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Interaction of Insulin-Like Growth Factor-II and Estradiol Directs Steroidogenesis in the Human Fetal Adrenal toward Dehydroepiandrosterone Sulfate Production*

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ABSTRACT

We examined the regulation of steroid production in fetal zone cells from midgestation (16–21 weeks) human fetal adrenal glands to elucidate the mechanism by which these cells secrete large quantities of dehydroepiandrosterone sulfate (DHAS) and little cortisol in response to ACTH. Our underlying hypothesis is that estrogen and insulin-like growth factor-II (IGF-II) modulate the steroidogenic response of fetal zone cells to ACTH, driving steroid production toward DHAS rather than cortisol. We also hypothesize that the effects of IGF-II and estrogen on steroidogenesis are achieved by modulating the expression of key enzymes in the steroidogenic pathway. Basal cortisol secretion by cultured fetal zone cells was below the limit of assay sensitivity (<0.54 pmol/10^5 cells·24 h), whereas basal DHAS secretion was 210.8 ± 41.0 pmol/10^5 cells·24 h (mean ± SE). ACTH-(1–24) increased the secretion of cortisol to 228.96 ± 6.75 pmol/10^5 cells·24 h and that of DHAS to 2039.8 ± 121.7 pmol/10^5 cells·24 h. Neither IGF-II nor estradiol (E_2) affected basal (no added ACTH) steroid secretion by fetal zone cells. IGF-II increased ACTH-stimulated cortisol and DHAS secretion by fetal zone cells in a dose-dependent fashion. In contrast, E_2 at high concentrations (1–10 μmol/L) decreased ACTH-stimulated cortisol production to basal levels, but increased ACTH-stimulated DHAS production 1.5- to 2-fold. Combinations of IGF-II (100 ng/mL) and E_2 (1 μmol/L) increased ACTH-stimulated cortisol and DHAS secretion by 1.5- to 2-fold compared with control values. However, compared with cultures exposed to IGF-II alone, inclusion of E_2 decreased ACTH-stimulated cortisol secretion by about 60% and increased ACTH-stimulated DHAS secretion by about 50%. IGF-II increased the abundance of ACTH-stimulated mRNAs encoding cholesterol side-chain cleavage cytochrome P450 (P450scc), 17α hydroxylase/17,20 lyase P450 (P450c17), and 3β-hydroxysteroid dehydrogenase (3βHSD). In addition, IGF-II increased the abundance of mRNA encoding P450c17 under basal conditions, but did not affect the basal expression of P450scc or 3βHSD. E_2 had no effect on basal expression of these steroidogenic enzymes, but increased the abundance of ACTH-stimulated mRNA encoding P450scc and P450c17. The abundance of mRNA encoding 3βHSD was not affected by E_2. The effect of IGF-II and E_2 in combination on steroidogenic enzyme mRNA abundance was not different from that of IGF-II alone.

These data indicate that IGF-II increases ACTH-stimulated steroid production in fetal zone cells by increasing the expression of key steroidogenic enzymes. Estrogen may increase ACTH-stimulated DHAS secretion by increasing the expression of P450scc and P450c17. Although the mechanism by which estrogen inhibits cortisol production is uncertain, our data clearly show that this effect is not due to an inhibition of 3βHSD expression. These data indicate that IGF-II and E_2 can influence the steroidogenic activity of fetal zone cells. As the human fetal adrenal is exposed to high concentrations of these substances, it is likely that they influence the steroidogenic response of fetal zone cells to ACTH *in vivo*, resulting in the production of DHAS rather than cortisol. (*J Clin Endocrinol Metab* **77:** 754–758, 1993)

THE HUMAN fetal adrenal cortex is composed of two morphologically distinct zones, the definitive zone and the fetal zone. The definitive zone is a narrow band of cells on the periphery of the cortex, whereas the fetal zone comprises the bulk of the cortex and, by midgestation, occupies 80–90% of the gland's mass (for reviews, see Refs. 1 and 2). Although steroid production in both zones can be regulated by ACTH secreted from the fetal pituitary, *in vitro* studies have shown that in response to ACTH, the definitive zone secretes predominantly cortisol, whereas the fetal zone secretes large quantities of dehydroepiandrosterone sulfate (DHAS) and little cortisol (3). In the present study, we have examined the mechanism by which fetal zone cells produce DHAS rather than cortisol in response to ACTH.

Our underlying hypothesis is that fetal zone cells are exposed to factors that modulate their steroidogenic response to ACTH in such a manner that steroidogenesis is directed toward DHAS, and not cortisol, synthesis. Two factors that may influence fetal zone steroid production are insulin-like growth factor-II (IGF-II) and estrogen. Several studies have indicated that the high concentrations of circulating estrogens in the human fetus may inhibit ACTH-stimulated cortisol and enhance ACTH-stimulated DHAS secretion by fetal zone cells. Fujieda *et al.* (4) and Voutilainen *et al.* (5) demonstrated that estradiol (E_2), albeit at high concentrations (1–10 μmol/L), inhibits ACTH-stimulated cortisol production by cultured fetal zone cells, whereas DHAS production is increased. Growth factors also may modulate steroidogenesis in fetal zone cells. In adult bovine adrenal cortical cells, IGF-I is required for maintenance of sensitivity to ACTH (6). In the human fetal adrenal, ACTH stimulates the expression of a closely related peptide, IGF-II (7–10), which we have shown to be a mitogen for fetal zone cells (10). It is possible, therefore, that IGF-II, like IGF-I in adult bovine adrenal cortical cells, may modulate the steroidogenic potential of human fetal zone cells.

As fetal zone cells are exposed to high circulating estrogen concentrations and produce large amounts of IGF-II, which

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probably act in an autocrine/paracrine fashion (10), we investigated whether these factors modulate the steroidogenic potential of fetal zone cells, possibly resulting in their production of DHAS rather than cortisol in response to ACTH. In addition, we examined the effects of IGF-II and E_2 on the expression of key steroidogenic enzymes in fetal zone cells in an effort to determine the mechanism by which these factors may modulate steroidogenesis.

Materials and Methods

Cell culture

Human fetal adrenal glands were obtained from second trimester fetuses (16–21 weeks gestation) after therapeutic termination of pregnancy by dilatation and evacuation. Gestational age was estimated by foot length. The study protocol was approved by the Committee on Human Research of the University of California, San Francisco (UCSF).

Primary human fetal adrenal cortical cell cultures, consisting mainly (90–95%) of fetal zone cells, were prepared as previously described (9). Briefly, the glands were decapsulated to remove most of the definitive zone, and the remaining fetal zone cells were dispersed by enzymatic digestion and plated onto plastic culture dishes (Falcon Plastics, Los Angeles, CA) in Dulbecco's Modified Eagle's H-16/Ham's F-12 (1:1) medium containing nonessential amino acids and supplemented with 10% fetal calf serum, 2 mmol/L glutamine, and 50 μg/mL gentamicin (Cell Culture Facility, UCSF) in a humidified environment at 37 C in 95% air-5% CO_2. For steroidogenic studies, cells were plated at a density of approximately 50,000 cells/well on 48-well plates. Cells used for mRNA analysis were plated on 6-cm dishes at a density of approximately 500,000 cells/dish. After 48 h in culture, the medium was changed to one containing 2.5% fetal calf serum, and test substances (IGF-II and/or E_2) were added in the doses shown in the figures. Human recombinant IGF-II was a generous gift from Dr. J. P. Merryweather, Chiron Corp. (Emeryville, CA), and E_2 (17β-estradiol) was obtained from Sigma (St. Louis, MO). Media were changed and test substances replenished 48 h later. At this point, some wells received ACTH-(1–24) (0.1 nmol/L; Organon, West Orange, NJ). After a further 48 h, the media were collected, and cells were harvested by trypsinization and counted in a particle counter (Coulter Electronics, Hialeah, FL). For mRNA studies, cells were treated in an identical fashion to those on 48-well plates. After exposure to test substances and ACTH, the cells were harvested, and total RNA was extracted using the method of Chomczynski and Sacchi (11).

RNA analysis

The abundance of mRNA for the enzymes cholesterol side-chain cleavage cytochrome P450 (P450scc), 17α hydroxylase/17,20 lyase P450 (P450c17), and 3β-hydroxysteroid dehydrogenase (3βHSD) was assessed by Northern hybridization analysis of total RNA extracted from cultured fetal adrenal cortical cells. Total RNA was denatured in formaldehyde and subjected to electrophoresis through a 1.2% agarose gel, then transferred to a Hybond-N nitrocellulose membrane (Amersham International, Aylesbury, Buckinghamshire, United Kingdom). Full-length cDNAs for P450scc and P450c17 were obtained from Dr. W. L. Miller, UCSF (12, 13), and the full-length cDNA for human type-II 3βHSD was obtained from Dr. F. Labrie, Centre de Recherche du Chul, University of Laval (Quebec, Canada) (14). [^{32}P]Deoxy-CTP-labeled cDNA probes for 3βHSD, P450scc, P450c17, and rat cyclophilin (15) were synthesized by random primer extension of full-length cDNAs. Prehybridization was performed in hybridization buffer (Quickhyb Buffer, Stratagene, La Jolla, CA) at 68 C for 15 min. Denatured radiolabeled probe was then added to the membranes and incubated at 68 C for 1 h. Membranes were washed in 2 × SSC (1 × SSC = 0.15 mmol/L NaCl/0.015 mmol/L Na citrate) 0.1% sodium dodecyl sulfate at room temperature for 15 min and then in 0.1 × SSC-0.1% sodium dodecyl sulfate at 60C for 30 min and subjected to autoradiography at −70 C. Probes were removed by washing the membranes in distilled water at 100 C. Complete removal of probe was confirmed by autoradiography before reprobing. Membranes were hybridized sequentially with the aforementioned cDNA probes in the order described.

RIAs

Cortisol and DHAS were measured in conditioned medium using specific RIAs, which we have previously described (3). Unconjugated cortisol was assayed using a kit purchased from Diagnostic Products Corp. (Los Angeles, CA). DHAS was assayed using an antiserum specific for DHAS purchased from ICN Biomedicals, Inc. (Costa Mesa, CA), with charcoal separation of free from bound steroid. All assays were validated for use on conditioned medium from fetal adrenal cortical cell cultures, and each had inter- and intraassay coefficients of variation of less than 10%.

Statistical analysis

All cortisol and DHAS data were normalized for cell number and time of exposure to ACTH-(1–24) and are presented as the mean ± SE. All experiments were performed in triplicate wells and repeated at least three times. Statistical analyses were conducted by analysis of variance, followed by Newman-Keuls *post-hoc* test for significance between groups. Differences were considered statistically significant when $P < 0.05$.

Results

Effects of IGF-II and E_2 on steroidogenesis

IGF-II increased ACTH-stimulated cortisol and DHAS production in cultured fetal zone cells in a dose-dependent fashion (Fig. 1). The magnitude of this effect was highly variable between experiments, probably due to variable plating efficiency. Despite this variation, a significant increase in ACTH-stimulated cortisol production was detected in wells containing IGF-II in all experiments (n = 5). In contrast, the effect of IGF-II on DHAS production was not as pronounced, and in some cases did not achieve statistical significance. In all experiments, IGF-II caused a 2- to 3-fold increase in cell number. There was no effect of IGF-II on basal cortisol or DHAS production.

At concentrations greater than 1 μmol/L, E_2 decreased ACTH-stimulated cortisol production by human fetal adrenal cortical cells to almost basal levels and increased ACTH-stimulated DHAS production almost 2-fold (Fig. 2). At concentrations below 1 μmol/L, E_2 had no effect on cortisol or DHAS production by fetal zone cells. There was no effect of E_2 on basal cortisol and DHAS production or cell proliferation.

The combination of E_2 (1 μmol/L) and IGF-II (100 ng/mL) increased ACTH-stimulated cortisol and DHAS production compared with control values. However, compared with that in wells exposed to IGF-II alone, the combination of IGF-II and E_2 significantly decreased ACTH-stimulated cortisol and increased ACTH-stimulated DHAS production in fetal zone cells (Fig. 3).

Effects of IGF-II and E_2 on steroidogenic enzyme expression

Under basal conditions (no ACTH, IGF-II, or E_2), mRNAs encoding P450scc, P450c17, and 3βHSD were undetected by Northern blotting. As expected, ACTH increased the abundance of mRNA encoding each enzyme, with mRNA encoding P450c17 being the most responsive to ACTH stimulation. The amounts of mRNA encoding P450scc and 3βHSD were

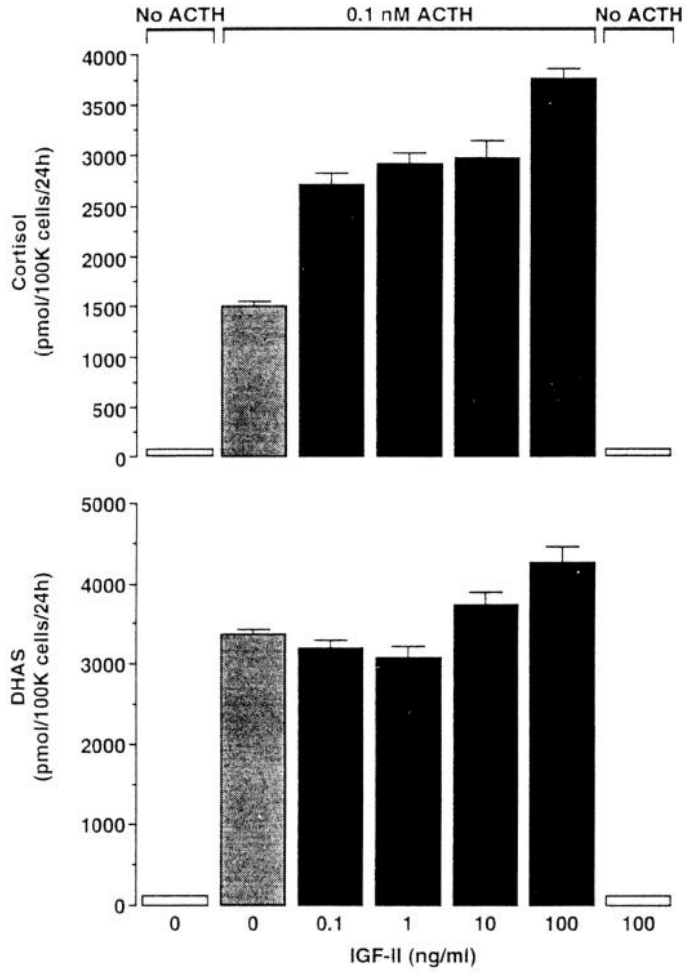

FIG. 1. Effects of IGF-II on basal and ACTH-stimulated cortisol and DHAS production by fetal zone cells. Cells were incubated with various concentrations of IGF-II as described in the text. Data are the mean ± SEM of five experiments.

about 10- and 100-fold lower (assessed by computer-imaged densitometry; Biosoft, Inc., Cambridge, England) respectively, than that for P450c17 in cells treated with ACTH alone.

IGF-II (100 ng/mL) increased the abundance of ACTH-stimulated mRNA encoding P450scc, P450c17, and 3βHSD. IGF-II also increased the basal abundance of mRNA encoding P450c17, but did not affect the basal abundance of mRNAs encoding P450scc and 3βHSD. E_2 (1 μmol/L) increased ACTH-stimulated accumulation of mRNA encoding P450scc and P450c17, but did not affect the abundance of mRNA encoding 3βHSD. E_2 had no effect on the basal abundance of these mRNA species. The combined effect of IGF-II (100 ng/ml) and E_2 (1 μmol/L) on each of these mRNA species was not different from the effect of IGF-II alone (Fig. 4).

Discussion

In this study, we examined the hypothesis that the steroidogenic activity of fetal zone cells in the midgestation human fetal adrenal cortex is modulated by IGF-II and estrogen such that steroid production is directed toward DHAS rather than cortisol. Our data show that IGF-II increases cortisol and DHAS production in fetal zone cells in response to ACTH. In contrast, E_2 inhibits cortisol production to basal levels and increases DHAS production. IGF-II and E_2 in combination increase DHAS production and decrease cortisol production by fetal zone cells. These data indicate that *in vivo*, both IGF-II and estrogen may influence the steroidogenic activity of fetal zone cells, causing them to produce large amounts of DHAS and little cortisol in response to ACTH.

To elucidate the mechanism of action of IGF-II and E_2, we examined whether changes in steroid production induced by these factors are due to alterations in the expression of key steroidogenic enzymes. We found that IGF-II increased the ACTH-stimulated abundance of mRNAs encoding P450scc, P450c17, and 3βHSD in fetal zone cells. In addition, IGF-II increased the basal abundance of mRNA encoding P450c17. E_2 also increased ACTH-stimulated accumulation of mRNA encoding P450scc and P450c17, but had no effect on the abundance of mRNA encoding 3βHSD. The basal abundance of mRNA encoding all three enzymes was not affected by E_2.

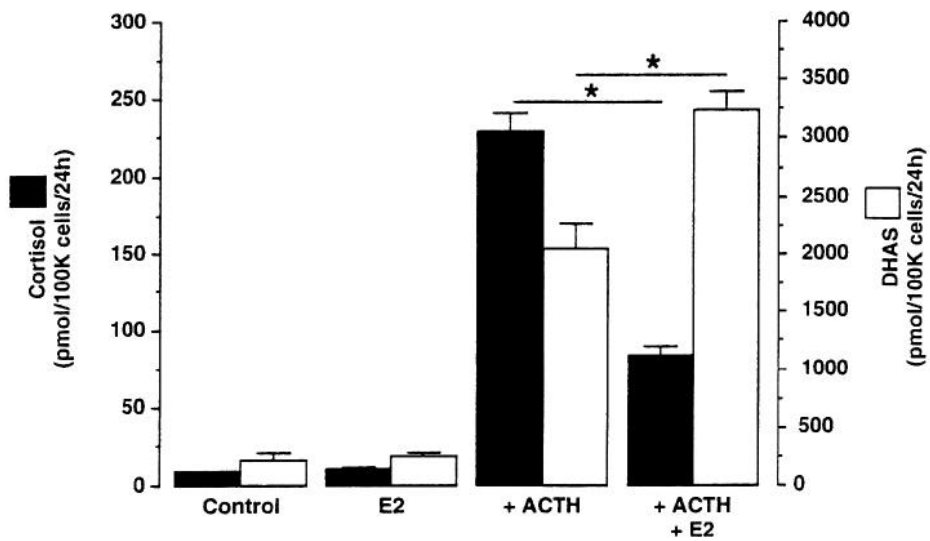

FIG. 2. Effects of E_2 (1 μmol/L) on basal and ACTH-stimulated (0.1 nmol/L) cortisol and DHAS production by fetal zone cells. Data are the mean ± SEM of three experiments. ★, $P < 0.05$.

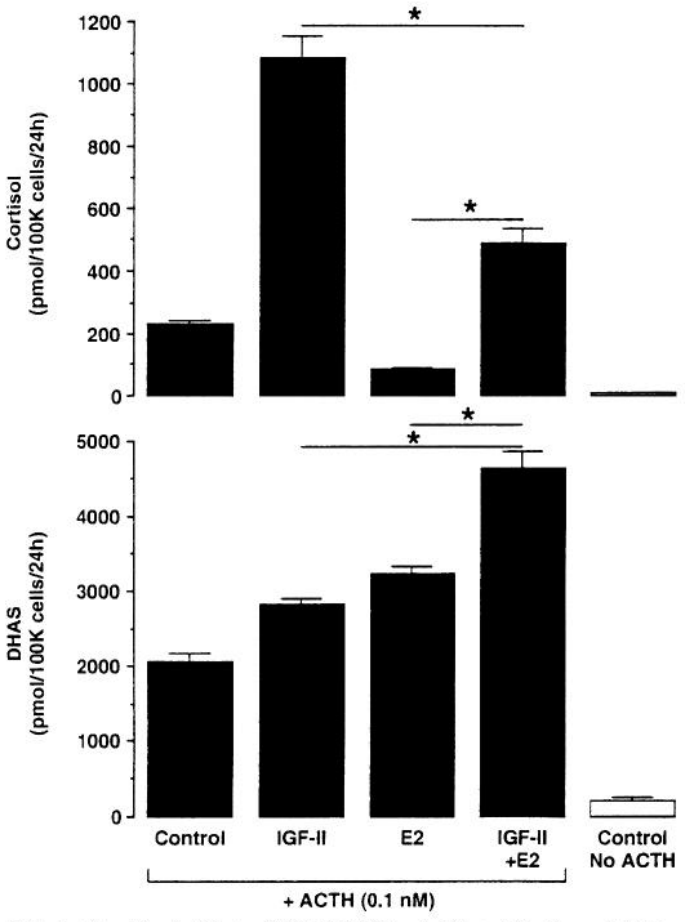

FIG. 3. Combined effects of IGF-II (100 ng/mL) and E_2 (1 μmol/L) on cortisol and DHAS production by fetal zone cells. Data are the mean ± SEM of three experiments. ★, $P < 0.05$.

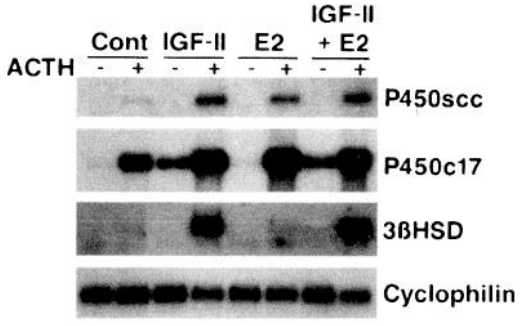

FIG. 4. Northern blot analysis of total RNA (10 μg) from IGF-II-, E_2-, and IGF-II/E_2-treated cells in the presence and absence of ACTH, showing the effects of these substances on the steady state abundance of mRNAs encoding P450scc (overnight exposure), P450c17 (2-h exposure), 3βHSD (2-day exposure), and cyclophilin (4-h exposure). Cont, Control.

The increase in steroidogenic enzyme gene expression caused by IGF-II is consistent with the effects of IGF-II on ACTH-stimulated cortisol and DHAS production in fetal zone cells. The enhancement of ACTH-stimulated expression of 3βHSD by IGF-II is consistent with the increased production of cortisol in response to ACTH, and the increased expression of P450c17 is consistent with the increase in ACTH-stimulated DHAS production. It is of interest that IGF-II stimulated P450c17 expression in the absence of ACTH without affecting steroid production. This direct effect of IGF-II on P450c17 expression indicates that expression of this enzyme can be regulated by factors other than ACTH. The physiological significance of this effect of IGF-II is uncertain. It appears that IGF-II is able to enhance the steroidogenic activity of fetal zone cells, such that when they are stimulated by ACTH they have an enhanced potential for steroid production.

The mechanism by which IGF-II increases ACTH-stimulated P450scc, P450c17, and 3βHSD expression is uncertain. In bovine adrenal cortical cells, a related peptide, IGF-I, has been shown to increase ACTH-stimulated cAMP production and maintain the cells' sensitivity to ACTH. Our data clearly show that IGF-II enhances the action of ACTH on fetal zone cells, possibly by increasing the sensitivity of the cells to ACTH. It is possible that IGF-II increases fetal zone cell sensitivity to ACTH by increasing the number and/or affinity of ACTH receptors on these cells. This effect of IGF-II would result in fetal zone cells with enhanced steroidogenic activity *in vivo* and, coupled with the inhibitory effect of E_2 on cortisol production, would lead to an increase in DHAS synthesis.

The changes in steroidogenic enzyme expression caused by E_2 do not fully explain its effects on steroid production. E_2 increased ACTH-stimulated expression of P450scc and P450c17. To our knowledge, this is the first time that such an effect of E_2 has been observed. The biological significance of this effect of E_2 is uncertain, particularly as the concentrations of E_2 required to cause this effect were in the micromolar range. However, this may explain why E_2 increased ACTH-stimulated DHAS production by fetal zone cells, but does not explain why E_2 descreased ACTH-stimulated cortisol production. The production of DHAS and not cortisol by the fetal zone has been attributed to a deficiency of 3βHSD activity in fetal zone cells (16, 17). As a consequence, fetal zone cells cannot convert Δ^5-steroids to Δ^4-steroids. This step in the steroidogenic pathway is essential for cortisol synthesis, but not for DHAS synthesis. Coupled with the high activity of P450scc and P450c17 enzymes, the deficiency in 3βHSD results in the conversion of pregnenolone to dehydroepiandrosterone and subsequently DHAS. Protein and mRNA analyses have shown that 3βHSD is lacking in fetal zone cells *in vivo* (16, 17). However, cultured fetal zone cells are able to synthesize cortisol in response to ACTH, indicating that *in vitro*, these cells can express 3βHSD and that its expression can be induced by ACTH. If ACTH induces 3βHSD expression *in vitro*, why is this enzyme not expressed by fetal zone cells *in vivo*? A possible answer is that 3βHSD activity in fetal zone cells is suppressed *in vivo*. Fujieda *et al.* (4) proposed that estrogens inhibit 3βHSD expression in fetal zone cells. Our data, however, show that although E_2 inhibits cortisol production, it does not inhibit the expression of 3βHSD. The inhibition of ACTH-stimulated cortisol production by E_2, therefore, was not due to decreased expression of 3βHSD, as previously suggested (4). Recently, Hirst *et al.* (18) have shown that fetal zone cells from rhesus monkey fetal adrenals lack estrogen receptors. Their findings suggest that the estrogens do not modulate the function of primate fetal adrenals by receptor-mediated mechanisms. Our findings

that 3βHSD expression was not affected by E_2, and the effects of E_2 only occurred at high concentrations (significantly higher than the K_d for the estrogen receptor) lend support to this hypothesis. The mechanism by which E_2 inhibited cortisol production in the present studies is not known.

Our findings suggest that 3βHSD expression by fetal zone cells *in vivo* is not inhibited by placental estrogens. By 30 weeks gestation in human fetal adrenals, 3βHSD is expressed in definitive zone cells, but is still lacking in fetal zone cells (16). Recently, we have investigated the functional zonation of the primate (human and rhesus monkey) fetal adrenal cortex and have found that late in gestation, 3βHSD also is expressed in cells lying between the fetal and definitive zones, which we refer to as the transitional zone. This zone may represent a third functional zone that is analogous to the zona fasciculata. It appears, therefore, that whatever the factor is which inhibits 3βHSD expression, it specifically exerts its effects on fetal zone cells. That 3βHSD is not expressed by fetal zone cells *in vivo* also suggests that the stimulation of its expression by IGF-II does not occur *in vivo*. It is possible that the factor(s) that inhibits 3βHSD expression in fetal zone cells *in vivo* is able to override any stimulation of 3βHSD expression by IGF-II.

Thus, we have shown that IGF-II increases ACTH-stimulated cortisol and DHAS production by fetal zone cells and that E_2, at high concentrations, inhibits ACTH-stimulated cortisol production by these cells. The actions of IGF-II can be explained by its increasing the sensitivity of fetal zone cells to ACTH and enhancing ACTH-stimulated expression of key steroidogenic enzymes. E_2 also increases ACTH-stimulated expression of P450scc and P450c17, but does not affect the expression of 3βHSD. Although the mechanism by which E_2 inhibits ACTH-stimulated cortisol production by fetal zone cells is not clearly understood, it does not appear to be mediated via the estrogen receptor. As fetal zone cells *in vivo* are exposed to both IGF-II and E_2 at relatively high concentrations (19), it is likely that both substances can modulate the steroidogenic potential of fetal zone cells, directing their activity toward the production of DHAS rather than cortisol. Thus, the availablity of DHAS for placental estrogen production may be regulated by a positive feedback loop, in which estrogens, in conjunction with IGF-II, enhance the production of DHAS from fetal zone cells, thereby increasing substrate available for placental estrogen production.

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References

1. **Serón-Ferré M, Jaffe RB.** 1981 The fetal adrenal gland. Annu Rev Physiol. 43:141–162.
2. **McClellan M, Brenner RM.** 1981 Development of the fetal adrenals in nonhuman primates: electron microscopy. In: Novy MJ, Resko JA, eds. Fetal endocrinology. New York: Academic Press; 383–400.
3. **Serón-Ferré M, Lawrence CC, Siiteri PK, Jaffe RB.** 1978 Steroid production by definitive and fetal zones of the human fetal adrenal gland. J Clin Endocrinol Metab. 47:603–609.
4. **Fujieda K, Faiman C, Reyes FI, Winter JSD.** 1982 The control of steroidogenesis by human fetal adrenal cells in tissue culture. IV. The effects of exposure to placental steroids. J Clin Endocrinol Metab. 54:89–94.
5. **Voutilainen R, Kahri AI, Salmenpera M.** 1979 The effects of progesterone, pregnenolone, estriol, ACTH and hCG on steroid secretion of cultured human fetal adrenals. J Steroid Biochem. 10:695–700.
6. **Penhoat A, Chatelain PG, Jaillard C, Saez JM.** 1988 Characterization of insulin-like growth factor I and insulin receptors on cultured bovine adrenal fasciculata cells. Role of these peptides on adrenal cell function. Endocrinology. 122:2518–2526.
7. **Voutilainen R, Miller WL.** 1987 Coordinate tropic hormone regulation of mRNAs for insulin-like growth factor II and cholesterol side-chain-cleavage enzyme, P450ssc, in steroidogenic tissues. Proc Natl Acad Sci USA. 84:1590–1594.
8. **Voutilainen R, Miller WL.** 1988 Developmental and hormonal regulation of mRNAs for insulin-like growth factor II and steroidogenic enzymes in human fetal adrenals and gonads. DNA. 7:9–15.
9. **Mesiano S, Mellon SH, Gospodarowicz D, Di Blasio AM, Jaffe RB.** 1991 Basic fibroblast growth factor expression is regulated by ACTH in the human fetal adrenal: a model for adrenal growth regulation. Proc Natl Acad Sci USA. 88:5428–5431.
10. **Mesiano S, Mellon SH, Jaffe RB.** 1993 Mitogenic action, regulation and localization of insulin-like growth factors in the human fetal adrenal gland. J Clin Endocrinol Metab. 90:142–149.
11. **Chomczynski P, Sacchi N.** 1987 Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156–159.
12. **Chung BC, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL.** 1986 Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. Proc Natl Acad Sci USA. 83:8962–8966.
13. **Chung BC, Picado-Leonard J, Haniu M, et al.** 1987 Cytochrome P450c17 (steroid 17α-hydroxylase/17,20 lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. Proc Natl Acad Sci USA. 84:407–411.
14. **Rheaume E, Lachance Y, Zhao H, et al.** 1991 Structure and expression of a new complementary DNA encoding the almost exclusive 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase in human adrenals and gonads. Mol Endocrinol. 5:1147–1157.
15. **Danielson PA, Forss-Petter S, Brow MA, et al.** 1988 p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. DNA. 7:261–267.
16. **Dupont E, Luu-The V, Labrie F, Pelletier G.** 1990 Ontogeny of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) in human adrenal gland performed by immunocytochemestry. Mol Cell Endocrinol. 74:R7–R10.
17. **Voutilainen R, Ilveskmaki V, Miettinen P.** 1991 Low expression of 3β-hydroxy-5-ene steroid dehydrogenase gene in human fetal adrenals *in vivo*; adrenocorticotropin and protein kinase C-dependent regulation in adrenocortical cultures. J Clin Endocrinol Metab. 72:761–767.
18. **Hirst JJ, West NB, Brenner RM, Novy MJ.** 1992 Steroid hormone receptors in the adrenal glands of fetal and adult rhesus monkeys. J Clin Endocrinol Metab. 75:308–314.
19. **Shutt DA, Smith ID, Shearman RP.** 1974 Oestrone, oestradiol-17β and oestriol levels in human foetal plasma during gestation and at term. J Endocrinol. 60:333–341.