Insulin-Like Growth Factors Enhance Steroidogenic Enzyme and Corticotropin Receptor Messenger Ribonucleic Acid Levels and Corticotropin Steroidogenic Responsiveness in Cultured Human Adrenocortical Cells*

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ABSTRACT

In several species, including the human fetus, insulin-like growth factors (IGF-I and IGF-II) have been reported to modulate adrenal steroidogenesis, thus contributing to adrenal cortical differentiation. In the present study, we examined the long term effects of IGF-I and -II on human adult adrenal fasciculata-reticularis cells cultured in a chemically defined medium and compared them to the effects of insulin, human GH, and ACTH. Treatment for 3 days with IGF-I or -II at nanomolar concentrations or with insulin at micromolar concentrations slightly increased the production of androstenedione, cortisol, and dehydroepiandrosterone about 1.5-fold over that by control cells.

Moreover, the acute steroidogenic response to ACTH of cells pretreated with IGF-I, IGF-II, or insulin was 3- to 6-fold higher than that of control cells. For each hormone, these effects of IGF-I and -II were dose dependent between 0.1–26 nmol/L (1–200 ng/mL). The secretion of androstenedione was more potently stimulated than that of dehydroepiandrosterone and cortisol, and this effect was more clearly yielded by pretreatment with IGF-II than with IGF-I or insulin. Human GH had no effect on these cells.

In cells treated with IGF-I or -II, the messenger ribonucleic acid (mRNA) levels of cytochrome P450 17α-hydroxylase and of 3β-hydroxysteroid dehydrogenase were increased, and the abundance of ACTH receptor mRNA was also slightly enhanced, but the mRNA of cytochrome P450 cholesterol side-chain cleavage enzyme was unchanged.

In conclusion, IGFs enhance the steroidogenesis and ACTH responsiveness of human adrenocortical cells in culture. We speculate, that by this mechanism, IGFs may contribute to clinical states with hyperandrogenemia. (*J Clin Endocrinol Metab* 81: 3892–3897, 1996)

THE FACTORS controlling adrenarche, differentiation of the zona reticularis of the human adrenal gland, and secretion of adrenal androgens are not entirely clear (1, 2). In addition to ACTH (1, 3), other factors can promote formation of the most important adrenal androgen, dehydroepiandrosterone (DHEA), and its sulfate, *e.g.* extraadrenal hormones such as PRL in high concentrations (1); however, a cortical androgen-stimulating hormone has not yet been identified (4). Several intraadrenal factors, of neuroendocrine origin (5), or growth factors, *e.g.* transforming growth factor-β (6), may be involved in the local regulation of DHEA formation. Insulin-like growth factors (IGFs), besides their mitogenic effects, maintain differentiated functions of various organs, *e.g.* in steroidogenic ovarian (7) and testicular (8) cells. Whereas IGF-I plays a major role postnatally, IGF-II is an important regulator of fetal development; in human fetal adrenal cells, IGF-II enhances steroidogenesis (9), and its effect on secretion of DHEAS is potentiated by estradiol (10). An auto- or paracrine, ACTH-stimulated, formation of IGF-II has been demonstrated in human fetal adrenal cells (11–13). In contrast, the effects and production of IGF-I in fetal adrenals are negligible (9, 12). As deduced from bovine adrenocortical cells (BAC) in culture (reviewed in Ref. 14), IGF-I and its binding proteins are locally secreted, and like the adrenal IGF-I receptors, they are controlled by ACTH and angiotensin II. On the other hand, IGF-I enhances the effects of ACTH and angiotensin II, increasing the respective receptors and their coupling proteins; it also modulates steroidogenesis, stimulating the activities of 3β-hydroxysteroid dehydrogenase (3βHSD), 11β-hydroxylase, and 21-hydroxylase. In human adrenocortical cells (HAC), it has been shown that 1) IGF-I is specifically bound, mainly in the zona reticularis, to a 135-kDa receptor species (15); and 2) IGF-I enhances the same enzyme activities as in BAC and, additionally, that of 17α-hydroxylase (16).

IGF data derived from adult human adrenals have been rare due to the scarce availability of human tissues. As adrenarche is unique in the human species, and the regulation of adrenal steroidogenesis is strictly species specific (17), and

as, on the other hand, the role of the IGF system varies among species (18), the results observed in other species are not transferable to humans. The aim of this work was to study the effects of both IGF-I and -II on the differentiated functions of the human adrenal cortex; therefore, using adult human fasciculata-reticularis cells cultured in a chemically defined medium, we investigated the long term effects of IGF-I and IGF-II on the secretion of steroids and the ACTH responsiveness as well as the messenger ribonucleic acid (mRNA) abundance of ACTH receptor mRNA and steroidogenic enzymes. The effects of IGFs were compared to those of other growth-promoting factors, such as human GH (hGH) and insulin, and to the chronic effects of ACTH.

Materials and Methods

Substances and hormones

Recombinant human IGF-I and hGH were kindly donated by Pharmacia (Erlangen, Germany), and recombinant human IGF-II was supplied by Lilly (Bad Homburg, Germany). Synthetic adrenocorticotropin [ACTH-(1–24), Synacthen] was purchased from Ciba (Wehr, Germany), Insulin, deoxyribonuclease, transferrin, ascorbic acid, and steroids [DHEA, Δ^4-androstenedione, cortisol, and 22(R)-hydroxycholesterol, pregnenolone, progesterone, and 17α-hydroxyprogesterone] were obtained from Sigma (Munich, Germany); media (DMEM and Ham's F-12), penicillin/streptomycin, gentamicin, nystatin, FCS, and horse serum were purchased from Life Technologies (Eggenstein, Germany), and collagenase was obtained from Serva (Heidelberg, Germany). All other chemicals were obtained from Sigma or Merck (Munich or Darmstadt, Germany).

Human adrenal primary cell culture and hormonal treatments

Human adrenals were obtained from 20 adult kidney or multiorgan transplantation donors, with the approval of the ethical committee of the Virchow-Klinikum, Humboldt-Universität (Berlin, Germany). Macroscopic dissection of the zona fasciculata-reticularis and dispersion by collagenase/deoxyribonuclease were performed as described previously (19). More than 95% of cultured cells were 3βHSD positive by histochemical staining. Moreover, as described before, less than 10% of these cells were glomerulosa cells (19). Cells (2×10^5 to $5 \times 10^5/1$ mL) were cultured for the first 16 h in DMEM-Ham's F-12 medium containing 10% FCS, transferrin (1.25 nmol/L), and vitamin C (0.1 mmol/L) in 12-well dishes. After two washes, cells were maintained in the same medium as that described above, but without serum, from the second to the sixth day of culture (controls), or submitted to 72- to 96-h treatments with IGF-I or IGF-II (0.013–26 nmol/L; 0.1–200 ng/mL), ACTH (10 nmol/L; 45 ng/mL), hGH (4.7 pmol/L; 100 ng/mL), or insulin (0.9 μmol/L; 5 μg/mL). Medium was replaced every 24 h and centrifuged, and the supernatant was frozen at -20 C in aliquots for steroid hormone determination. Control and treated cells were then washed with medium and incubated for 2 h with ACTH (1 nmol/L) or 5×10^{-5} mol/L steroid precursors. The reaction was stopped on ice, and the medium was stored at -20 C until hormone determination. At the end of the experiments, cells of each condition, tested in at least three wells, were counted and tested for viability by trypan blue exclusion, which was always 85% or more.

Hormone measurements

Steroid hormone secretion into adrenocortical cell-conditioned medium was measured by direct ^{3}H RIA (^{3}H-labeled steroids from Amersham, Braunschweig, Germany) with specific antisera to cortisol, DHEA (Steranti, St. Albans, UK), and Δ^4-androstenedione (a kind gift from Dr. S. Lewicka, Heidelberg, Germany) as described previously (20). All relevant comparisons were made within the same assay. Results for steroid hormones were calculated for 5×10^5 cells and related to the values of the corresponding control cells of each preparation.

RNA extraction and Northern blotting

Total RNA was extracted from cells harvested in guanidium thiocyanate-containing buffer, followed by ultracentrifugation through a cesium-trifluoroacetate layer (Pharmacia Biosystems, Freiburg, Germany). Ten to 20 μg RNA were loaded onto a 1% agarose gel with 2.2 mol/L formaldehyde and transferred by capillarity to a Hybond-N nylon membrane (Amersham). Equal loading of RNA was checked by scanning densitometrically (Hirschmann laser densitometer) the photo negatives (Kodak type 55 film, Eastman Kodak, Rochester, NY) of the ethidium-bromide stained membranes. Differences in RNA loading could be corrected by the corresponding values for the 28S ribosomal RNA. Using human placental 3βHSD complementary DNA (cDNA) (21), bovine 17α-hydroxylase cytochrome P450 (P45017α) cDNA (22), bovine cholesterol side-chain cleavage enzyme (P450scc) cDNA (23), and a 955-bp cDNA fragment of the human ACTH receptor (19) containing the whole coding sequence as probes, prehybridization and hybridization were performed as previously indicated (3, 19). cDNA probe-containing vectors, kindly donated by the authors, were transformed and amplified by G. Gutschmidt (Institut f. Humangenetik, Berlin, Germany) and inserts [^{32}P]deoxy-CTP radiolabeled by random priming (Multiprime Kit, Amersham).

Statistical evaluation

Differences between groups were examined by the nonparametric Wilcoxon rank sum test for paired samples and were judged as significant at $P < 0.05$.

Results

Effects of IGF-I, IGF-II, and insulin on basal and ACTH-stimulated steroid production by HAC

In the first series of experiments we investigated the long term effects of the three peptides alone as well as those of hGH and ACTH on DHEA, androstenedione, and cortisol secretion by HAC. Cells were treated for 3 days, and the steroids were measured in the medium on the last day (Table 1). hGH had no significant effects on any of the three steroids, whereas IGF-I and IGF-II at nanomolar concentrations slightly, but significantly, enhanced the secretion of the three steroids. There were no significant differences among the effects of IGF-I, IGF-II, and insulin. However, the stimulatory action of ACTH (10 nmol/L) on the secretion of the three steroids was severalfold higher than that of the three peptides. The effects of IGF-I and IGF-II on the production of the three steroids was dose dependent, with an ED_{50} between 0.4–1.2 nmol/L (3 and 9 ng/mL) and a maximum velocity at about 13 nmol/L (100 ng/mL).

Next, we investigated whether the above pretreatments modified the steroidogenic capacity of HAC, i.e. the acute responsiveness to ACTH. Again, pretreatment with hGH had no effect, whereas pretreatment with IGF-I, IGF-II, and insulin clearly enhanced the production of the three steroids after acute ACTH stimulation (Table 2). The effects on androstenedione formation were greater than those on DHEA and cortisol. Moreover, the effects of IGF-I and insulin on the three steroids tended to be lower than those of IGF-II, which, in turn, were not significantly different from those produced by ACTH pretreatment. The effects of IGF-I or IGF-II priming on ACTH-stimulated steroid secretion were dose dependent (Fig. 1); no significant effects were detected below 0.13 nmol/L (1 ng/mL) IGF-I or IGF-II. The maximum DHEA or androstenedione secretion was observed after pretreatment doses between 6.6 and 13 nmol/L (50 and 100 ng/mL) of each IGF, respectively.

TABLE 1. Secretion of adrenal androgens and cortisol by HAC in culture under several experimental conditions

Treatment for 72 or 96 h	n	Steroid hormone production of HAC during the last 24 h of pretreatment, as multiple of control		
		DHEA	Androstenedione	Cortisol
Control	20	1.0^a	1.0^a	1.0^a
IGF-I, 26.2 nmol/L (200 ng/mL)	13	$1.31 \pm 0.13^{b,c}$	$1.47 \pm 0.13^{b,d}$	$1.55 \pm 0.19^{b,c}$
IGF-II, 26.8 nmol/L (200 ng/mL)	9	$1.35 \pm 0.18^{b,c}$	$1.58 \pm 0.24^{b,c}$	$1.55 \pm 0.18^{b,c}$
Insulin, 0.9 μmol/L (5 μg/mL)	12	$1.30 \pm 0.18^{b,c}$	$1.48 \pm 0.13^{b,d}$	$1.22 \pm 0.13^{a,b}$
hGH, 4.7 pmol/L (100 ng/mL)	4	1.06 ± 0.08^a	1.09 ± 0.07^a	0.97 ± 0.09^a
ACTH, 10.0 nmol/L (45 ng/mL)	17	$6.51 \pm 1.48^{e,f,g}$	$10.4 \pm 2.09^{c,f,g}$	$23.4 \pm 4.44^{e,f,g}$
Control basal steroid hormone production, as pmol/500,000 cells · 24 h	20	224 ± 72	777 ± 115	$10,479 \pm 1.903$

Cells on the second day of culture were incubated in the absence (control) or presence of IGF-I, IGF-II. insulin, hGH, or ACTH for 3 days, and medium and hormones were replaced daily. DHEA, androstenedione, and cortisol were measured in the medium on the last day (*cf. Materials and Methods*). The results, expressed as multiple of control cells, are the mean ± SEM of n adrenal preparations, in which each condition was performed as triplicate.

^{a,b,c} Different letters indicate significant differences within the same column.
^{c} $P < 0.05$ *vs.* controls.
^{d} $P < 0.01$ *vs.* controls.
^{f} $P < 0.001$ *vs.* controls.
^{g} Significant differences between the columns were found only after treatment with ACTH 10 nmol/L: cortisol > androstenedione ($P < 0.05$), cortisol > DHEA ($P < 0.01$), androstenedione> DHEA ($P < 0.01$).

TABLE 2. Steroid hormone response to ACTH stimulation of HAC

Pretreatment for 72 or 96 h	n	Steroid hormone production of HAC after stimulation with ACTH 1 nmol/L for 2 h, as multiple of control		
		DHEA	Androstenedione	Cortisol
Control	20	1.0^a	1.0^a	1.0^a
IGF-I, 26.2 nmol/L (200 ng/mL)	13	$3.12 \pm 0.93^{b,c,d}$	$4.14 \pm 1.02^{b,d,e,f}$	$2.97 \pm 1.10^{b,c,d}$
IGF-II, 26.8 nmol/L (200 ng/mL)	10	$4.92 \pm 1.35^{b,c,g}$	$6.89 \pm 1.51^{c,d,h,i}$	$3.78 \pm 1.50^{b,c,g}$
Insulin, 0.9 μmol/L (5 μg/mL)	13	$2.45 \pm 0.62^{b,g}$	$3.96 \pm 1.75^{b,c,d}$	$2.31 \pm 0.43^{b,d}$
hGH, 4.7 pmol/L (100 ng/mL)	4	1.11 ± 0.16^a	1.15 ± 0.18^a	0.96 ± 0.08^a
ACTH, 10.0 nmol/L (45 ng/mL)	17	$4.49 \pm 1.29^{c,j}$	$6.12 \pm 2.73^{c,h,j}$	$5.36 \pm 1.43^{c,j}$
Control basal steroid hormone production, as pmol/500,000 cells · 2 h, with ACTH (1 nmol/L)	20	132 ± 46	397 ± 99	$2,782 \pm 578$

HAC were pretreated as indicated in Table 1, and after 2 h of subsequent acute stimulation with ACTH (1 nmol/L), steroid hormones secreted into the medium were measured as indicated in *Materials and Methods*, and related to the ACTH-stimulated steroids of HAC without pretreatment (control).

^{a,b,c,e,h} Different letters within the same column indicate significant differences.
^{d} $P < 0.01$ *vs.* controls.
^{g} $P < 0.05$ *vs.* controls.
^{j} $P < 0.001$ *vs.* controls.
Significant differences between the columns, *e.g.* the production of ACTH-stimulated steroid, were found after the pretreatment indicated in brackets: ^{f}, Androstenedione [IGF-I] > cortisol [IGF-I] ($P < 0.05$), androstenedione [IGF-I] > DHEA [IGF-I] ($P < 0.01$); ^{i}, androstenedione [IGF-II] > cortisol [IGF-II] ($P < 0.05$).

To determine which enzymatic step was stimulated by IGF, at the end of the pretreatment period, cells were incubated for 2 h with several precursors, and the amounts of steroidal end products formed were measured (Table 3). Control cells incubated with 22(R)-hydroxycholesterol increased their production of the three steroids. Pretreatment with IGF-I, which was only investigated with this precursor, further increased DHEA 2-fold, androstenedione 3.3-fold, and cortisol 2.8-fold, but the stimulatory effects of IGF-II were still higher. Pregnenolone was a better substrate than 22(R)-hydroxycholesterol, but again, IGF-II pretreatment enhanced the secretion of the three steroids. These results suggest that IGFs enhanced at least the activation of 3βHSD and of 17α-hydroxylase. The results observed using progesterone, a poor substrate, and 17-hydroxyprogesterone confirmed that IGFs enhanced the activity of the 17α-hydroxy- lase/17-20-lyase as well as that of 21-hydroxylase and/or 11β-hydroxylase.

Effects of IGFs on steroidogenic enzyme and ACTH receptor mRNA levels

Northern blot analysis of RNA extracted from cultured HAC revealed single transcripts for P450scc, P45017α, and 3βHSD of 2.0, 1.9, and 1.7 kilobases (kb), respectively, and two major ACTH receptor transcripts of 1.8 and 3.4 kb (Fig. 2). To define the mechanism underlying the increased steroidogenic responsiveness of HAC after pretreatment with IGFs, we investigated their effects on the mRNA levels of these genes. As previously described, ACTH increased the levels of all ACTH receptor transcripts (19) as well as the mRNA levels of the three enzymes (3). IGF-I
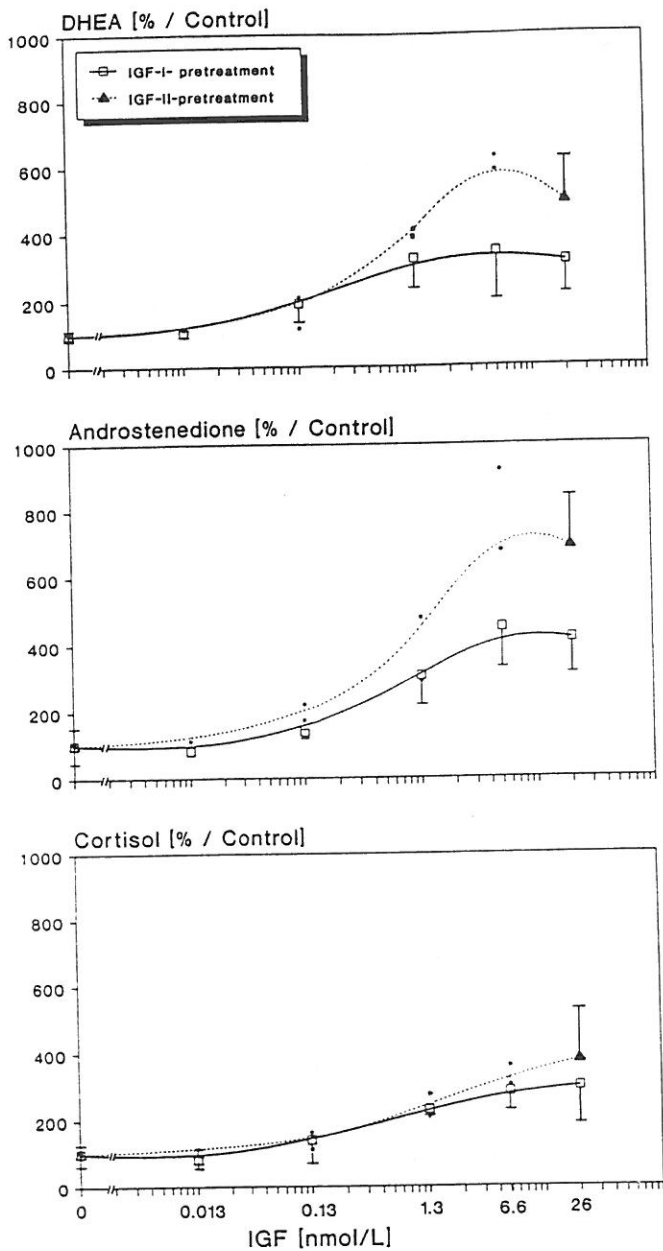
FIG. 1. Steroid hormone response to acute ACTH stimulation: effects of pretreatment with increasing concentrations of IGF-I or IGF-II. On day 2 of culture, HAC were incubated with the indicated IGF concentrations for 4 days, washed, and subsequently stimulated with ACTH (1 nmol/L). After 2 h, DHEA, androstenedione, and cortisol were determined in the medium. Steroid hormone secretion is indicated as a percentage of that in control cells, for 3 (mean ± SEM) or, with IGF-II, 2 different HAC preparations, each measured in triplicate. Note that 13 experiments were performed with a dose of 26.2 nmol/L IGF-I (200 ng/mL) and 10 with a dose of 26.8 nmol/L IGF-II (200 ng/mL).

also significantly enhanced the mRNA levels of ACTH receptor, 3βHSD, and P45017α, but not those of P450scc. Similar effects were observed after IGF-II treatment, but because only two RNA-yielding experiments were performed with IGF-II, no statistical analysis could be conducted.

Discussion

The present results show that IGFs, but not GH, have several effects on human adrenal steroidogenesis. At first they slightly, yet significantly, increase the secretion of the three main steroids, DHEA, androstenedione, and cortisol. Secondly, and more important, pretreatment with IGFs markedly enhances the steroidogenic responsiveness to ACTH and the ability of cells to transform several precursors into these three steroids. This enhanced steroidogenic capacity, which was dose dependent and observed at physiological concentrations, was associated, and probably related, to increased mRNA levels of genes encoding for adrenal cell differentiated functions. Thus, both IGFs increased the mRNA levels of ACTH receptor, P45017α, and 3βHSD, but not those of P450scc. Although in the present work we did not measure the effects of IGFs on ACTH receptor number, previous studies, using the same model, have shown that an increase in ACTH receptor mRNA was associated with an increase in ACTH receptor number (19). The stimulatory effects of IGF-I on P45017α and 3βHSD mRNA levels were associated with an increased activity of both enzymes as inferred by the enhanced metabolism of several steroid precursors. However, although the main steroid produced by human adrenal fasciculata-reticularis cells was cortisol, the relative stimulatory effect of IGFs on androstenedione production after either ACTH stimulation or incubation with several steroid precursors was higher than that on cortisol or DHEA. The differences between the effects of IGF on DHEA and androstenedione can be explained by the fact that both peptides increased 3βHSD mRNA and activity and, therefore, the conversion of Δ^5- to Δ^4-steroids. On the other hand, the difference between cortisol and androstenedione stimulation suggests that IGFs, in addition to increase P45017α mRNA and activity, also enhanced the levels and/or activity of cytochrome-$b5$, the coenzyme that increases the 17,20-lyase activity of 17α-hydroxylase (24). Previous studies have shown that IGFs also increased the steroidogenic capacity of bovine adrenal cells (14, 25–27); as in HAC, IGFs enhance ACTH receptor mRNA (27) and binding sites (14) as well as 3βHSD mRNA, protein, and activity (14, 27). However, whereas in HAC, IGFs increase P45017α mRNA and activity (present results), in BAC, IGFs increase the mRNA and protein, but not the activity, of this enzyme (14, 25, 27). In addition, in HAC, but not in BAC, IGFs enhance the 17,20-lyase activity.

The stimulatory effects of IGF-II on the steroidogenic capacity of HAC tended to be higher than that of IGF-I, in particular for androstenedione. Similar results have been reported recently using BAC (26). As BAC secrete several IGF-binding proteins (14) with higher affinity for IGF-I than for IGF-II, it has been suggested that these binding proteins may cause a greater inhibition of the effects of IGF-I than those of IGF-II. This hypothesis was confirmed by using IGF analogs with reduced affinity for the IGF-binding proteins (18). However, this explanation probably cannot be extrapolated to HAC for the following reasons: 1) the type and the amounts of IGF-binding proteins secreted by these cells are different from those secreted by BAC (18); 2) the effects of an IGF-I analog with reduced affinity for IGF-binding proteins

TABLE 3. Effects of IGF-II pretreatment on steroidogenesis in HAC

Incubation (2 h)	Control (pmol/5 × 10⁵ cells · 24 h)			IGF-II (multiple of control)		
	DHEA	Δ^4-AD	Cortisol	DHEA	Δ^4-AD	Cortisol
Basal	10.1 ± 3.1	35.3 ± 14.3	46.5 ± 9.3			
22(R)Hydroxycholesterol	23.9 ± 2.1	81.0 ± 3.5	174.0 ± 10.8	2.61 ± 0.09	6.29 ± 0.10	7.34 ± 0.39
Δ^5-Pregnenolone	142.5 ± 21.8	150.1 ± 38.1	467.5 ± 188.7	2.31 ± 0.16	4.32 ± 0.14	4.33 ± 0.14
Progesterone		67.4 ± 3.1	108.7 ± 10.8		4.76 ± 0.18	5.55 ± 0.21
17-Hydroxyprogesterone		480.8 ± 85.2	2442.2 ± 660.1		1.90 ± 0.27	2.10 ± 0.45

HAC were incubated for 3 days in the absence (control) or presence of 26.8 nmol/L (200 ng/mL) IGF-II. Then the medium was replaced by fresh medium (basal) or medium with the indicated precursors all at 5 × 10⁻⁵ mol/L. After 2 h, DHEA, androstenedione (Δ^4-AD), and cortisol secretion was measured. The results are the mean ± SEM of triplicate incubations of a single cell preparation.
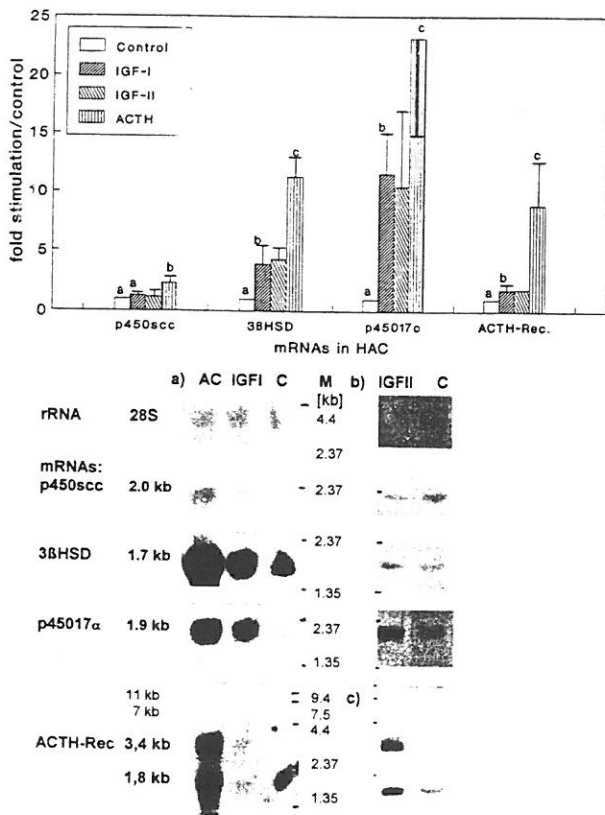

FIG. 2. Effects of various treatments on mRNA levels of the steroidogenic enzymes P450scc, 3βHSD, and P45017α and of the ACTH receptor in human adrenocortical cells. On the second day of culture, HAC were supplied with medium (controls) or treated with IGF-I (26.2 nmol/L) or IGF-II (26.8 nmol/L; 200 ng/mL) or ACTH (10 nmol/L; 45 ng/mL) for 3 days. Medium and hormones were renewed daily. *Top*, Results are the mean ± SEM, expressed as multiples of control cells of Northern blots from eight (ACTH or IGF-I) adrenal preparations (n = 2 for IGF-II-treatment). ACTH receptor mRNA is the sum of the two major transcripts (1.8 and 3.4 kb). Significant differences between the bars are indicated by different letters (P < 0.05). *Bottom*, Representative Northern blot analyses of approximately 15 μg total RNA (*cf.* photo negative of ethidium-bromide stained 28S ribosomal RNA) of HAC of three individual experiments (a, b, and c) incubated from days 2–6 with IGF-I (6.6 nmol/L; 50 ng/mL), ACTH (10 nmol/L), and IGF-II (26.8 nmol/L; 200 ng/mL), compared to that of control cells (artifact in a, ACTH-Rec.blot, aside of "C" RNA). M, Marker.

were similar to those induced by IGF-I (26); and 3) insulin, acting at micromolar concentrations not only through the insulin receptor, but also through the adrenal IGF type 1 receptor (15), had effects similar to those of IGF-I; however

insulin does not bind to IGF-binding proteins. Another possibility to explain the differences between the effects of IGF-I and IGF-II is that some of the effects of IGF-II could be mediated through the IGF type 2 receptor, present in normal adult adrenals (28) and producing some metabolic effects in certain cell types (29). Clearly, further studies are required to explain the differences between IGF-I and IGF II.

The effects of insulin on human adrenal steroidogenesis *in vitro* have not previously been established. In the present work, most experiments were performed at micromolar insulin concentrations, like those used in standard culture medium for other experiments (3, 19, 27). At this concentration, which occupied both IGF type 1 and its own receptors, the effects were similar to those produced by IGF-I at nanomolar concentration. In five experiments preincubation with physiological concentrations of insulin (8.6 nmol/L; 50 ng/mL), it increased both basal and ACTH-stimulated androstenedione and DHEA production at the same level as equimolar concentrations of IGF-I. These results suggest that activation of insulin or IGF type 1 receptor produced similar effects and that simultaneous activation of both receptors by micromolar concentrations of insulin was not additive. Together, the *in vitro* effects of IGFs and insulin on the functions of HAC substantiate the hypothesis that in hyperandrogenemic women, who frequently present with insulin excess, the observed exaggerated adrenal secretion of 17-hydroxylated C_{21} steroids and androgens (30) is not due to a defect in P45017α, but, rather, to a generalized, insulin- or IGF-induced, adrenal hyperresponsiveness to ACTH. Whereas some effects of IGFs mimic the changes observed at the onset of adrenal androgen secretion, the adrenarche, characterized by a relative increase in the 17,20-lyase activity of the 17α-hydroxylase, the stimulatory effects of IGFs on 3βHSD activity are opposite those observed at the time of adrenarche and, therefore, cannot explain the increased plasma levels of DHEA and its sulfate. The initiation of adrenarche probably needs an interplay of several factors besides IGF, such as ACTH, endorphins, and steroids (1) as well as transforming growth factor-β (6).

In conclusion, the present results demonstrate that IGFs, which have a weak steroidogenic effect, markedly enhance the steroidogenic capacity of HAC by promoting the expression of genes encoding for their differentiated function. In addition to their endocrine role, IGFs may play a paracrine/autocrine role in the regulation of adrenal function, as previous studies (11, 12, 14, 27, 31) in several species have demonstrated the presence of IGFs mRNAs and peptides.

Acknowledgments

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