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ENHANCED ANDROGEN PRODUCTION BY RABBIT ADRENOCORTICAL CELLS STIMULATED CHRONICALLY WITH CORTICOTROPIN: EVIDENCE FOR INCREASED 17α-HYDROXYLASE ACTIVITY

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Summary—The effects of prolonged treatment with corticotropin ($ACTH_{1-24}$, 200 μg s.c. daily during 12 days) on the production of androgens and glucocorticoids were studied on rabbit dispersed adrenocortical cells. The steroidogenic capacity of adrenocortical cells, expressed in terms of the maximal response to ACTH of glucocorticoid (i.e. corticosterone and cortisol) production, was significantly increased after treatment with ACTH. This was associated with a loss of sensitivity to this peptide: indeed, the concentration of ACTH required to induce a half maximal secretory response was one order of magnitude higher with cells from ACTH-treated animals.

Among the C_{21} steroids measured the changes observed involved the 17α-hydroxylated compounds (cortisol, cortisone, 11-deoxycortisol) while corticosterone production was significantly depressed. This effect of prolonged ACTH treatment on steroidogenic pathways involving 17α-hydroxylation, was further evidenced by a clear-cut enhancement in androgen secretion (dehydroepiandrosterone, androstenedione and testosterone) by adrenocortical cells from ACTH-treated animals. The changes observed after treatment of the animal with ACTH were equally obvious, whether the adrenocortical cells were incubated with ACTH or with dibutyryl-c-AMP.

INTRODUCTION

Several studies on the regulation of adrenocortical steroidogenesis by corticotropin (ACTH) have suggested that besides the well known acute effect of this peptide on cholesterol side-chain cleavage leading to increased pregnenolone production [for references, see 1], ACTH exerts prolonged effects on the capacity of adrenocortical cells to generate pregnenolone as well as on the conversion of this precursor into cortisol. The increase in activity of post-pregnenolone steroidogenic pathways was demonstrated in several species including man [2–6]. Among enzymatic steps involved in the conversion of pregnenolone into cortisol it has been suggested that prolonged exposure of adrenocortical cells to ACTH stimulates in a lasting way the activity of 11β- and 17α-hydroxylases [5–10].

That ACTH exerts a prolonged stimulatory influence on 17α-hydroxylase was first demonstrated in rabbits by Kass *et al.*[11]; the observation was repeatedly confirmed [5, 11–15]. Rabbits proved especially suitable for assessment of the postulated effect of ACTH on 17α-hydroxylase activity since in this species the adrenal cortex secretes large amounts of cortisol only after a prolonged ACTH treatment. We confirmed this observation on adrenocortical cells harvested from control and ACTH-treated rabbits [5]. Furthermore, in addition to the reduction of corticosterone secretion, the production of aldosterone by adrenocortical cells was also strongly depressed as a result of previous *in vivo* treatment with ACTH.

The present study was designed to further investigate features of the action of ACTH on adrenocortical 17α-hydroxylase activity in the rabbit. Since this enzyme is also involved in androgen synthesis, the production of dehydroepiandrosterone, dehydroepiandrosterone-sulfate, androstenedione and testosterone was therefore measured in control and ACTH-treated rabbits.

EXPERIMENTAL

Animals and experimental procedure

The experiments were performed on dispersed adrenocortical cells harvested from 24 male white rabbits weighing 3.0 kg on average (range: 2.4 to 3.9 kg) and maintained during several days before the experiment in individual cages on a standard diet. Twelve rabbits served as a control group while 12 others were

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The following trivial names are used: Cortisol: 11β,17α,21-Trihydroxy-4-pregnene-3,20-dione; Cortisone: 17α,21-Dihydroxy-4-pregnene-3,11,20-trione; 11-Deoxycortisol: 17α,21-Dihydroxy-4-pregnene-30,30-dione; Corticosterone: 11β,21-Dihydroxy-4-pregnene-3,20-dione; Dehydroepiandrosterone: 3β-Hydroxy-5-androsten-17-one; Dehydroepiandrosterone sulfate: 17-oxo-5-androsten-3β-yl sulfate; Androstenedione: 4-androstene-3,17-dione; Testosterone: 17β-hydroxy-4-androsten-3-one.

treated during 12 days with a long-acting form of corticotropin ($ACTH_{1-24}$: Cortrosyn-depot, Organon-Holland, 200 μg s.c. daily).

Animals were sacrificed by exsanguination under anesthesia (Hypnorm-Duphar Holland, 6 mg/kg b.wt.i.m.); the adrenal glands were quickly removed, cleaned of fat, weighed and cut in small fragments that were pooled for a given rabbit and subsequently submitted to cell dispersion, according to Sayers *et al.* [16]. After five 20 min periods of mechanical dispersion and tryptic digestion at 37°C, the dispersed cells were separated by centrifugation (500 *g*, 4°C) and resuspended in Krebs–Ringer–bicarbonate solution containing 200 mg/dl glucose, bovine serum albumin (0.5 g/dl), 7.65 mM calcium and LBI (Lima bean trypsin inhibitor, Worthington).

Aliquots of cell suspension (0.9 ml) were transferred into 10 ml Teflon beakers before addition of 0.1 ml solution containing either $ACTH_{1-24}$ (in all experiments), N^6-2-0 dibutyryl-adenosine monophosphate (dbc-AMP, Boehringer-Mannheim) (in 5 experiments for each experimental group), or vehicle only which consisted in isotonic saline containing 0.1 g/dl bovine albumin (Sigma) acidified to pH 2.5 with 0.1 M hydrochloric acid. The concentration of ACTH ranged from 10^{-12} to 10^{-7} M and that of dbc-AMP, from 0.01 to 10 mM. The aliquots of adrenocortical cells were placed in a Dubnoff shaker incubator for 2 h in an atmosphere of 95% O_2 and 5% CO_2, at 37°C and 60 oscillations per min. All samples were run in duplicate. The concentration of adrenocortical cells submitted to incubation averaged 115,000 ± 11,000 (SE) cells per ml in control group ($n = 12$) and 105,000 ± 19,000 (SE) cells per ml in the *in vivo* ACTH-treated group ($n = 12$).

Steroid measurements

Except for dehydroepiandrosterone sulfate (DHA-S) measured in the crude cell suspension samples, the measurement of other steroids was performed after methylene chloride extraction. To this end, the cell suspension samples were extracted with 5 ml methylene chloride during 60 s; the phases were separated by centrifugation during 5 min at 1500 *g*. The methylene chloride extracts were evaporated under air and the dry residue was suspended in ethanol with subsequent chromatography as will be detailed.

Reagents, steroids and antibodies used for steroid measurements. Non radioactive steroids were purchased from Sigma and tritium labelled steroids (cortisol, 11 deoxycortisol, corticosterone, cortisone, DHA and DHA-S, androstenedione and testosterone) from New England Nuclear (Boston, MA, U.S.A.). The antibodies used for androgen radioimmunoassays were purchased from radioassay Systems Laboratories, Carson-California (for DHA and DHA-S), from Endocrine Sciences, Torrance, California (for androstenedione) and from Wien Laboratories, Saccasunna, NJ (for the measurement of testosterone). All solvents and reagents were analytical grade (from Merck-Germany).

Technics used. The measurement of the sum of corticosterone and cortisol, referred to in the results as "steroid production", was performed by protein-binding competitive assay using human plasma saturated with [^{3}H]corticosterone, according to the procedure described previously [17]. In addition, the extracts of cell aliquots incubated in the presence of 10^{-7} M ACTH were submitted to thin-layer chromatography (1st system: ethylene acetate–methylene chloride 7:3, v/v, and 2nd system: chloroform/acetone 7:3, v/v) for isolation of 11-deoxycortisol and cortisone (after the first TLC) and corticosterone and cortisol (after the 2nd TLC) and these 4 steroids were measured isolately by specific protein-binding assays [17, 18].

Measurement of androgens was performed by radioimmunoassay using specific rabbit antisera and Dextran-coated charcoal to separate bound from free steroids [19].

Analysis and presentation of results. All measurements were performed in duplicate. The results are expressed as mean values ± S.E.M., and the statistical significance of differences between mean values of two experimental groups was estimated by the Student's t-test. When, as was the case for androgen production in the presence of dbc-AMP, groups differed by wide margins with large discrepancy in terms of scatter of the data, the non-parametric Wilcoxon test was applied.

Steroid production is expressed in terms of net output (thus after subtracting the amount produced by cells incubated in the vehicle only) per 1×10^5 adrenocortical cells incubated for 2 h. The maximal net steroid production in response to ACTH (V_{max}) as well as the concentration of ACTH inducing a half-maximal effect (A_{50}), were calculated for each experiment by the last square non-linear method [20]. The mean values of V_{max} and A_{50} given in results represent average values computed from data estimated for each individual experiment, while the log–dose response curves indicated in Fig. 1 were calculated from mean values of net steroid production in response to different concentrations of ACTH.

RESULTS

Effect of in vivo *ACTH treatment on adrenal weight and on glucocorticoid production by adrenocortical cells.*

The weight of adrenal glands went from 217 ± 18 mg in control rabbits to 974 ± 119 mg in ACTH-treated animals ($P < 0.001$). The cellular steroid content (mainly corticosterone and cortisol, see methods) before incubation was identical in both groups averaging 24 ± 4 pmol in control and 26 ± 7 pmol after ACTH treatment, per 10^5 cells. There was some spontaneous production of C_{21} ste-

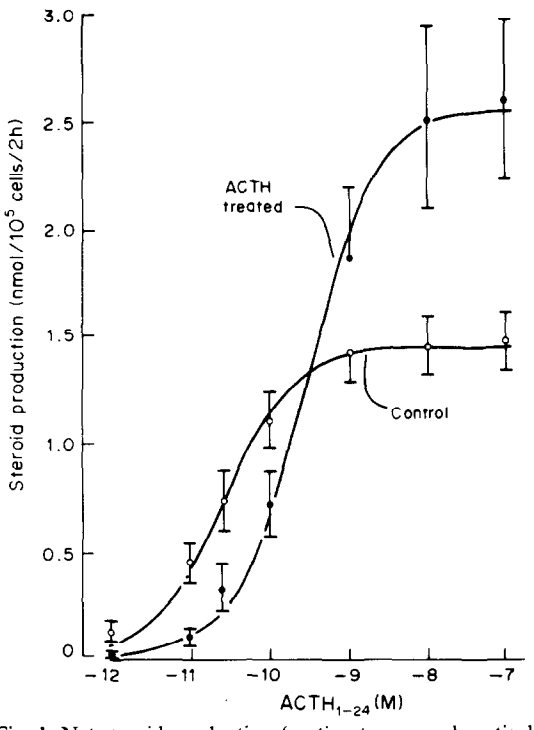

Fig. 1. Net steroid production (corticosterone and cortisol, see methods) by rabbit adrenocortical cells from control ($n = 12$) and ACTH-treated ($n = 12$) animals in response to $ACTH_{1-24}$ *in vitro*. The dots represent the mean observed values and vertical bars the standard error of the means. The curves describing the response to ACTH were computed by non-linear least square method [20], from mean values of net steroid production in response to different concentration of $ACTH_{1-24}$.

roids in both groups, averaging 63 pmol ± 22 and 34 pmol ± 7, respectively (n.s.), over 2 h of incubation in the presence of vehicle only.

On the other hand, when the cells were incubated with ACTH, the maximal steroidogenic response was markedly enhanced for cells from ACTH-treated rabbits. V_{max} for steroid production averaged in this group 2.64 ± 0.41 nmol (per 1×10^5 cells and 2 h incubation) vs 1.47 ± 0.13 nmol in controls ($P < 0.005$). This increase in steroidogenic response to ACTH *in vitro* induced by previous *in vivo* treatment with ACTH, was demonstrable only at peptide concentrations higher than 10^{-9} M, as illustrated in Fig. 1. By contrast, at ACTH concentrations lower than 10^{-10} M, the steroidogenic response was reduced for cells from ACTH-treated animals, hence the shift to the right of the log-dose response curve. Thus, the mean A_{50} values (concentration of ACTH inducing a half maximal effect) was 60 ± 17 pM in the case of the control group and 528 ± 202 pM for the ACTH-treated group. When the maximal steroidogenic response to ACTH (at $\geqslant 10^{-8}$ M concentration of the peptide) is expressed in terms of specific net production of corticosterone and cortisol, as well as cortisone and 11-deoxycortisol (Fig. 2), a sharp increase in the production of 17-hydroxylated steroids was noted with concomitant reduction in corticosterone production by 50% (Fig. 2).

Androgen production by adrenocortical cells of control and ACTH-treated rabbits

Production of dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulfate (DHA-S). Before incubation, cells from control animals contained small amounts of DHA, averaging 110 ± 29 fmol per 1×10^5 cells. There was a 10-fold increase (to 1,139 ± 419 fmol) in cells from animals treated with ACTH *in vivo* ($P < 0.01$). The corresponding values for DHA-S were 110 ± 17 and 717 ± 216 fmol, respectively ($P < 0.01$). Net production in baseline conditions was negligible for both compounds.

The production of these steroids in response to ACTH added to the incubation medium was considerably increased when adrenocortical cells were har-

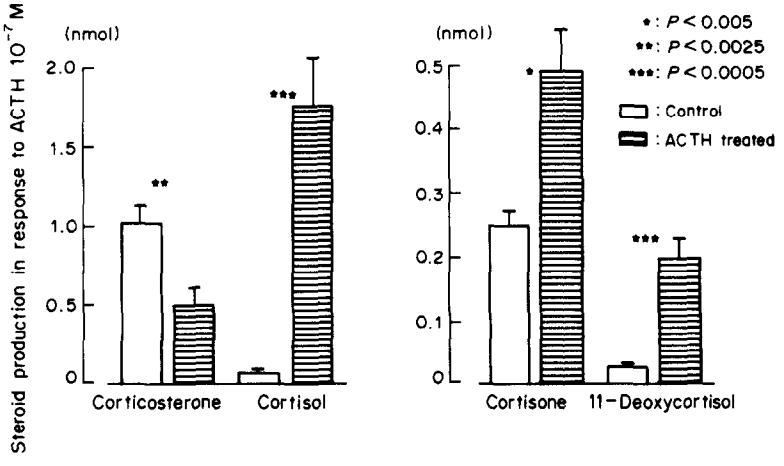

Fig. 2. Net production of corticosterone, cortisol, cortisone and 11-deoxycortisol (in nmol per 10^5 cells and 2 h) by rabbit adrenocortical cells harvested from control ($n = 12$) and ACTH-treated ($n = 12$) animals, and incubated during 2 h in the presence of $ACTH_{1-24}$, 10^{-7} M. Values are means ± SEM.

Table 1. Dehydroepiandrosterone (DHA) and dehydroepiandrosterone sulfate (DHA-S) production by adrenocortical cells harvested from control ($n = 12$) and ACTH-treated ($n = 12$) rabbits, as a function of concentration of ACTH during incubation

		ACTH concentration in the incubation medium (mol/litre)						
		10^{-12}	10^{-11}	2.5×10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
DHA	Control	12 ± 7	24 ± 5	31 ± 6	78 ± 17	94 ± 14	104 ± 12	125 ± 21
	ACTH treated	92 ± 38	92 ± 34	161 ± 43	767 ± 263	2,019 ± 553	3,289 ± 830	4,097 ± 1,252
	$P <$	0.025	0.025	0.005	0.01	0.0025	0.0005	0.0005
DHA-S	Control	57 ± 12	91 ± 25	116 ± 29	143 ± 38	176 ± 39	214 ± 43	229 ± 42
	ACTH treated	61 ± 26	316 ± 82	743 ± 185	2,095 ± 438	3,532 ± 925	4,409 ± 1,159	4,506 ± 926
	$P <$	n.s	0.0025	0.0005	0.0005	0.0005	0.0005	0.0005

Results are in fmol per 10^5 cells and 2-h incubation; means ± SEM.
P values (Student's t-test) indicate the significance of differences between cells from control and ACTH-treated animals.

Table 2. Dehydroepiandrosterone (DHA) and dehydroepiandrosterone-sulfate (DHA-S) production by adrenocortical cells harvested from control ($n = 5$) and ACTH-treated ($n = 5$) rabbits, incubated in the presence of dbc-AMP

		Dbc-AMP concentration in the incubation medium (mmol/litre)				ACTH†
		0.01	0.1	1.0	10.0	10^{-7} M
DHA (fmol)	Control	3 ± 3	6 ± 3	111 ± 59	145 ± 53	144 ± 40
	ACTH treated	122 ± 48	139 ± 35	871 ± 279	5,212 ± 2,605	4,760 ± 2,096
	$P^* <$	0.0025	0.0025	0.0025	0.0025	0.0025
DHA-S (fmol)	Control	9 ± 9	24 ± 24	90 ± 67	243 ± 104	231 ± 58
	ACTH treated	31 ± 19	64 ± 37	669 ± 216	2,139 ± 932	3,452 ± 732
	$P^* <$	n.s.	n.s.	0.005	0.005	0.005

Results are in fmoles per 10^5 cells and 2-h incubation; means ± SEM.
†Maximal steroidogenic effect of ACTH determined for the same cell aliquots. *P-values estimated by the non-parametric Wilcoxon test.

vested from ACTH-treated animals, as indicated in Table 1. The production of DHA-S slightly exceeded that of DHA; for both androgens it was much lower than the production of corticosterone or cortisol.

The production of DHA and DHA-S in response to dbc-AMP, determined in 5 experiments for each experimental group, is summarised in Table 2. As was previously demonstrated for glucocorticoid and aldosterone production, dbc-AMP (at 10 mM concentration) quantitatively reproduced the maximal effect of ACTH. Furthermore, the magnification of DHA and DHA-S production by cells from ACTH-treated animals (Table 1), was observed both with ACTH and dbc-AMP (Table 2).

Production of androstenedione and testosterone. The content of androstenedione in adrenocortical cells before the incubation averaged 961 ± 283 fmol and 3,841 ± 1,024 fmol ($P < 0.005$) for cells from control and ACTH-treated group, respectively.

The cellular content of testosterone before incubation was lower yet, amounting to 335 ± 53 fmol for control and 722 ± 422 fmol for ACTH-treated group (n.s.).

The net production of these 2 steroids in baseline conditions, thus in absence of ACTH *in vitro*, was again negligible for both experimental groups.

When the cells were incubated in the presence of ACTH (Table 3) the production of both androgens increased in a dose-dependent fashion. As for DHA and DHA-S, the ACTH-induced enhancement in androgen production by cells from ACTH-treated rabbits.

DISCUSSION

The results of the present study strongly support the notion that prolonged stimulation of the adrenal cortex with ACTH not only exerts a trophic effect (as evidenced here by a more than 4-fold increase in adrenal weight), but also enhances the steroidogenic capacity of adrenocortical cells. Indeed, the total production of C_{21} steroids (essentially cortisol) in ACTH-treated rabbits [5, 14], was clearly enhanced (Fig. 1). This enhancement in steroid production could be reproduced by incubation of adrenocortical cells with dbc-AMP [5]. This indicates that the pro-

Table 3. Androstenedione and testosterone production by adrenocortical cells harvested from control ($n = 12$) and ACTH-treated ($n = 12$) rabbits, as a function of the concentration of ACTH during incubation.

		ACTH concentration in the incubation medium (Mol/litre)						
		10^{-12}	10^{-11}	2.5×10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}
Androstenedione	Control	156 ± 72	130 ± 37	302 ± 60	600 ± 101	854 ± 110	861 ± 124	901 ± 123
	ACTH treated	656 ± 231	1,662 ± 345	3,107 ± 767	9,119 ± 2,235	20,426 ± 5,936	29,050 ± 7,578	30,063 ± 7,367
	$P <$	0.025	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
Testosterone	Control	21 ± 10	50 ± 18	92 ± 25	287 ± 103	370 ± 68	395 ± 72	474 ± 76
	ACTH treated	33 ± 27	74 ± 49	137 ± 29	709 ± 208	1,014 ± 227	1,302 ± 446	1,646 ± 529
	$P <$	n.s.	n.s.	n.s.	0.05	0.025	0.0025	0.0025

P-values (Student's t-test) indicate the significance of differences between cells from control and ACTH-treated animals.
Results are in pmol per 10^5 cells and 2-h incubation; means ± SEM.

Table 4. Androstenedione and testosterone production by adrenocortical cells harvested from control ($n = 5$) and ACTH-treated ($n = 5$) rabbits, incubated in the presence of dbc-AMP.

		Dbc-AMP concentration in the incubation medium (mmol/litre) 0.01	0.1	1.0	10.0	ACTH† 10^{-7} M
Androstenedione	Control	100 ± 47	195 ± 62	275 ± 135	682 ± 308	1,003 ± 226
	ACTH treated	968 ± 310	1,449 ± 445	7,263 ± 2,592	28,924 ± 10,350	28,367 ± 10,310
	$P^*<$	0.0025	0.0025	0.0025	0.0025	0.0025
Testosterone	Control	11 ± 10	13 ± 13	157 ± 20	359 ± 79	462 ± 97
	ACTH treated	83 ± 45	89 ± 41	292 ± 108	739 ± 200	1,152 ± 420
	$P^*<$	0.0025	0.0025	0.05	0.05	0.025

Results are in pmol per 10^5 cells and 2-h incubation; means ± SEM.
*P values estimated by the non-parametric Wilcoxon test.

longed ACTH treatment stimulates in a lasting way steroidogenic steps distal to binding of ACTH to its receptor and subsequent activation of the adenylate cyclase system. In addition, the present study confirms several previous observations [4–6, 10] indicating that the increase in the steroidogenic response to ACTH *in vitro*, which results from previous chronic exposure of adrenocortical cells to the peptides is associated with a decrease in the sensitivity of adrenocortical cells to ACTH. While the mechanism of the latter effect was not elucidated it may be a consequence of a reduced number or altered function of ACTH receptors. Even if the changes in adrenocortical function documented in the present study in chronically ACTH-treated animals were induced by administration of $ACTH_{1\text{-}24}$, it can be assumed that the natural form of corticotropin exerts a similar influence on adrenocortical cells. Indeed, it has been previously demonstrated in man [21] and in guinea-pig [6] that the stress-induced release of endogenous ACTH, as well as prolonged corticotropin hypersecretion in patients with Cushing's disease [4], are followed by an enhancement of the steroidogenic potency of adrenocortical cells quite comparable to that documented in the present study as a result of chronic treatment with $ACTH_{1\text{-}24}$.

The present data also confirm that prolonged stimulation with ACTH influences the pattern of glucocorticoid synthesis [5, 11–15] in the rabbit since the production of 17α-hydroxylated compounds was considerably enhanced while that of corticosterone decreased. In fact, adrenocortical cells from ACTH-treated animals reacted to the peptide *in vitro* much more in terms not only of cortisol production but also of production of 11-deoxycortisol as well as of cortisone (Fig. 2).

The present study extends this kind of finding to the case of androgens produced by adrenocortical cells from control and ACTH-treated rabbits. Production of these steroids either in absence or in the presence of ACTH was very small compared to the production of C_{21} steroids. Indeed, in normal rabbits the sum of all androgens measured amounted to 1.73 pmol (per 10^5 cells and 2-h incubation) in response to maximal concentration of ACTH, which is three orders of magnitude smaller than glucocorticoid production. This contrasts sharply with the quantitative importance of androgen (mainly DHA-S) production in other species such as dog [22] or man [23, 24]. Another surprising finding was the fact that the dose-related increase in androgen secretion in response to ACTH *in vitro* did not fit the sigmoid curve, which describes glucocorticoid production (Fig. 1) in response to ACTH. Another feature of androgen production in normal rabbit consists in the fact that the production of androstenedione and testosterone is much higher than that of Δ5-androgens i.e. DHA and DHA sulfate.

As to the production of androgens by adrenocortical cells of rabbits treated *in vivo* with ACTH, it was in general much more enhanced with respect to normal rabbits, compared to what occurred for the total production of C_{21} steroids. As may be concluded from Tables 1 and 3, the increase in the maximal response to ACTH ranged from more than 3-fold (for testosterone) to 33-fold (for DHA) while total steroidogenesis (Fig. 1) nearly doubled with respect to control rabbits. As to the relatively small enhancement in testosterone production by adrenocortical cells from ACTH-treated animals (all males) it might reflect the limited conversion of androstenedione into testosterone since in both experimental groups androstenedione was quantitatively the most important among the androgens measured.

The data presented in Tables 2 and 4 clearly indicate that the ACTH-induced enhancement in androgen production may be quantitatively reproduced by incubation of adrenocortical cells with dbc-AMP. This indicates again that the prolonged effect of ACTH on steroidogenesis stimulates the sites distal to the receptor action of the peptide and cyclic AMP generation [3–5]. In the case of androgen production by rabbit adrenocortical cells, this prolonged effect of ACTH consisted in all likelihood essentially in a stimulation of 17α-hydroxylase activity. Indeed, enhancement in androgen production documented in ACTH-treated rabbits is much more than could be expected from increased generation of pregnenolone.

The present data therefore provide an additional significant argument for a prolonged stimulatory effect of ACTH on 17α-hydroxylase activity. This is observed not only in the rabbit [5, 11–15] but also in other species, including man. Indeed, we reported previously that repeated administration of ACTH to

normal subjects, led to a progressive increase in cortisol and 11-deoxycortisol plasma levels, which stood in contrast with corticosterone, the secretion of which did not change [25]. The effect of ACTH on androgen secretion, documented here for the rabbit, may also occur in man. This particular aspect of the influence of ACTH on human adrenal cortex has not been carefully studied to date: it may have potential clinical implications, essentially in female patients undergoing chronic treatment with ACTH or suffering from Cushing's disease.

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REFERENCES

1. Gill G. N.: Mechanism of ACTH action. *Metabolism* **21** (1972) 571–588.
2. Dubois R. N., Simpson E. R., Kramer R. E. and Waterman M. R.: Induction of synthesis of cholesterol side chain cleavage cytochrome P-450 by adrenocorticotropin in cultured bovine adrenocortical cells. *J. biol. Chem.* **256** (1981) 7000–7005.
3. McKenna T. J., Island D. P., Nicholson W. E., Miller R. B., Lacroix L. and Liddle G. W.: ACTH stimulates late steps in cortisol biosynthesis. *Acta endocr., Copenh.* **90** (1979) 122–132.
4. Kolanowski J. and Crabbé J.: Characteristics of the response of human adrenocortical cells to ACTH. *Molec. cell. Endocr.* **5** (1976) 255–267.
5. Llano M., Kolanowski J., Ortega N. and Crabbé J.: Changes in corticosteroid secretory pattern induced by prolonged corticotropin treatment in the rabbit. *J. steroid Biochem.* **17** (1982) 631–638.
6. Lambert F., Lammerant J. and Kolanowski J.: The stimulatory effect of corticotropin on cortisol biosynthetic pathway in guinea-pig adrenocortical cells. *J. steroid Biochem.* **18** (1983) 731–735.
7. Kowal J.: Adrenal cells in tissue culture, II Effect of adrenocorticotropin and 3′5′ cyclic adenosine monophosphate on 11β-hydroxylase and other steroidogenic enzymes. *Biochemistry* **8** (1969) 1821–1831.
8. Ganguly A., Meikle A. W., Tyler F. H. and West C. D.: Assessment of 11β-hydroxylase activity with plasma corticosterone, deoxycorticosterone, cortisol and deoxycortisol: role of ACTH and angiotensine. *J. clin. Endocr. Metab.* **44** (1977) 560–568.
9. Kramer R. E., Simpson E. R. and Waterman M. R.: Induction of 11β-hydroxylase by corticotropin in primary cultures of bovine adrenocortical cells. *J. biol. Chem.* **258** (1983) 3000–3005.
10. Lambert F., Lammerant J. and Kolanowski J.: The prolonged stimulatory effect of ACTH on 11β-hydroxylation, and its contribution to the steroidogenic potency of adrenocortical cells. *J. steroid Biochem.* **20** (1984) 863–868.
11. Kaas E. N., Hechter O., Macchi I. M. and Mou J. W.: Changes in patterns of secretion of corticosteroids in rabbits after prolonged treatment with ACTH. *Proc. Soc. exp. Biol.* **85** (1954) 583.
12. Krum A. A. and Glenn R. E.: Adrenal steroid secretion in rabbit following prolonged ACTH administration. *Proc. Soc. exp. Biol.* **118** (1965) 255–258.
13. Fevold H. R.: Regulation of adrenal cortex secretory pattern by adrenocorticotropin. *Science* **156** (1967) 1753–1755.
14. Slaga T. J. and Krum A.: Modification of rabbit adrenal steroid biosynthesis by prolonged ACTH administration. *Endocrinology* **93** (1973) 517–526.
15. Fevold H. R. and Drummond H. B.: Factors affecting the adrenocorticotropic hormone stimulation of rabbit adrenal 17α-hydroxylase activity. *Biochim. biophys. Acta* **313** (1973) 211–220.
16. Sayers G., Swallow R. L. and Giordano N. D.: An improved technique for the preparation of isolated rat adrenal cells: a sensitive, accurate and specific method for the assay of ACTH. *Endocrinology* **88** (1971) 1062–1068.
17. Kolanowski J.: Stimultaneous determinations of cortisol, deoxycortisol and corticosterone in plasma and urine by competitive protein binding assay. Response of normal subjects to ACTH and metyrapone. *J. steroid Biochem.* **5** (1974) 55–64.
18. Kolanowski J. and Ortega N.: Sécrétion de cortisone par le cortex surrénal humain. *Ann. Endocr., Paris* **37** (1976) 403–404.
19. Bardin C. W. and Santen R. J.: Androgen assay in blood and other biological fluids. In *Methods in Investigative and Diagnostic Endocrinology* (Edited by A. S. Berson and R. F. Yalow). North Holland, Vol. III (1975) pp. 53–67.
20. Cleland W. W.: The statistical analysis of enzyme kinetic data. *Adv. Enzym.* **29** (1967) 1–32.
21. Kolanowski J., Salvador G. and Crabbé J.: Influence de l'agression chirurgicale sur la sensibilité du cortex surrénal à la corticotropine exogène. *Ann. Endocr., Paris* **33** (1972) 211–215.
22. Parker L. and Odell W.: Control of adrenal androgen secretion. *Endocr. Rev.* **1** (1980) 392.
23. Nieschlag E., Loriaux D. L., Ruder H. J., Zucker I. R., Kirschner M. A. and Lipsett M. B.: The secretion of hydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J. Endocr.* **57** (1973) 123–134.
24. Brooks R. V.: Biosynthesis and metabolism of adrenocortical steroids. In *The Adrenal Gland* (Edited by V. H. T. James). Raven Press, New York (1979) pp. 67–92.
25. Kolanowski J., Pizarro M. A. and Crabbé J.: Potentiation of adrenocortical response upon intermittent stimulation with corticotropin in normal subjects. *J. clin. Endocr. Metab.* **41** (1975) 453–465.