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EFFECTS OF INSULIN-LIKE GROWTH FACTOR I (IGF-I) ON ENZYMATIC ACTIVITY IN HUMAN ADRENOCORTICAL CELLS. INTERACTIONS WITH ACTH

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Summary—Cells obtained from 6 adult human adrenals or adrenal fragments were cultured in serum-free synthetic medium (McCoy's) in order to study the isolated effects of IGF-I on steroidogenesis and its interactions with ACTH. After addition of peptide, changes in the activities of steroidogenic enzymes were assessed by measuring certain steroids in the spent medium. These included pregnenolone, 17-hydroxypregnenolone (17-OH-Preg), dehydroepiandrosterone (DHA), 17-hydroxyprogesterone (17-OH-P), androstenedione (AD), 11-deoxycortisol and glucocorticoids (chiefly cortisol and its immediate precursors, 11-deoxycortisol and 17-OH-P) and cortisol itself.

The steroid responses obtained with repeated doses of IGF-I (40 ng/ml $\approx 10^{-9}$ M), added at 0, 48 and 72 h, over 4 days' culture were quite different from those obtained with repeated doses of ACTH (0.25 ng/ml $\approx 10^{-10}$ M). All the steroids measured increased with time of culture under the influence of ACTH and, apart from pregnenolone which peaked, tended to reach a plateau. With IGF-I, by contrast, DHA, AD, 11-deoxycortisol and glucocorticoid production increased initially, then decreased progressively, whereas pregnenolone, 17-OH-Preg and 17-OH-P production was either absent or negative.

Cumulative steroid production over 4 days reached similar levels in response to a single dose of IGF-I and/or ACTH, with two major exceptions: pregnenolone dropped significantly with IGF-I [46% ± 6 (SEM) as opposed to 93% ± 11 with ACTH, $P < 0.005$, $n = 5$], as did 17-OH-P (48% ± 11 vs 113% ± 8 with ACTH, $P < 0.001$, $n = 6$). Increased formation of down-stream metabolites (DHA, AD, 11-deoxycortisol and glucocorticoids) would suggest that IGF-I induced stimulation of the 17α-, 21- and 11β-hydroxylases.

The responses to ACTH stimulation of cells which 4 days previously had been pre-treated with an initial and single dose of IGF-I and/or ACTH emphasized the impact of IGF-I on the 3-hydroxylation steps in cortisol biosynthesis. Compared with ACTH pre-treatment, the effects of which faded in the long term, pre-treatment with IGF-I resulted in a significantly increased steroidogenic response (P between < 0.05 and < 0.01). With the single exception of pregnenolone (43% ± 4.7), production of all the metabolites was amplified: 17-OH-Preg: 348% ± 88; DHA: 643% ± 127; 17-OH-P: 193% ± 36; AD: 725% ± 200; 11-deoxycortisol: 573% ± 110; cortisol: 1000%.

Our findings strongly suggest that IGF-I plays a major rôle in the regulation of steroidogenesis by promoting and maintaining enzymatic activity (17α, 21- and 11β-hydroxylases) via which the function of ACTH is achieved, viz., biosynthesis of cortisol.

INTRODUCTION

The biological action of Insulin-like Growth Factor I (IGF-I), whose biosynthesis is regulated by growth hormone, is pivotal in skeletal growth, but also extends over a broad range of cell types, stimulating proliferation and/or maturation. Gonads, and particularly the ovaries, are target organs of IGF-I and also sites of production [1]. IGF-I and insulin receptors have been identified in bovine [2] and human [3] adrenocortical cells and a rôle for both peptides in maintaining the specific functions of these cells has been described [2]. However, significantly more insulin is required than IGF-I in obtaining the same stimulatory effects on

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enzymatic activity. IGF-I acts synergistically with ACTH, but we have found that in bovine adrenocortical cells it is also capable of inducing steroidogenesis on its own [4]. This dual rôle of IGF-I has been clearly demonstrated in human granulosa cells [5]. In this study, we have used human adrenocortical cells cultured in serum-free defined medium to investigate both the isolated effects of IGF-I and its interactions with ACTH. Steroidogenesis was evaluated by measuring the steroids which form the major biosynthetic pathway for cortisol in man, i.e. pregnenolone, 17-hydroxypregnenolone (17-OH-Preg), 17-hydroxyprogesterone (17-OH-P), 11-deoxycortisol and cortisol or the glucocorticoids taken together (cortisol and its immediate precursors, 11-deoxycortisol and 17-OH-P), as well as the androgens, dehydroepiandrosterone (DHA) and androstenedione (AD).

EXPERIMENTAL

Six either whole adrenals or adrenal fragments were collected for this study, five during expanded nephrectomy for non-invasive kidney cancer and one during an adrenalectomy for a case of Conn's Syndrome. In this last case, there were micro-sites of hyperplasia in the glomerulosa, but the adrenal tissue was used, since in an earlier study [6] we had demonstrated that, unlike aldosterone, cortisol and corticosterone production in cells responsible for primary hyperaldosteronism (i.e. characterized adenoma cells and cells from tissues exhibiting hyperplasia of the glomerulosa) was very similar to that in cells taken from control adrenals.

Adrenals or fragments were transported to the laboratory in serum-free McCoy's culture medium on ice. Fat and connective tissue were removed and the adrenal tissue treated with collagenase according to a technique previously established for human adrenocortical cells [7, 8]. The cells were seeded in 24-well plates (at $3–4 \times 10^5$ cells per well and per ml McCoy's medium) (Eurobio). The medium was supplemented with 10% foetal calf serum (Gibco) and contained 100 U/ml penicillin, 50 μg/ml streptomycin and 20 μg/ml gentamycin. The cells were cultured for 48 h in a moist atmosphere containing 5% CO_2, then washed three times in 0.9% NaCl in order to remove all traces of serum. Thereafter, they were incubated in serum-free McCoy's medium with or without ACTH (0.25 ng/ml $\approx 10^{-10}$ M), IGF-I (40 ng/ml $\approx 5 \times 10^{-9}$ M) or ACTH + IGF-I (at the same concentrations). Each concentration of hormone was tested in triplicate. Following the transfer to serum-free medium, three types of experiment were carried out: (1) time-course studies were done of steroidogenesis in the absence or presence of ACTH or IGF-I added at 0, 48 and 72 h of culture; (2) cumulative steroid production was measured after 4 days of culture in the absence or presence of a single dose of ACTH, IGF-I or ACTH + IGF-I added at 0 h; (3) in 4 experiments, the cells were pre-treated with IGF-I, ACTH or ACTH + IGF-I and 4 days later thoroughly washed three times with 0.9% NaCl so as to remove peptide, and then submitted to further stimulation (3 h) with ACTH (25 ng/ml $\approx 10^{-8}$ M). Thereafter, cortisol, its precursors and the androgens were measured in the culture medium.

Following extraction of the steroids with dichloromethane (Merck) and evaporation of the organic layer, the various steroids were measured using techniques previously established in our laboratory. Glucocorticoids were determined by competitive binding using transcortin [9]. This method measures principally cortisol (which is the major glucocorticoid in man), its immediate precursors (11-deoxycortisol and 17-OH-P) and corticosterone (which is secreted in very small quantities). 17-OH-P was measured directly by RIA using a highly specific antibody [10]. The remaining steroids were first isolated by chromatography on celite (pregnenolone, 17-OH-Preg, DHA and AD [11, 12]) or Sephadex LH-20 (11-deoxycortisol and cortisol [13]) and then assayed by RIA.

Student's t-test was used to test the statistical significance of variations in steroid production induced by the peptides studied.

RESULTS

Time-course of changes in steroidogenesis: comparison of the effects of IGF-I and ACTH

After 48 h of culture in the presence of 0.25 ng/ml ACTH ($\approx 10^{-10}$ M), glucocorticoids (comprising 80% cortisol) reached 1240 pmol $\pm$ 69 (per well and per $3–4 \times 10^5$ cells: mean of triplicates $\pm$ SEM) (Fig. 1). This was similar to the levels obtained in response to 40 ng/ml IGF-I ($\approx 5 \times 10^{-9}$ M): 1170 pmol $\pm$ 2.76. After 3 and 4 days' culture in the presence of ACTH, glucocorticoid production increased (1840 pmol $\pm$ 179 and 1840 pmol $\pm$ 161), but after 4 days, the proportion of cortisol dropped to 40%.

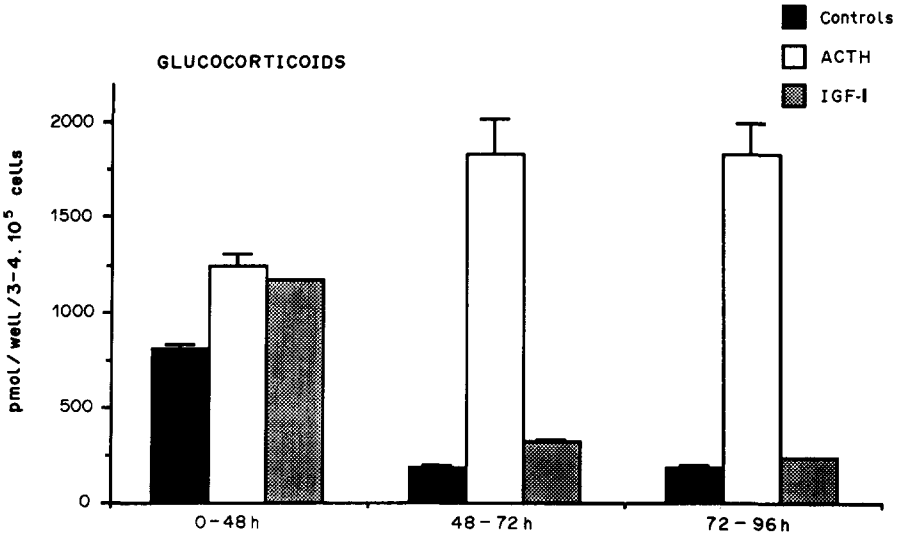

Fig. 1. Time-course of glucocorticoid production under the influence of ACTH (0.25 ng/ml $\approx 10^{-10}$ M) and IGF-I (40 ng/ml $\approx 5 \times 10^{-9}$ M). Cells were seeded at 3–4 $\times 10^5$ cells/ml/well and cultured for 48 h in McCoy's medium containing 10% foetal calf serum and thereafter in serum-free McCoy's medium. Peptides were added at 0, 48 and 72 h and culture media collected at 48, 72 and 96 h. Each column represents the mean for 3 wells ± SEM. Where the SEM bar is omitted, the 3 values were practically identical.

With IGF-I, by contrast, glucocorticoid production decreased (326 pmol ± 12.1 and 235 pmol ± 2.76) and the proportion of cortisol remained the same.

As regards Δ-5 metabolites, pregnenolone (Fig. 2a) was stimulated by ACTH to 79 pmol after 3 days of culture, but dropped to 21 pmol after 4 days. 17-OH-Preg and DHA rose to 82.5 pmol and 8.5 pmol, respectively, after 3 days and remained at these levels after 4 days (87.6 and 10.1 pmol).

The responses of the Δ-4 metabolites to ACTH were as follows: 17-OH-P (Fig. 2b) reached 475 pmol ± 72.6 after 3 days and remained unchanged (454 pmol ± 3.03) after 4 days. AD production was 185 pmol after 3 days and 314 pmol after 4 days. 11-deoxycortisol rose to 201 pmol after 3 days and further to 345 pmol (30 times base-line levels) after 4 days.

IGF-I, by contrast, failed to stimulate pregnenolone, 17-OH-Preg (Fig. 2a) or 17-OH-P (Fig. 2b). In the case of 17-OH-P, slight inhibition was consistently observed: 169 pmol ± 14.8 as opposed to a base-line level of 201 pmol ± 8.96 after 48 h, 41.5 pmol ± 8.62 as opposed to 60.5 pmol ± 6.05 after 3 days and 18.2 pmol ± 4.54 as opposed to 34.8 pmol ± 1.51 after 4 days. DHA, AD and 11-deoxycortisol, however, reached levels at 48 h (4.92, 182 and 105 pmol, respectively) which were similar to those obtained with ACTH (3.47, 168 and 114 pmol). After 3 and 4 days, there was a progressive drop in the production of these three steroids, unlike the case with ACTH.

Cumulative effects of a single dose of IGF-I and/or ACTH on steroidogenesis

In these experiments a single dose of IGF-I (40 ng/ml) and/or ACTH (0.25 ng/ml) was used and steroid production measured after 4 days of culture, since the experiments above had shown that IGF-I had no further effect after 4 days and, at the same time, had confirmed the results of an earlier study using bovine adrenocortical cells. Results are expressed here as percentages of the corresponding base-line levels (Fig. 3).

IGF-I provoked marked decreases in pregnenolone (46% ± 6) and 17-OH-P (48% ± 11) production. The differences from the levels obtained with ACTH (93% ± 11 for pregnenolone and 113% ± 8 for 17-OH-P) were highly significant: $P < 0.005$ and $P < 0.001$, respectively.

Despite the lack of stimulation of pregnenolone, 17-OH-Preg and 17-OH-P by IGF-I, DHA [200% ± 32 (SEM)], AD (132% ± 24), 11-deoxycortisol (121% ± 32) and glucocorticoid (141% ± 12) production reached levels which were comparable to those obtained in response to ACTH (143% ± 16, 113% ± 9, 141% ± 18 and 135% ± 10, respectively).

IGF-I + ACTH did not induce changes much different from IGF-I alone. In further experiments where steroidogenesis was studied using three concentrations of IGF-I (10, 40 and

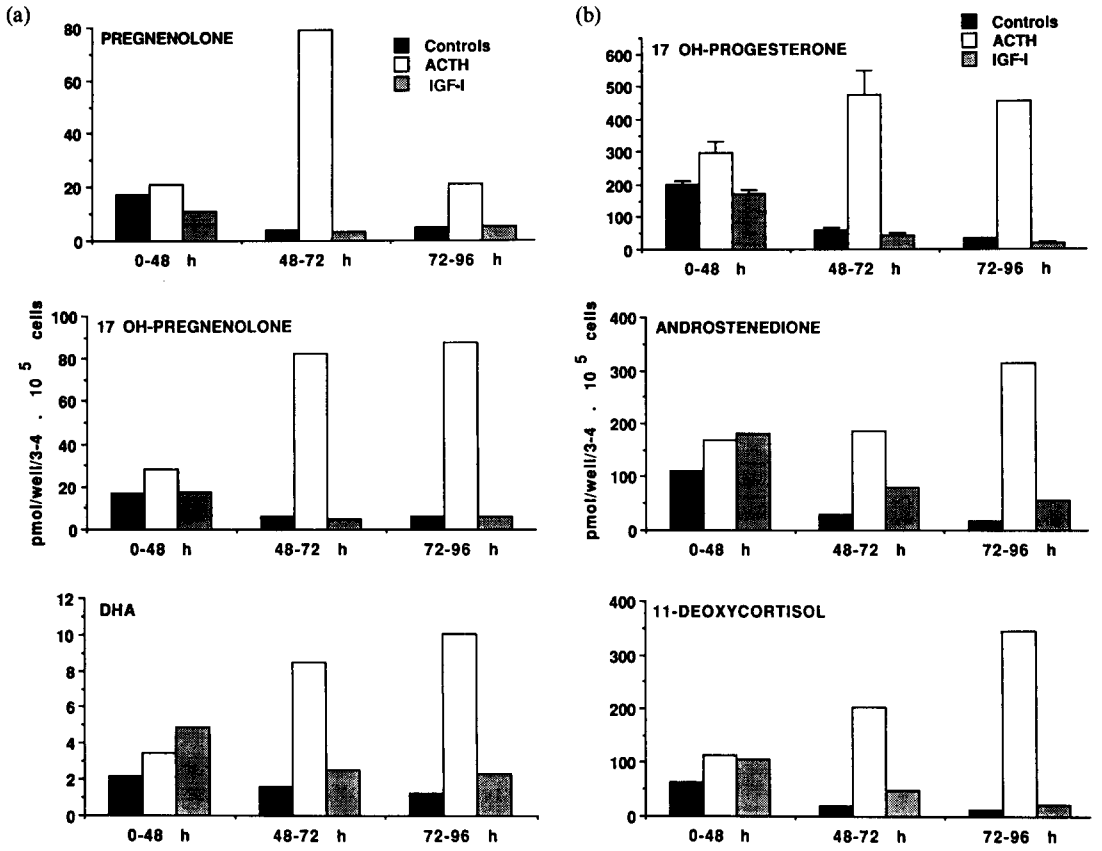

Fig. 2. Time-course of the production of the Δ-5 (2a) and Δ-4 (2b) steroids, as in Fig. 1. Culture conditions were the same as those described in Fig. 1. Except in the case of 17-OH-P (triplicates ± SEM), chromatography was necessary prior to RIA of the various metabolites: each column represents mean production in two wells.

160 ng/ml), no dose–response effects were seen. Nevertheless, whichever the concentration used, pregnenolone failed to be stimulated and there were significant decreases in 17-OH-P, in contrast to the increases in DHA, AD, 11-deoxycortisol and glucocorticoids.

Effects of IGF-I- and ACTH-pre-treatment on ACTH stimulation of steroidogenesis

Steroid responses to 3 h stimulation with ACTH differed depending on the peptide to which the cells had been subjected 4 days previously.

The various metabolites in the culture media of pre-treated cells subsequently stimulated with 25 ng/ml ACTH ($\approx 10^{-8}$ M) were analyzed and the results expressed as a percentage of the corresponding response obtained in control cells (Table 1). Pre-treatment with ACTH caused a drop in 17-OH-Preg (63% ± 7.6) and a rise in DHA (142% ± 16) after the second ACTH stimulation. The other steroid responses were unaffected. Pre-treatment with IGF-I, by contrast, resulted in markedly enhanced responses to ACTH in all the steroids except for pregnenolone which was reduced to 43% ± 4.7. Stimulation of all the other metabolites was significantly amplified (P between <0.05 and 0.01): 17-OH-Preg: 348% ± 88; DHA 643% ± 127; 117-OH-P: 193% ± 36; AD: 725% ± 200; 11-deoxycortisol: 573% ± 110. In two of the four experiments, where despite 7 days' culture the cells retained the ability to secrete cortisol, this was also considerably increased after the pre-treatment with IGF-I (1000%).

Pre-treatment with ACTH + IGF-I had little additional effect over that with IGF-I alone on any of the metabolites studied.

DISCUSSION

Our results are based on experiments done in serum-free synthetic medium. The culture conditions and methodology used in estimating steroid output were very different from those used by Hornsby and Aldern for foetal definitive zone adrenocortical cells [14], which would explain our being able to detect the major

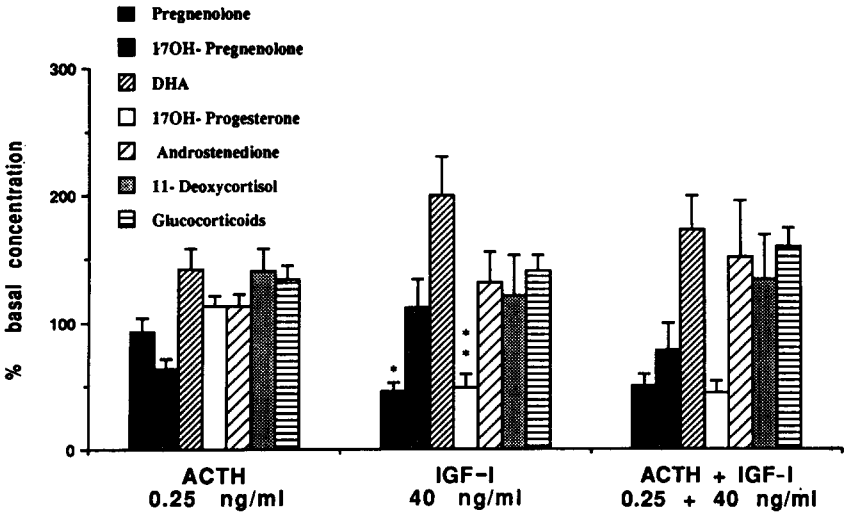

Fig. 3. Effects of a single dose of ACTH (0.25 ng/ml) and/or IGF-I (40 ng/ml), after 4 days' culture in serum-free McCoy's medium, on the production of glucocorticoids, androgens and their precursors. Each column represents the mean response, expressed as a percentage of base-line production ± SEM, in 5 (Δ-5 derivatives) or 6 (Δ-4 derivatives) experiments. Base-line production (in pmol/well of 3–4 × 10^5 cells) ranged from 5.72 to 17.4 for pregnenolone; 3.99–17.1 for 17-OH-Preg; 2.05–6.69 for DHA; 136–369 for 17-OH-P; 112–656 for AD; 31.7–288 for 11-deoxycortisol and 717–4220 for glucocorticoids. *$P < 0.005$; **$P < 0.001$ compared with the effects of ACTH.

metabolites of the cortisol and androgen biosynthetic pathways under base-line, and even more so under stimulated, conditions. The steroidogenic responses to IGF-I were studied in comparison with ACTH as reference stimulatory hormone, using concentrations corresponding to approximately half-maximal doses [4]. The action of ACTH in initiating steroidogenesis is known to involve promotion of cholesterol binding to the cleavage enzyme, $P\text{-}450_{scc}$, which transforms it to pregnenolone. The long-term action of ACTH, beyond this rate-limiting step, is to stimulate the biosynthesis and activation of the P-450 cytochromes specific for the 17α- 21- and 11β-hydroxylases [15]. In addition to these intracellular events, ACTH also exerts a positive regulation over its own receptors [16]. In the 4-day period studied here, a generalized increase in steroidogenesis was apparent, involving the glucocorticoids, pregnenolone, 17-OH-Preg and the androgens. This could be interpreted as reflecting increases in both ACTH receptors and levels of RNA messengers encoding the enzymes governing steroidogenesis [17–19] in response to repeated, but moderate, doses of ACTH. Similarly to the case in foetal human adrenocortical cells [18], there was no evidence of refractoriness in our experiments. The bell-shaped profile of pregnenolone secretion would be explained by the absence of serum in the culture medium and consequently the limited supply of cholesterol.

Table 1. Steroidogenic responses to 3 h stimulation with ACTH (25 ng/ml ≈ 10^{-8} M) in cells pre-treated 4 days previously with a single dose of ACTH (0.25 ng/ml ≈ 10^{-10} M), IGF-I (40 ng/ml ≈ 5 × 10^{-9} M) or ACTH + IGF-I

Pre-treatment → 0		ACTH	IGF-I	ACTH + IGF-I
Metabolite	pmol/well/3 h	% of control cell response ± SEM		
Pregnenolone	20.5–70.8	90 ± 9	43 ± 4.7[b]	47.3 ± 8.4
17-OH-Preg	6.92–20.0	63 ± 7.6	348 ± 88[a]	315 ± 75
DHA	0.94–1.87	143 ± 16	643 ± 127[b]	688 ± 186
17-OH-P	103–297	107 ± 5.3	193 ± 36[a]	200 ± 38
AD	20.9–59.4	89 ± 11	725 ± 200[b]	729 ± 220
11-deoxycortisol	5.76–71.9	95 ± 5	573 ± 110[c]	702 ± 144
Cortisol	ND–27.6	100*	1000*	1200*

The response of the cells pre-treated with either ACTH and/or IGF-I is expressed as a percentage ± SEM of the response (pmol/well/3 h) in control cells (0) ($n = 3$ *for* Δ-5 steroids, $n = 4$ for Δ-4 steroids). In the case of cortisol, the results (*) represent the means of two very similar values (in the two other experiments, no cortisol production was seen, whichever type of pre-treatment had been applied). (a) $P < 0.05$; (b) $P < 0.02$; (c) $P < 0.01$, compared with ACTH pre-treatment.

The massive increase in 11-deoxycortisol at 4 days is a symptom of 11β-hydroxylase depletion [20] which accounts for the diminished cortisol yields at this stage of the experiments.

Like ACTH, IGF-I provoked production of glucocorticoids, 11-deoxycortisol and androgens over the same period investigated. However, the patterns of secretion were quite different from those observed in response to ACTH. This would suggest that the mechanisms initiated by ACTH do not apply to IGF-I. Even with repeated doses of IGF-I, pregnenolone failed to be stimulated. This apparently conflicts with the notion of IGF-I's affecting the action of cholesterol side-chain cleavage enzyme, as has been described for ovarian cells [20], and reflects a major difference between the actions of IGF-I and ACTH. However, the fact that 17-OH-Preg did not change and that 17-OH-P production tended to drop, whereas the production of DHA, AD, 11-deoxycortisol and glucocorticoids increased, would rather suggest that the 17α-hydroxylase/17, 20-lyase, 21-hydroxylase and 11β-hydroxylase systems were activated. Activation of these enzymes was probably relatively greater than that of the cleavage enzyme, which would account for the lack of change in pregnenolone production.

Comparison of the effects of single doses of IGF-I and ACTH on cumulative steroid production after 4 days' culture confirmed that under our experimental conditions IGF-I is capable of stimulating glucocorticoids and the androgens to the same extent as ACTH. The fact that pregnenolone (46% $\pm$ 6) and 17-OH-P (48% $\pm$ 11) production was heavily depressed indicates that these metabolites were being used as substrates for 17α-hydroxylase/17, 20-lyase and 21-hydroxylase in forming DHA, AD and 11-deoxycortisol. It could be inferred from the stimulation of glucocorticoids that 11β-hydroxylase was also involved. No conclusions could be drawn as regards induction of 3β-dehydrogenase-isomerase, in spite of the fact that DHA and AD were produced.

The effects of IGF-I on steroidogenesis did not seem to be closely related to the doses used. There was very little difference between the stimulatory effects of 10, 40 or 160 ng on glucocorticoid or androgen production. IGF-I concentrations below 10 ng/ml had no effect, probably because they were bound by the IGF binding proteins produced by the cells themselves (unpublished results). In addition, the fact that steroidogenesis was limited could suggest that the IGF-I receptors were saturated. It might also mean that IGF-I is not an initiator of steroidogenesis but rather a factor promoting and facilitating it. To this extent, IGF-I's interaction with ACTH is conclusively demonstrated.

Whereas insulin acts at pharmacological doses, and synergistically with ACTH [2, 4, 22], the action of IGF-I at physiological levels was particularly evident when combined with ACTH. In view of the relative levels of production of the various metabolites (pregnenolone, 17-OH-Preg, DHA, 17-OH-P. AD, 11-deoxycortisol and cortisol) in response to ACTH, it was evident that, with ACTH pre-treatment, steroid production stopped at DHA which was moderately elevated (143% $\pm$ 16) at the expense of 17-OH-Preg (63% $\pm$ 7.6). In the case of cells pre-treated with IGF-I or ACTH + IGF-I, ACTH stimulation of all steroids was obviously more efficient, with the exception of pregnenolone (43% $\pm$ 4.7). Significantly reduced pregnenolone production ($P < 0.02$) in conjunction with markedly enhanced down-stream metabolite production would point towards stimulated activity of 17α-hydroxylase/17, 20-lyase, 21- and 11β-hydroxylases. The DHA, AD and 11-deoxycortisol responses in IGF-I-pre-treated cells were 5-, 8- and 6-fold those in ACTH-pre-treated cells. The effects of IGF-I on 11β-hydroxylation were less consistent. 11β-hydroxylase is a fragile enzyme which does not survive well under conditions of long-term *in vitro* culture [20]. In only two experiments did cell samples, with high levels of steroidogenesis at the outset, retain their 11β-hydroxylase activity and this increased 10-fold after pre-treatment with IGF-I. Apart from IGF-I's rôle in maintaining differentiated cell function, it can be envisaged that it has a positive regulatory action on ACTH receptors, amplifying the biological effects of ACTH [23].

Conflicting results have been obtained concerning the production of IGF-I *in situ* in adrenocortical cells. Penhoat *et al.* have identified IGF-I in bovine adrenals and considered its levels to be regulated by ACTH and angiotensin II [24]. However, in specific assays for IGF-I and IGF-II [25, 26] in the culture media of bovine adrenocortical cells, we have found essentially IGF-II, the concentrations of which appeared to be modulated by ACTH and angiotensin II (unpublished results). Furthermore, messenger RNAs encoding IGF-II (but not IGF-I) have been found in foetal human adrenal

cells [27, 28]. Further research is needed in order to clarify this issue. Nevertheless, the present findings suggest that IGF-I plays an important part in the regulation of adrenocortical steroidogenesis by facilitating enzymatic activity (17α-, 21- and 11β-hydroxylases) and by maintaining differentiated functions through which ACTH may fulfill its rôle, the stimulation of cortisol production.

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REFERENCES

1. Adashi E. Y., Resnick C. E., D'Ercole A. J., Svoboda M. E. and Van Wyk J. J.: Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocrine Rev.* **6** (1985) 400–420.
2. Penhoat A., Chatelain P. G., Jaillard C. and Saez J. M.: Characterization of insulin-like growth factor I and insulin receptors on cultured bovine adrenal fasciculata cells. Rôle of these peptides on adrenal cell function. *Endocrinology* **122** (1988) 2518–2526.
3. Pillion D. J., Arnold P., Yang M., Stockard C. R. and Grizzle W. E.: Receptors for insulin and insulin-like growth factor-I in the human adrenal gland. *Biochem. Biophys. Res. Commun.* **165** (1989) 204–211.
4. Pham-Huu-Trung M. T. and Binoux M.: Insulin-like growth factor-I (IGF-I) induces cortisol production in bovine adrenocortical cells in primary culture. *J. Steroid Biochem.* **36** (1990) 583–588.
5. Erickson G. F., Garzo V. G. and Magoffin D. A.: Insulin-like growth factor I regulates aromatase activity in human granulosa and granulosa luteal cells. *J. Clin. Endocr. Metab.* **69** (1989) 716–724.
6. Pham-Huu-Trung M. T., Duclos J. M., Pagny J. Y., Bogyo A., Leneuve P. and Girard F.: *In vitro* studies in primary aldosteronism: base-line steroid production and aldosterone responses to ACTH and angiotensin II. *Acta Endocr. (Copenh.)* **117** (1988) 135–144.
7. Pham-Huu-Trung M. T., Bogyo A., De Smitter N. and Girard F.: Effects of pro-opiomelanocortin peptides and angiotensin II on steroidogenesis in isolated aldosteronoma cells. *J. Clin. Endocr. Metab.* **61** (1985) 467–471.
8. Pham-Huu-Trung M. T., Bogyo A., Leneuve P. and Girard F.: Comparative effects of ACTH, angiotensin II and POMC peptides on isolated human adrenal cells. *J. Steroid Biochem.* **24** (1986) 345–348.
9. Pham-Huu-Trung M. T., Gourmelen M. and Girard F.: The simultaneous assay of cortisol and 17α-hydroxyprogesterone in the plasma of patients with congenital adrenal hyperplasia. *Acta Endocr. (Copenh.)* **74** (1973) 316–330.
10. Fiet J. and Pham-Huu-Trung M. T.: Radioimmunoassay of 17-hydroxyprogesterone: physiological variations and pathological values on man. *J. Steroid Biochem.* **10** (1979) 569–570.
11. Fiet J., Gourmel B., Villette J. M., Brérault J. L., Julien R., Cathelineau G. and Dreux C.: Simultaneous radioimmunoassay of androstenedione, dehydroepiandrosterone and 11β-hydroxyandrostenedione in plasma. *Horm. Res.* **13** (1980) 133–149.
12. Lacroix C., Fiet J., Benais J. P., Gueux B., Bonete R., Villette J. M., Gourmel B. and Dreux C.: Simultaneous radioiommunoassay of progesterone, androstenedione, pregnenolone, dehydroepiandrosterone and 17-hydroxyprogesterone in specific regions of human brain. *J. Steroid Biochem.* **3** (1987) 317–325.
13. Murphy B. E. P.: Sephadex column chromatography as adjunct to competitive protein binding assays of steroids. *Nature* **232** (1973) 21–24.
14. Hornsby P. J. and Aldern K. A.: Steroidogenic enzyme activities in cultured human definitive zone adrenocortical cells: comparison with bovine adrenocortical cells and resultant differences in adrenal androgen synthesis. *J. Clin. Endocr. Metab.* **58** (1984) 121–127.
15. Simpson E. R. and Waterman M. R.: Regulation by ACTH of steroid hormone biosynthesis in the adrenal cortex. *Can. J. Biochem. Cell Biol.* **61** (1983) 692–707.
16. Penhoat A., Jaillard C. and Saez J. M.: Corticotropin positively regulates its own receptors and cAMP response in cultured bovine adrenal cells. *Proc. Natn. Acad. Sci., U.S.A.* **86** (1989) 4978–4981.
17. Simpson E. R. and Waterman M. R.: Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. *Am. Rev. Physiol.* **50** (1988) 427–440.
18. Di Blasio A. M., Voutilainen R., Jaffe R. B. and Miller W. L.: Hormonal regulation of messenger ribonucleic acids for P450scc (cholesterol side-chain cleavage enzyme) and P450c17 (17α-hydroxylase/17–20-lyase) in cultured human fetal adrenal cells. *J. Clin. Endocr. Metab.* **65** (1987) 170–175.
19. Boggaram V., John M. E., Simpson E. R. and Waterman M. R.: Effect of ACTH on the stability of mRNAs encoding bovine adrenocortical P-450scc, P-45011β, P-450 17α, P-450c21 and adrenodoxin. *Biochem. Biophys. Res. Commun.* **160** (1989) 1227–1232.
20. Hornsby P. J.: Regulation of cytochrome P-450-supported 11β-hydroxylation of deoxycortisol by steroids, oxygen and antioxydants in adrenocortical cell cultures. *J. Biol. Chem.* **255** (1980) 4020–4027.
21. Veldhuis J. D., Rodgers R. J., Dee A. and Simpson E. R.: The insulin-like growth factor, somatomedin-C, induces the synthesis of cholesterol side-chain cleavage cytochrome P-450 and adrenodoxin in ovarin cells. *J. Biol. Chem.* **261** (1986) 2499–2502.
22. Ill C. R., Lepine J. and Gospodarowicz D.: Permissive effect of insulin on the adenosine 3′5′-monophosphate-dependent up-regulation of low density lipoprotein receptors and the stimulation of steroid release in bovine adrenal cortical cells. *Endocrinology* **114** (1984) 767–775.
23. Penhoat A., Jaillard C. and Saez J. M.: Synergistic effects of corticotropin and insulin-like growth factor I on corticotropin receptors and corticotropin responsiveness in cultured bovine adrenocortical cells. *Biochem. Biophys. Res. Commun.* **165** (1989) 355–359.
24. Penhoat A., Naville D., Jaillard C., Chatelain P. G. and Saez J. M.: Hormonal regulation of insulin-like growth factor I secretion by bovine adrenal cells. *J. Biol. Chem.* **264** (1989) 6858–6862.
25. Binoux M., Seurin D., Lassarre C. and Gourmelen M.: Preferential measurement of insulin-like growth factor-I-related peptides in serum with the aid of IGF-binding proteins (IGF BPs) produced by rat liver in culture. Estimation of serum IGF BP levels. *J. Clin. Endocr. Metab.* **59** (1984) 453–462.
26. Binoux M., Lassarre C. and Gourmelen M.: Specific assay for insulin-like growth factor (IGF) II using the IGF binding proteins extracted from human cerebrospinal fluid. *J. Clin. Endocr. Metab.* **63** (1986) 1151–1155.
27. Voutilainen R. and Miller W. L.: Coordinate hormone regulation of mRNA for insulin-like growth factor II and the cholesterol side-chain cleavage enzyme, P-450scc, in human steroidogenic tissues. *Proc. Natn. Acad. Sci., U.S.A.* **84** (1987) 1590–1594.
28. Voutilainen R. and Miller W. L.: Developmental and hormonal regulation of mRNAs for insulin-like growth factor II and steroidogenic enzymes in human fetal adrenals and gonads. *DNA* **7** (1988) 9–15.