 150 d of gestation, CYP11B is localized predominantly in the TZ and 3βHSD is localized predominantly in the DZ, whereas after 155 d gestation, CYP11B and 3βHSD are localized extensively in both the DZ and TZ of the fetal primate adrenal gland (12, 13). The premature expression of CYP11B in the DZ and 3βHSD in the TZ can be induced by increasing fetal pituitary ACTH (12, 13). We also found that the IP and IA administration of EGF stimulated an increase in adrenal content of 3βHSD protein primarily by inducing expression of 3βHSD protein in the TZ (14). In the current study, the administration of EGF via the IP route alone was sufficient to stimulate the functional maturation of the fetal adrenal as indicated by the premature induction of expression of CYP11B protein in the DZ and 3βHSD protein in the TZ. However, if EGF was administered via the IA route alone, there was no change in the pattern of localization of either CYP11B or 3βHSD, consistent with the lack of a significant effect of EGF IA administration on adrenal weight and DZ growth measurements. These data indicate that the effective route of action of EGF on adrenal gland growth and functional maturation is achieved via the IP route alone but not via the IA route.

The CRF antagonist successfully inhibited the EGF-activated induction of 3βHSD in the TZ. These data suggest that the effects of EGF on functional maturation and induction of 3βHSD are also mediated via the hypothalamic-pituitary axis. It is interesting that whereas administration of the competitive CRF antagonist blocked the effects of EGF on inducing 3βHSD in the TZ, we still observed increased 3βHSD staining and induction of CYP11B-ir in the DZ, which is the putative zona glomerulosa of the adult adrenal gland. We know from studies in the adult adrenal gland that the zona glomerulosa and zona fasciculata are differentially regulated via angiotensin and ACTH, respectively. Our data suggest that in the fetal adrenal gland there may be a direct action of EGF on the DZ. Previous studies have shown that EGF receptors are present on human fetal adrenal cells (16) and that EGF stimulates aldosterone secretion from the zona glomerulosa (29). Alternatively EGF may modulate its effects via angiotensin II, as EGF has been shown to potentiate angiotensin II-induced aldosterone secretion (29). Clearly, the role of EGF in mediating functional maturation of the DZ requires further investigation.

In summary, we have shown that IP administration of EGF is effective in stimulating the growth and functional maturation of the fetal primate adrenal gland. Administration of a competitive CRF antagonist, [D-phenylalanine¹², Norleucine^{21,38}] CRF¹²⁻⁴¹, blocked the action of EGF on stimulating adrenal

growth and the induction of 3β HSD in the TZ but did not diminish the increase in 3β HSD staining or induction of CYP11B in the DZ. These data suggest that EGF acts via the hypothalamic-pituitary axis to modulate the growth of the DZ (the putative zona glomerulosa) and growth and functional maturation of the TZ (the putative zona fasciculata), whereas EGF acts independently of the hypothalamic-pituitary axis to stimulate functional maturation of the DZ.

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