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EFFECT OF ACTH AND PROLACTIN ON DEHYDROEPIANDROSTERONE, ITS SULFATE ESTER AND CORTISOL PRODUCTION BY NORMAL AND TUMOROUS HUMAN ADRENOCORTICAL CELLS

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Summary—The effect of ACTH and prolactin on the synthesis of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) was studied in cell suspensions of "normal" and tumorous (adenoma) human adrenal cortex. A stimulation of DHEA and no response of DHEAS production by ACTH in "normal" adrenocortical cell suspension was observed. However ACTH stimulated both DHEA and DHEAS synthesis in tumorous adrenocortical cells. Prolactin did not influence either the basal or the ACTH stimulated DHEA and DHEAS production of adrenocortical cells irrespective of their origin. Our results are compatible with the concept that the biosynthesis of DHEA is under ACTH control, while other factor(s) regulate(s) the sulfate pathway of DHEA secretion under normal conditions. In tumorous adrenocortical cells DHEA may be regulated—at least partly—by ACTH. Prolactin seems to have no direct effect on DHEA and DHEAS synthesis. It is postulated that the relationship between serum prolactin and DHEAS (or DHEA) levels observed by several authors might be an extraadrenal effect of prolactin on adrenal androgens.

INTRODUCTION

The regulation of adrenal androgen production is not well understood. In an early study [1] a 20- to 100-fold rise of the DHEA level in human adrenal venous effluent following ACTH administration was observed, but, there was no change in the DHEAS level. It has been demonstrated [2] that adrenal DHEA is secreted episodically, and synchronously with cortisol throughout the entire day, while DHEAS displays a different daily pattern. There is a much more significant rise in the peripheral circulation of DHEA relative to that of DHEAS after ACTH stimulation in normal and hyperprolactinaemic subjects [3–5], and there is a more pronounced decrease of the serum free steroid relative to its sulfate following dexamethasone suppression [6, 7]. Although there are a cortisol like diurnal and day-to-day variations of serum DHEA concentration, such a fluctuation of DHEAS is lacking [8]. These moderate changes of the DHEAS level in the circulation relative to the parent free adrenal secretory product and its ACTH independent glandular production might be attributed to an extraglandular interconversion of DHEA and DHEAS and a slow metabolic clearance pattern of DHEAS [9].

Several investigators postulated a separate pituitary factor acting in addition to, or in place of, ACTH in the control of adrenal DHEA secretion [10–12]. Recently, it has been hypothesized, mainly from morphological findings, that an adrenal androgen-stimulating hormone does not exist [13].

Many authors have found a greater than normal DHEAS level in the peripheral blood of women with hyperprolactinaemia [14–17] and a normalization of its level after the treatment of the hyperprolactinaemic state [18, 19]. These findings led to the conclusion that, although the mechanism is still unknown, prolactin has an influence on the adrenal androgen (at least DHEAS) synthesis. However, the relationship between adrenal androgen and prolactin is still controversial, since many reports could not demonstrate a correlation between prolactin and DHEAS [4, 20–22].

To gain additional information on the regulation of adrenal androgen synthesis, an *in vitro* study has been performed on "normal" and tumorous human adrenocortical cell suspensions. The production of DHEA, DHEAS and as a comparison of cortisol by isolated adrenocortical cells was stimulated by adding ACTH and/or prolactin to the medium.

EXPERIMENTAL

For presumably normal human adrenocortical tissue the adjacent adrenal cortex of an adrenal phaeochromocytoma from a 26-year old male was used. The tumorous adrenocortical tissue was removed from a 27-year old woman, for whom a diagnosis of adrenal hyperandrogenism due to unilateral adrenocortical adenoma was established.

After the removal of the glands, fragments of adrenal cortex (of approx 1–2 g) were placed in ice-cold Krebs–Ringer bicarbonate solution containing 2 g/l glucose and 40 g/l human serum albumin (Phylaxia, Hungary), [KRBGA] and immediately transferred to the laboratory. The fragments were cleaned from fat and adjacent connective tissue, cut into small pieces and digested with 2 mg/ml collagenase (type I, Worthington Chemical Co., U.S.A.) and 0.15 mg/ml DNase I (Sigma Chemical Co., U.S.A.) in 3 ml/100 mg KRBGA solution [23, 24]. The mixture was incubated at 37°C under an atmosphere of 95% O_2 and 5% CO_2 in a shaking incubator (100 cpm) for 2 × 25 min. During and after incubation, dispersion of the cells was enhanced with mechanical disruption by repeated (25 ×) aspiration into glass pipettes. The supernatant containing the dispersed cells was decanted, filtered through nylon gauze (60 μm pore size), washed with KRBGA and centrifuged (100 *g*) 3-times for 10 min. The cell pellets were resuspended in KRBGA containing 5 g/l albumin.

Incubation for assessing steroid hormone production of the isolated adrenocortical cells was carried out in 10 ml Teflon beakers. Multiple 0.9 ml aliquots of the cell suspension (approx 10^5 cells/ml) were incubated as above for 2 h. Corticotrophin (α_h^{1-39} ACTH, Hormone Distribution Office, N.I.A.M.D.D., National Institutes of Health, Bethesda, Maryland, U.S.A.) was added in concentrations of 2.0×10^{-11} M to 5.1×10^{-9} M dissolved in 0.05 ml vol of physiological saline containing 0.5% albumin (pH 3.5). Samples were assayed in duplicates or triplicates. Additional 0.05 ml vol of the ACTH free solution served as control.

Prolactin (NIAMDD Rat Prolactin-B-Z) in amounts of 10.0, 100.0 and 1000.0 ng/ml was added to ACTH free beakers, and 10.0 ng prolactin was added to those containing ACTH in amounts indicated above. The ACTH contamination of prolactin was measured by radioimmunoassay [25].

Steroids in the incubates were determined by radioimmunological methods.

To a 200 μl aliquot, 800 μl phosphate buffer (0.05 mol, pH 7.3) and a tracer amount of [1,2,6,7-^{3}H] DHEA (Amersham, sp. act. 60–100 Ci/mmol) to monitor recovery of the extraction, were added. The incubate was extracted with 8 ml ether and frozen at −20°C, then the ether phase was decanted and evaporated. The residue was dissolved in 1.0 ml phosphate buffer, and the DHEA content in two 200 μl samples determined [26]. In brief, to samples the above tracer and the antiserum were added, thereafter it was incubated. An antiserum raised by ourselves against DHEA-7-carboxymethyloxime-BSA in rabbits was employed. (Titer 1:50.000; cross reactivities: DHEAS, androst-5-en-3β, 17β-diol, androstanediols, androsterone, whatever C_{18}-, C_{19}- and C_{21}-steroid <0.3%).

The above residue of ether extraction was worked out for the determination of DHEAS. Two 20 and 200 μl aliquots, respectively, were assayed as described previously [27]. Tritiated DHEA and an antiserum raised in our laboratory against DHEA-3-hemisuccinate-BSA in rabbits (Titer 1:10.000) were used [27].

Cortisol was determined in 200 μl aliquots of the incubate. In our ether extraction technique [1,2,6,7-^{3}H] cortisol (Amersham; sp. act. 59.3 Ci/mmol) was used either to control recovery or for the assay. Our anti-cortisol-21-hemisuccinate-BSA serum raised in rabbits were employed (Titer 1:2.500). For details we refer to our previous communication [27].

Dextran-charcoal technique for the separation of free and bound fraction, and a Packard Tri-Carb 3380 liquid scintillation spectrometer for radioactivity measurements were used for all assays.

An intraassay coefficient of variation below 7.9% and an interassay variance below 13.4% for the 3 steroid assays were calculated.

The significance of the data were analyzed using two-way analysis of variance.

RESULTS

Adrenocortical cells obtained at surgery for phaeochromocytoma were regarded from functional aspects as "normal". Figure 1 demonstrates the DHEA, DHEAS and cortisol production by normal

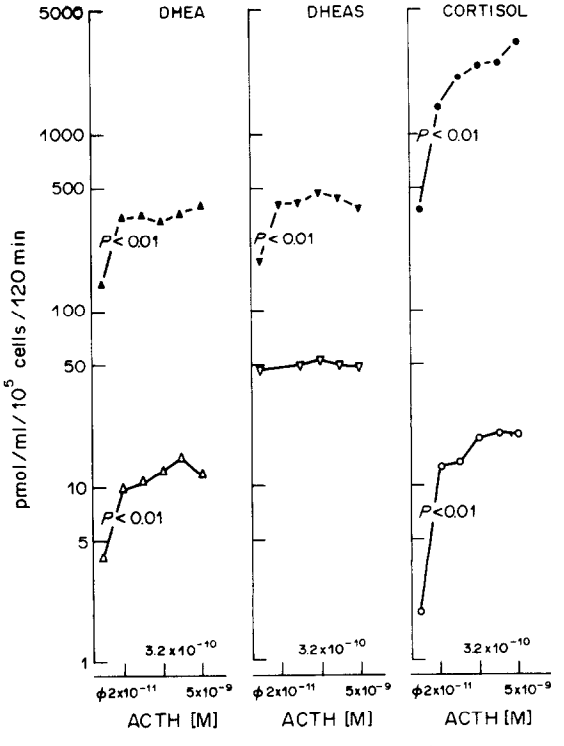

Fig. 1. Dose–response curves of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and cortisol production by normal adrenocortical cells (△——△) and by adrenocortical adenoma cells (▲——▲). Points represent the mean of 2–4 incubations.

Table 1. Effect of prolactin on basal and ACTH stimulated steroid secretion of isolated normal human adrenal cortical cells (nmol/l/3 × 10^5 cells/2^h)

	DHEA	DHEA-S	Cortisol
Vehicle	4.0 ± 0.21*	46.9 ± 5.88	19.3 ± 2.93
Prolactin 10 ng	4.4 ± 1.09	42.2 ± 3.82	22.7 ± 2.40
Prolactin 100 ng	3.7 ± 0.43	43.7 ± 2.97	20.3 ± 1.35
Prolactin 1000 ng	7.3 ± 0.26	43.3 ± 1.58	49.4 ± 2.40
ACTH 2×10^{-11} M	9.8 ± 0.10	—	130.2 ± 13.40
ACTH 2×10^{-11} M + Prolactin 10 ng	9.6 ± 0.75	47.6 ± 2.17	142.1 ± 7.31
ACTH 8×10^{-11} M	10.5 ± 0.38	49.9 ± 2.09	135.1 ± 10.58
ACTH 8×10^{-11} M + Prolactin 10 ng	10.5 ± 0.45	51.5 ± 2.32	152.4 ± 9.74

*Mean ± SEM of 2–4 incubates.
— not measured.

and tumorous adrenocortical cells in response to increasing concentrations of ACTH. The basal steroid production without any precursor in pmol/ml/10^5 cells/120 min was 4.0 ± 0.2 for DHEA, 46.9 ± 5.9 for DHEAS and 19.3 ± 2.9 for cortisol ($\bar{x}$ ± SEM) in normal adrenocortical cells (Table 1). Tumorous adrenocortical cells produced 144.2 ± 8.0 DHEA, 196.8 ± 8.5 DHEAS and 383 ± 38.9 cortisol (Table 2). Corticotrophin increased DHEA and cortisol production of both normal and tumorous adrenal cells, it stimulated DHEAS biosynthesis only in tumorous cells.

Prolactin in a dose of 10 or 100 ng/ml medium did not affect the basal and ACTH stimulated steroid production in normal adrenocortical cells (Table 1). A statistically insignificant increase of DHEA synthesis was observed in tumorous adrenocortical cells when ACTH and prolactin in a dose of 10 ng/ml were added to the suspension. Interestingly, such a mild increase of cortisol synthesis was also demonstrable in the same samples (Table 2).

Although prolactin in a dose of 100 ng/ml stimulated steroid production in normal as well as in tumorous adrenocortical cells, this effect may be attributed to the ACTH contamination of prolactin. (The ACTH content of the prolactin preparation was 24 fmol/l μg prolactin as determined by radioimmunoassay.)

DISCUSSION

In the present *in vitro* experiments the results gave an evidence that ACTH did not stimulate DHEAS secretion in cell suspension of normal human adrenals, whereas, like that of cortisol, free DHEA synthesis appeared to be under ACTH controls. These results confirm the earlier *in vivo* observations [1] demonstrating a sharp increase of free DHEA and no change of DHEAS levels in adrenal venous blood following exogenous ACTH in subjects with normal adrenocortical function.

The mechanism of ACTH action on unconjugated DHEA in normal adrenal appears to be a complex chain of events, and is not well understood. Although previous studies [1–5] and the present observation demonstrated that cortisol and DHEA synthesis is under ACTH control, the enzymes which operate through the 5-ene pathway to produce DHEA, or through the 4-ene route resulting in cortisol and other corticosteroids, are under the influence of multiple control mechanisms. Corticotropin administration generally causes a more pronounced increase in serum cortisol than in DHEA levels [3–5]. Although angiotensin II in synergism with ACTH stimulated cortisol and DHEA synthesis alike in adrenocortical cells *in vitro*, the rate of production of the two steroids was again not identical [28]. The slope of the dose–response curves for the suspension of normal as well as tumorous adrenocortical cells in the present study showed a more marked cortisol stimulation than that of DHEA by ACTH, indicating different responses and diverse mechanisms involved in the ACTH action. The different mode and rate of the ACTH effect on the adrenal DHEA and cortisol synthesis are still unknown, and their characterization, requires further studies.

Table 2. Effect of prolactin on basal and ACTH stimulated steroid secretion of isolated tumorous human adrenal cortical cells (nmol/l/3 × 10^5 cells/2^h)

	DHEA	DHEA-S	Cortisol
Vehicle	144.2 ± 7.99*	196.4 ± 8.49	383.8 ± 38.95
Prolactin 10 ng	142.2 ± 4.55	161.2 ± 8.60	340.8 ± 22.95
Prolactin 100 ng	182.7 ± 10.50	198.2 ± 5.25	332.8 ± 22.30
Prolactin 1000 ng	301.7 ± 18.95	390.1 ± 22.60	791-5 ± 67.55
ACTH 2×10^{-11} M	335.9 ± 37.10	409.2 ± 32.91	1438.0 ± 316.10
ACTH 2×10^{-11} M + Prolactin 10 ng	425.8 ± 46.00	369.7 ± 9.45	2356.3 ± 76.05
ACTH 8×10^{-11} M	348.0 ± 23.17	408.9 ± 20.35	2104.0 ± 56.35
ACTH 8×10^{-11} M + Prolactin 10 ng	448.8 ± 39.55	322.2 ± 55.50	2352.6 ± 106.65
ACTH 3.2×10^{-10} M	328.1 ± 9.46	468.6 ± 51.10	2452.0 ± 75.68
ACTH 3.2×10^{-10} M + Prolactin 10 ng	400.4 ± 50.40	394.9 ± 1.75	2734.7 ± 91.05
ACTH 1.3×10^{-9} M	367.1 ± 13.11	438.1 ± 16.90	2495.5 ± 200.62
ACTH 1.3×10^{-9} M + Prolactin 10 ng	326.8 ± 18.05	410.1 ± 25.05	2473.1 ± 59.90

*Mean ± SEM of 2–4 incubates.

The present *in vitro* experiments with normal adrenocortical cell suspension clearly showed that ACTH did not influence the sulfate pathway of DHEAS synthesis. This is in accordance with a previous finding demonstrating the lack of DHEAS response to ACTH, by analysis of adrenal venous blood of 6 patients with no evidence of abnormal androgen metabolism [1]. There was invariably no response in the DHEAS synthesis to ACTH stimulation in the adrenal venous effluent of two patients with adrenal androgen hyperproduction due to Cushing's syndrome [1]. It may be assumed that the slow increase of the DHEAS level in peripheral blood frequently observed following exogenous ACTH [3–5] is a result of extraglandular conversion of DHEA to DHEAS, rather than an increase in glandular DHEAS secretion.

Our observations showed an ACTH stimulation of DHEAS synthesis in tumorous adrenocortical cells. From this surprising observation we hypothesize that *in situ* sulfation of free DHEA was triggered by ACTH, a stimulatory effect of ACTH which did not occur in normal adrenocortical cells.

Previous data revealed a correlation between elevated DHEAS and prolactin levels [14–19]. In an attempt to elucidate a possible regulatory role of prolactin in the production of DHEAS and/or DHEA by the human normal or tumorous adrenocortical tissue obtained from adult subjects, we examined the effect of prolactin on adrenal androgen synthesis. Our results could not confirm a direct prolactin effect, or a potentiation of the ACTH stimulation in suspension of normal as well as tumorous adrenocortical cells. A concentration of prolactin in the medium up to 100 ng/ml was evaluated, since at 1000 ng/ml the prolactin preparation used contained 24 fmol/ml of ACTH as a contaminant. The slight increase of DHEA could be attributed to an ACTH effect, since in these media a moderate increase in cortisol synthesis was also observed.

Recently, it has been reported that prolactin has a regulatory role in the DHEAS and DHEA synthesis by the human fetal adrenal [29]. Prolactin alone and in the presence of ACTH in incubates of minced human fetal adrenals enhanced significantly the adrenal androgen production. It is known that DHEAS and DHEA are produced by the fetal zone of the adrenal, and there is an involution of this zone as well as in prolactin production after delivery [29, 30]. From these we postulate that prolactin is involved in the regulation of steroid synthesis by the adrenal gland in the fetal life, and if there exists a correlation between blood prolactin and DHEAS level in adults, suggested by several investigators, it appears to be an extraadrenal prolactin effect.

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