Mechanism of Dissociation of Cortisol and Adrenal Androgen Secretion after Removal of Adrenocortical Adenoma in Patients with Cushing's Syndrome

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Abstract

We investigated the mechanism of dissociation of cortisol and dehydroepiandrosterone sulfate (DHEA-S) secretion by the adrenal glands after the removal of an adrenal gland containing an adrenocortical adenoma in a patient with Cushing's syndrome. After removal of the adrenocortical adenoma, the serum cortisol rapidly decreased from 24.6±6.4 μg/dl (mean±SD, n=6) to 0.7 ±0.5 μg/dl. Serum DHEA-S levels were 15±14 μg/dl and 6±9 μg/dl before and after surgery, respectively, and significantly lower than the control values. Serum cortisol levels reverted to normal levels 1.5 to 3 years after the surgery. On the other hand, DHEA-S levels reverted to normal 5 to 7 years after the serum cortisol levels had normalized.

Monolayer cultures of normal human adrenal cells obtained at adrenalectomy in patients with advanced breast cancer and atrophic adrenal cells adjacent to the adrenocortical adenoma in patients with Cushing's syndrome were used to study the mechanism of the dissociation of cortisol and DHEA-S secretion. ACTH caused significant increases in the productions of pregnenolone (P₅), progesterone (P₄), 17-hydroxyprogrenolone (17-OH-P₅), 17-hydroxyprogesterone (17-OH-P₄), DHEA, DHEA-S, androstenedione (Δ₄-A), and cortisol. The amounts of 17-OH-P₅ and 17-OH-P₄ produced by ACTH in atrophic adrenal cells were significantly greater than those in normal adrenal cells. The amounts of DHEA, DHEA-S and Δ₄-A produced by ACTH in atrophic adrenal cells were significantly smaller than those of normal adrenal cells. The conversion rate of 17-OH-[³H]P₅ to 17-OH-[³H]P₄ and 11-deoxy-[³H] cortisol was higher in atrophic adrenal cells than in normal adrenal cells, but the conversion rate to [³H]DHEA, [³H]DHEA-S and [³H]Δ₄-A was significantly lower in atrophic adrenal cells than in normal adrenal cells.

These results suggest that the dissociation of cortisol from DHEA-S after the removal of adrenocortical adenoma is probably due to diminished C₁₇,₂₀-lyase activity in the remaining atrophic adrenal gland.

Although ACTH is a major regulator of both adrenal androgens and cortisol in man (Rosenfeld et al., 1971; Nieschlag et al., 1973; Sekihara et al., 1974), the dissociation of adrenal androgens from cortisol secretion was seen in many clinical situations, such as fetal adrenal (Easterling et al., 1966), adrenarche (Parker et al., 1978), puberty
(Hopper et al., 1975), aging (Ibayashi and Kato, 1984), fasting and anorexia nervosa (Zumoff et al., 1983; Sehultz et al., 1964), hyperprolactinemia (Higuchi et al., 1984), and recovery from adrenal suppression (Kappas et al., 1956; Cutler et al., 1979; Albertson et al., 1984; Yamaji et al., 1984). Additional factors explaining this independent regulation of adrenal androgens and cortisol include estrogen (Fujieda et al., 1982), prolactin (Higuchi et al., 1984), and adrenal androgen stimulating factor (Parker et al., 1983).

The dissociation of adrenal androgens from cortisol on the recovery from adrenal suppression was reported by Kappas et al., (1956), Cutler et al., (1979), and Yamaji et al., (1983). We attempted to determine the mechanism of dissociation of adrenal androgens from cortisol secretion in the recovery from adrenal suppression, using primary cultures of atrophic adrenal cells.

**Subjects and Methods**

**Materials**

Fetal calf serum and collagenase (type 1) were purchased from Gibco-Biocult (Grand Island, NY) and Sigma (St Louis, MO), respectively. Plastic culture dishes were obtained from Falcon Plastics (Oxnard, CA). $a^{1-18}$ACTH was obtained from Shionogi Co. (Osaka, Japan). Thin layer chromatography (TLC) plates silica gel 60 F$_{254}$ were purchased from Merk (Darmstadt, Germany). [1, 2, 6, 7-$^3$H]P$_5$ (19.3 Ci/mmol), [7-$^3$H]P$_4$ (97 Ci/mmol), 17-$\alpha$-OH-[1, 2-$^3$H]P$_4$ (50 Ci/mmol), [1, 2-$^3$H]DHEA (58.6 Ci/mmol), [7-$^3$H]DHEA-S (22.1 Ci/mmol) and [1, 2-$^3$H]$\Delta^4$-A (41 Ci/mmol) were purchased from New England Nuclear (Boston, Mass). 17-$\alpha$-OH-[7-$^3$H]P$_5$ (29 Ci/mmol) was purchased from Amersham (Buckinghamshire, England).

**Subjects**

Six patients with Cushing's syndrome due to adrenocortical adenoma (6 women, aged 19–50) were diagnosed as cases of Cushing's syndrome with adrenocortical adenoma, based on symptoms and signs of hypercortisolism, high plasma cortisol, low plasma ACTH, increased urinary 17-OHCS excretions, resistance to adrenal suppression by dexamethasone, and no plasma ACTH response to metyrapone. Adrenocortical adenoma was diagnosed by computed tomography and/or $^{131}$I adosterol scintigraphy of the adrenal gland. In each patient there was a unilateral and solitary adenoma. Histological diagnosis was confirmed by surgery. Serum cortisol and DHEA-S levels were determined at intervals after the removal of the adrenocortical adenoma. The patients were followed up for 1 to 9 years after surgery. Until normalization of serum cortisol, the patients were treated with maintenance doses of hydrocortisone (10–20 mg/day). Hydrocortisone was changed to dexamethasone 5 days before blood sampling to eliminate interference with measurement of serum cortisol. Blood samples were obtained between 0800 and 0900 h after an overnight fast. Serum was separated immediately and stored at $-20^\circ$C until assayed.

**Primary monolayer culture of adrenal cells**

The normal adrenal glands obtained by adrenalectomy in patients with advanced breast cancer and the adrenal glands with adrenocortical adenoma of patients with Cushing's syndrome were preserved under aseptic conditions immediately after surgery. Atrophic adrenal glands adjacent to the adrenocortical adenomas were separated from adrenocortical adenomas using fine scissors. Primary monolayer culture of adrenal cells was carried out as described (Higuchi et al., 1984). Normal adrenal glands and atrophic adrenal glands adjacent to adrenocortical adenoma were both minced with scissors, placed in modified Eagle's medium (MEM) containing 0.25% collagenase (type I), and incubated with agitation in enzyme solution, at room temperature for 60 min. The dispersed adrenal cells were collected, filtered through three layers of gauze and centrifuged at 2000 rpm for 10 min. The cells were washed three times with MEM. The washed dispersed adrenal cells were placed in 35×10 mm plastic culture dishes (Falcon 3001) at a density of 1×10$^6$ cells per dish and incubated in 1 ml MEM containing 10% fetal calf serum (FCS) at 37°C under 5% CO$_2$ in air. The culture medium was changed daily in the presence or absence of ACTH ($10^{-7}$ M) for 7 consecutive days beginning 24th after plating. All media were stored at $-20^\circ$C and then analyzed together by specific RIA for cortisol, $\Delta^4$-A, DHEA, DHEA-S, P$_5$, P$_6$, 17-OH-P$_5$, and 17-OH-P$_6$. 

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Assay
Serum cortisol was measured using Daiichi Radioisotope RIA kits (Tokyo). Interassay coefficient variation (CV) was 14%. Serum DHEA-S was extracted with ethyl alcohol. Serum DHEA, Δ⁴-A, 17-OH-P₅, 17-OH-P₄, P₅, and P₄ were extracted with ethyl ether containing tracer doses of [³H]DHEA, [³H]Δ⁴-A, 17-OH-[³H]P₅, 17-OH-[³H]P₄, [³H]P₅, and [³H]P₄ to correct the results for procedural losses, and separated as described (Higuchi et al., 1984). Extracted steroids were separated by LH-20 column chromatography with n-hexane-benzene-methanol (80: 10: 10, vol/vol/vol). The fractions containing each steroid were collected separately and subjected to RIAs. 17-OH-P₅ was separated by LH-20 column chromatography with benzene:methanol (95:5, vol/vol). Serum DHEA, DHEA-S, Δ⁴-A, 17-OH-P₅, 17-OH-P₄, P₅, and P₄ were measured by RIA using specific antisera to DHEA-11α-succ-BSA, 11-deoxy-17-KS, 4-Δ⁴-A-11α-succ-BSA, 17-OH-P₅-3-CMO-BSA, 17-OH-P₄-3-CMO-BSA, P₅-3-CMO-BSA, and P₄-3-CMO-BSA. Anti DHEA, anti 17-OH-P₅, anti 17-OH-P₄, anti P₅ and anti P₄ antibodies were obtained from Teikoku Hormone Co. (Tokyo, Japan). Anti Δ⁴-A antibody was obtained from Endocrine Sciences (Tarzana CA). Anti DHEA-S antibody was kindly supplied by Dr. H. Sekihara (1974). Interassay CV values were 13% for DHEA-S, 11% for DHEA 14% for Δ⁴-A, 9% for 17-OH-P₅, 11% for 17-OH-P₄, 10% for P₅ and 14% for P₄. Intraassay CV values were 6% for DHEA-S, 8% for DHEA, 5% for Δ⁴-A, 8% for 17-OH-P₅, 7% for 17-OH-P₄, 8% for P₅ and 8% for P₄.

Conversion of 17-OH-[³H]P₅ to [³H] products in cultured adrenal cells
Primary culture of dispersed adrenal cells (0.5×10⁵ cells/dish) from normal adrenal glands and atrophic adrenal glands was carried out. Culture medium was changed daily for 7 consecutive days in the presence of ACTH (10⁻⁸M). In a 7-day culture period 17-OH-[³H]P₅ (1.3×10⁶ cpm) and 10 nmol of 17-OH-P₅ were added to cultured adrenal cells in 1 ml MEM in the presence of ACTH (10⁻⁸ M) and incubation was carried out for 2 hs. The culture medium and adrenal cells detached from dishes by 0.05% trypsin and 0.02% EDTA were collected and homogenized in a Potter's homogenizer. Twelve ml of dichloromethane was added to the homo- genized cells which were then vortexed for 2 min and centrifuged at 2,000 rpm for 5 min. The water phase was collected to determine [³H] DHEA-S. The dichloromethane phase was collected and evaporated at 40°C under nitrogen gas. [³H]products in dichloromethane phase were isolated by two-dimensional TLC. Products were diluted in ethanol containing standard markers of 17-OH-P₅, 17-OH-P₄, Δ⁴-A, 11-OH-Δ⁴-A, DHEA, 11-deoxy cortisol and cortisol, chromatographed three times in chloroform: ethyl acetate (10:1) at the first dimension and then chromatographed twice in chloroform: ethanol (98:2) at the second dimension. Recovery ranged from 80 to 90%. Δ⁴-A, 11-OH-Δ⁴-A, 17-OH-P₅, cortisol and 11-deoxycortisol were detected with a short-wave ultraviolet light. DEHA and 17-OH-P₄ were detected with a sulfuric acid spray. DHEA-S extracted in water phase was solvolysed by the method of Ando et al., (1977). The solvolysed DHEA-S was extracted by dichloromethane and chromatographed on two-dimensional TLC and detected with a sulfuric acid spray. These products were scraped from the plate into scintillation vials and measured for radioactivity with a liquid scintillation counter after the addition of 10 ml of Scintisol (Wako, Tokyo, Japan).

Results
Change in serum cortisol and DHEA-S levels after removal of adrenal gland containing adrenocortical adenoma
Clinical features of 6 patients with adrenocortical adenoma of Cushing's syndrome are given in Table 1. Serum cortisol levels in the patients with Cushing's syndrome (26.6 ± 6.4 μg/dl) were significantly higher than those of normal women (13.3 ± 3.1 μg/dl). After the removal of the adrenocortical adenoma, the serum cortisol markedly decreased (0.7 ± 0.5 μg/dl). On the other hand, serum DHEA-S levels were significantly lower than those of normal women of corresponding age before and after surgery (15 ± 14 μg/dl and 6 ± 9 μg/dl, respectively) in the patients with Cushing's syndrome.
Changes in serum cortisol and DHEA-S levels after the removal of an adrenocortical adenoma are shown in Fig. 1. Following removal of the adrenocortical adenoma, high serum cortisol levels dropped markedly to low levels. After the surgery, maintenance doses of hydrocortisone (10–20 mg/day) were given once each morning. Serum cortisol began to rise 6 months to 1 year after the surgery and reverted to normal levels 1.5 to 3 years after surgery. In one patient, serum cortisol has not reverted to a normal level even 3.5 years after the surgery. On the other hand, serum DHEA-S levels in patients with Cushing's syndrome were lower than those in normal women before surgery and remained low after surgery, and even after the suppressed serum cortisol levels had normalized. Then serum DHEA-S levels gradually increased 3 to 9 years after the surgery.
Table 1. Clinical features of patients with adrenocortical adenomas of Cushing’s syndrome.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Serum cortisol (µg/dl)</th>
<th>Serum DHEA-S (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before surgery</td>
<td>After surgery</td>
</tr>
<tr>
<td>1. TM</td>
<td>19</td>
<td>F</td>
<td>36.0</td>
<td>0.2</td>
</tr>
<tr>
<td>2. MM</td>
<td>50</td>
<td>F</td>
<td>26.7</td>
<td>0.2</td>
</tr>
<tr>
<td>3. SA</td>
<td>28</td>
<td>F</td>
<td>24.0</td>
<td>1.2</td>
</tr>
<tr>
<td>4. MT</td>
<td>42</td>
<td>F</td>
<td>23.7</td>
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</tr>
<tr>
<td>5. NK</td>
<td>30</td>
<td>F</td>
<td>19.4</td>
<td>0.3</td>
</tr>
<tr>
<td>6. AA</td>
<td>30</td>
<td>F</td>
<td>17.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Normal women</td>
<td>24.6±6.4^a</td>
<td>0.7±0.5</td>
<td>15±14</td>
<td>6±9</td>
</tr>
</tbody>
</table>

(n=24)

a. Each value represents mean±1SD

Fig. 2. Change in cortisol and DHEA-S secretions from cultured normal adrenal cells (upper graph) and atrophic adrenal cells (lower graph) during 6 days of culture in the presence (closed column) and absence (open column) of ACTH (10^{-7}M). Each bar represents the mean±SD of three replicate dishes.
surgery. The recovery of serum DHEA-S levels was delayed over those of the serum cortisol by 5 to 7 years.

Change in cortisol and DHEA-S secretions from cultured normal and atrophic adrenal cells (Fig. 2).

To clarify the mechanism of dissociation between cortisol and DHEA-S secretions after removal of the adrenocortical adenoma, primary culture of dispersed normal human adrenal cells and atrophic adrenal cells adjacent to adrenocortical adenoma was carried out. Daily secretions of cortisol and DHEA-S were determined in the presence and absence of ACTH for 7 consecutive days. In normal adrenal cells, ACTH caused significant increases in the production of both cortisol and DHEA-S. Production of cortisol and DHEA-S by ACTH reached a plateau 3 to 5 days after the culture and was maintained for at least 7 days. On the other hand, in atrophic adrenal cells much smaller amounts of cortisol and DHEA-S were detected on the first day of culture than in normal adrenal cells. Dissociated production of cortisol and DHEA-S by ACTH was observed. ACTH caused a

![Diagram showing steroid production by normal and atrophic adrenal cells](image-url)

Fig. 3. Patterns of steroids produced by $1 \times 10^5$ normal adrenal cells (upper graph) and atrophic adrenal cells (lower graph) over a 7-day culture period in the presence (closed column) and absence (open column) of ACTH ($10^{-7}$M). Each bar represents the mean ± SD of three replicate dishes.
significant increase in the production of cortisol, but not in DHEA-S, in atrophic adrenal cells observed for 7 consecutive days.

Patterns of steroids produced by cultured normal and atrophic adrenal cells (Fig. 3).

The patterns of steroids produced by cultured normal and atrophic adrenal cells (A.A.) in the presence and absence of ACTH were determined over a 7-day culture period. ACTH caused significant increases in P₅, P₄, 17-OH-P₅, 17-OH-P₄, DHEA and Δ⁴-A, in both normal and atrophic adrenal cells.

The amounts of 17-OH-P₅ and 17-OH-P₄ produced by ACTH in atrophic adrenal cells were significantly greater than those in normal adrenal cells, while the amounts of DHEA, DHEA-S and Δ⁴-A produced by ACTH in atrophic adrenal cells were significantly smaller than those of normal adrenal cells. In the atrophic adrenal cells, production of DHEA and DHEA-S by ACTH was much lower than that of Δ⁴-A.

Conversion of 17-OH-[³H]P₅ to [³H] products in cultured normal and atrophic adrenal cells (Fig. 4).

Fig. 4. Conversion of 17-OH-[³H]P₅ to [³H] products in 0.5×10⁵ normal adrenal cells (open column) and atrophic adrenal cells (closed column) in the presence of ACTH (10⁻⁷M) over a 7-day culture period. Conversion rate (%) was found by dividing the % of [³H] product by 17-OH-[³H]P₅. Each bar represents the mean of two replicate dishes.
Conversion of $17\text{-OH-}[^3\text{H}]P_5$ to $[^3\text{H}]$ products in cultural normal and atrophic adrenal cells in the presence of ACTH was determined over a 7-day culture period. Conversion of $17\text{-OH-}[^3\text{H}]P_5$ to $17\text{-OH-}[^3\text{H}]P_4$, 11-deoxy-$[^3\text{H}]$cortisol, $[^3\text{H}]$DHEA, $[^3\text{H}]$DHEA-S, $[^3\text{H}]J^4\text{-A}$ and 11-OH-$[^3\text{H}]J^4\text{-A}$ occurred in both normal and atrophic adrenal cells. Conversion of $17\text{-OH-}[^3\text{H}]P_5$ to $17\text{-OH-}[^3\text{H}]P_4$ and 11-deoxy-$[^3\text{H}]$ cortisol was significantly greater in atrophic adrenal cells than in normal adrenal cells. However the conversion of $17\text{-OH-}[^3\text{H}]P_5$ to $[^3\text{H}]$DHEA, $[^3\text{H}]$DHEA-S, $[^3\text{H}]J^4\text{-A}$ and 11-OH-$[^3\text{H}]J^4\text{-A}$ was significantly less in the atrophic adrenal cells.

**Discussion**

We found a dissociation between cortisol and DHEA-S secretion on the recovery from long standing adrenal suppression after unilateral adrenalectomy for adrenocortical adenoma in patients with Cushing’s syndrome. Yamaji et al., (1984) also noted a delayed recovery of suppressed DHEA-S secretion, compared with cortisol after removal of the adrenocortical adenoma in such patients. The dissociated recovery of cortisol and DHEA-S from adrenal suppression was also found in clinical studies after prolonged glucocorticoid therapy (Kappas et al., 1956; Cutler et al., 1979). The low cortisol secretion was due to a decrease in ACTH release by a negative feedback mechanism with long lasting hypercortisolism and atrophic adrenal glands resulting from deficient ACTH secretion. Although serum DHEA-S levels remained low after the serum cortisol levels had normalized, serum DHEA-S levels reverted to normal during the long term follow up. Cutler et al., (1979) demonstrated that one month after discontinuation of prednisolone in one patient on prednisolone therapy with pharmacological doses for 2.8 years the plasma cortisol response normalized, but not DHEA or DHEA-S, following ACTH infusion and that both plasma cortisol and DHEA-S response to ACTH infusion normalized 4 months after discontinuation of the prednisolone. These results strongly suggest that the enzymes or cell populations involved in androgen secretion were suppressed to a greater extent than those of the cortisol pathway in atrophic adrenal glands due to chronic ACTH deficiency.

Therefore primary culture of atrophic adrenal cells adjacent to adrenocortical adenoma in patients with Cushing’s syndrome was done to clarify the mechanism of the dissociation between cortisol and DHEA-S on the recovery from adrenal suppression. The same dissociated production of cortisol and DHEA-S in the presence of ACTH was observed in atrophic adrenal cells, as reported in clinical studies on the recovery from adrenal suppression. ACTH caused significant increase in the production of cortisol, but not DHEA-S, in the atrophic adrenal cells. Determination of the amounts of steroids and conversion rate of $17\text{-OH-}[^3\text{H}]P_5$ to $[^3\text{H}]$ products produced by cultured adrenal cells revealed that ACTH caused significantly higher production of $17\text{-OH-P}_5$ and $17\text{-OH-P}_4$, and significantly lower production of DHEA, DHEA-S and $J^4\text{-A}$ in atrophic adrenal cells than in normal adrenal cells. These results demonstrated that $C_{17,20}\text{-lyase activity was markedly diminished over the cortisol pathway. The main cause of long lasting decreases in adrenal androgen secretions on recovery from adrenal suppression (Kappas et al., 1956; Cutler et al., 1979; Albertson et al., 1984; Yamaji et al., 1984) is indicated to be the diminished $C_{17,20}\text{-lyase activity. The finding that serum 17-OH-P}_5$ was within the normal range when DHEA, DHEA-S and $J^4\text{-A}$ levels were low in one patient with Cushing’s syndrome after the removal of an adrenocortical adenoma (Yamaji et al.,) suggests a decrease in $C_{17,20}\text{-lyase activity}$
in atrophic adrenal glands. Schiebinger et al., (1981) reported that a rise in plasma adrenal androgen levels during adrenarche is coincident with a rise in C17,20-lyase and 17-hydroxylase activity in the adrenal gland. Ibayashi and Kato (1984) reported that plasma DHEA-S, DHEA and Δ4-A levels show a significantly age-related linear drop from 20 years of age with no change in plasma cortisol levels. With regard to the prominent deposition of lipofuscin in the adrenocortical reticular cells in the aged, in vitro lipoperoxidation of human adrenal microsome caused a marked decrease in C17,20-lyase and 17-hydroxylase activities (Ibayashi and Kato, 1984). These results indicate that C17,20-lyase plays an important role in adrenal androgen secretion in many clinical situations.

From the observation that adrenal atrophy due to hypothalamic pituitary diseases or glucocorticoid therapy (Cutler et al., 1979) and hypophysectomized adreno-corticotropin-replaced chimpanzee (Albertson et al., 1984) are associated with a greater impairment of the secretion of DHEA and DHEA-S than in those of cortisol and Δ4-A, and from the finding that most children and adolescents with Cushing’s disease have normal plasma DEHA-S and DHEA levels for both chronological and bone age, despite elevated Δ4-A and cortisol levels (Hauffa et al., 1984), the maturation and activation of zona reticularis cells of adrenal glands may require a second factor in addition to ACTH. Our in vitro studies demonstrated that the main cause of the dissociation between cortisol and adrenal androgen secretions on the recovery from adrenal suppression is the markedly diminished C17,20-lyase activity in atrophic adrenal glands. But the amounts of DHEA-S and DHEA from atrophic adrenal cells after chronic ACTH stimulation were lower than Δ4-A, thereby suggesting another factor in addition to ACTH. Recently Parker et al. (Parker et al., 1983) identified a 60,000 dalton adrenal adrogen stimulating factor from human pituitary adrenal gland.

The rate of conversion of 17-OH-[3H]-P₅ to [3H]product by cultured adrenal cells revealed that ACTH caused significantly higher production of [3H]S and [3H]F in atrophic adrenal cells than in normal adrenal cells. These results suggest that the pathway of 17-OH-P₅ to S and F is rapidly improved in atrophic adrenal cells, because F is quite important to maintain life.

Current studies did not identify the intraadrenal sites of the observed enzymatic changes. ACTH-caused morphological changes in the zones (Mitshke et al., 1973) and changes in the cell population in addition to enzymes may also occur.

References


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