Adrenocorticotropic Hormone Production

E. G. BIGLIERI, B. CHANG, J. HIRAI, N. BRUST, C. R. ROST AND M. SCHAMBELAN

Medical Service, San Francisco General Hospital Medical Center, and Department of Medicine, University of California, San Francisco, California, U.S.A.

Summary

1. Adrenocorticotropic (ACTH)-induced steroidogenesis, obtained by continuous administration of ACTH for 3 days, produces in man (a) sustained elevations of plasma deoxycorticosterone and cortisol concentrations, (b) transient elevations of plasma aldosterone and 18-hydroxy-corticosterone concentrations that return to near-control values, and (c) brisk initial increases in plasma 18-hydroxydeoxycorticosterone and corticosterone concentrations that fall to 20–68% of peak values 30 h thereafter.

2. Dexamethasone (8 mg/day, orally) treatment for 2 days in man permits a greater postural increase in plasma aldosterone and 18-hydroxy-corticosterone concentrations. A dampening effect of ACTH is suggested.

3. An ACTH-initiated inhibition of 11β- and 18-hydroxylation is proposed to be operative in regulation of mineralocorticoid hormones.

Key words: adrenocorticotropic, aldosterone, corticosterone, cortisol, deoxycorticosterone, mineralocorticoid hormone.

Abbreviations: ACTH, adrenocorticotropic; DOC, deoxycorticosterone; 18-OHB, 18-hydroxycorticosterone; 18-OHDOC, 18-hydroxydeoxycorticosterone; PRA, plasma renin activity.

Introduction

Continuous superphysiological administration of ACTH in man induces a sustained increase in cortisol production but only transient elevation in aldosterone production (Liddle, Duncan & Bartter, 1956; Newton & Laragh, 1968; Biglieri, Schambelan & Slaton, 1969). Under similar conditions, urinary metabolites of other steroids in the mineralocorticoid pathway, corticosterone and deoxycorticosterone (DOC) also increase above the normal range (Rauh, Levine, Gottesdiener & New, 1978; Biglieri et al., 1969). Both plasma concentrations and urinary metabolites of 18-hydroxydeoxycorticosterone (18-OHDOC) and 18-hydroxycorticosterone (18-OHB) increase promptly in response to acute administration of ACTH (Melby, Dale, Grekin, Gaunt & Wilson, 1972; Williams, Braley & Underwood, 1976; Tuck, Chandler & Mayes, 1977; Martin, Edwards, Biglieri, Vinson & Bartter, 1975). The fact that the biphasic aldosterone response to ACTH occurs (1) during salt restriction (Newton & Laragh, 1968; Biglieri et al., 1969), (2) in the low renin state of primary aldosteronism and (3) without significant changes in serum potassium concentration (Biglieri et al., 1969; Rauh et al., 1978) suggests an induced intra-adrenal event (Biglieri et al., 1969). A more detailed investigation of the response to ACTH requires frequent assessment of plasma concentrations of the mineralocorticoid hormones, the activity of the renin–angiotensin system and potassium concentration.

Methods

Three groups were studied: group 1, ACTH in normal subjects (three normal women, 28–38 years of age, with normal adrenal and renal function were studied during exogenous administration of ACTH); group 2, ACTH in patients with limited adrenal function (two women: one 26 years of age...
with partial adrenal insufficiency, normal plasma steroid concentrations, and elevated ACTH at 715 pg/ml, and one 50 years of age with iatrogenic Cushing's syndrome secondary to daily ingestion of 7.5-10.0 mg of prednisone for over 20 years for eczema; group 3, ACTH suppression in patients with Cushing's disease and normal subjects (three women, 28-54 years of age, with Cushing's disease, established by removal of a microadenoma of the pituitary gland, and three women, 28-54 years of age, with normal adrenal and renal function).

All patients ingested a diet of fixed electrolyte composition (20 mmol of sodium and 50-70 mmol of potassium daily). Two grams of sodium chloride were added to each meal for the patient with partial adrenal insufficiency and all patients in group 3. Test manoeuvres were not performed until equilibration on the diet had been achieved. Recumbency was maintained from 23.00 to 08.00 hours.

All patients in groups 1 and 2 received superphysiological amounts of ACTH for 3 days. Blood samples were obtained before and every 4-6 h throughout the 72 h of ACTH administration and for 24 h after cessation of ACTH. A subject in group 1 and the patient with partial adrenal insufficiency in group 2 received 40 units of ACTH/24 h by constant intravenous infusion for 3 days. A second subject in group 1 received 40 units of ACTH intravenously for the first 24 h and then 20 units of ACTH gel intramuscularly every 6 h for the remainder of the 72 h. The third subject in group 1 and the second patient in group 2 received 40 units of ACTH gel every 6 h.

In the six subjects in group 3, control blood samples were obtained after overnight recumbency at 08.00 hours and again after 4 h in the upright posture for measurement of plasma aldosterone, 18-OHB, and plasma renin activity (PRA). The procedure was repeated after 2 days of administration of 2 mg of dexamethasone every 6 h.

Blood samples were analysed for plasma concentrations of DOC (Biglieri, 1977), aldosterone (Mayes, Furuyama, Kem & Nugent, 1970), corticosterone (Murphy, 1967), 18-OHB (Martin et al., 1975), 18-OHDOC (Edwards, Biglieri, Martin, Taylor & Bartter, 1974), cortisol (Murphy, 1967), and sodium and potassium (by internal standard flame photometry). PRA was assessed by a previously reported method (Stockigt, Collins & Biglieri, 1971).

All studies were approved by the Committee on Human Research, University of California School of Medicine, and performed in the Clinical Study Center at San Francisco General Hospital Medical Centre. Informed consent was obtained from all patients and normal subjects.

**Results**

**Group 1: ACTH in normal subjects (Fig. 1)**

An 8-30-fold increase above control values occurred in all steroid concentrations during the first 24 h of ACTH treatment. Thereafter, frequent measurements of plasma concentrations of cortisol, DOC, corticosterone, 18-OHB, aldosterone and 18-OHDOC showed three distinct patterns of steroid production.

In pattern 1, a progressive increase or sustained elevated plateau occurred only in DOC and cortisol concentrations. The DOC response correlated significantly with the cortisol response ($r = 0.84-0.91$, $P < 0.001$) but not with those of the other steroids.

In pattern 2, both corticosterone and 18-OHDOC decreased to 28-60% of the initial peak values observed on day 1. The corticosterone and 18-OHDOC responses correlated significantly with each other ($r = 0.78-0.90$, $P < 0.001$) and with aldosterone and 18-OHB ($r = 0.64-0.84$, $P < 0.001$), but not with those of DOC or cortisol.

In pattern 3, both the 18-OHB and aldosterone concentrations increased but returned to virtually control values. The aldosterone response correlated with the responses of 18-OHB ($r = 0.91-$
0.98, \( P < 0.001 \), corticosterone and 18-OHDOC but not with those of DOC and cortisol. No steroid showed any correlation with plasma potassium concentrations or PRA values, which were not significantly altered by ACTH administration. Cessation of ACTH treatment resulted in the virtual obliteration of corticosterone, 18-OHB, aldosterone, and 18-OHDOC 24 h after the last dose.

**Group 2: ACTH in patients with limited adrenal function**

In the patients with prolonged ACTH suppression, a limited but slowly progressive increase in steroid secretory capacity occurred for all steroids without the appearance of the three divergent patterns observed in Group 1. All steroid concentrations in the patient with partial adrenal insufficiency remained unchanged. No significant changes in potassium or PRA were observed in this group.

**Group 3: ACTH suppression in patients with Cushing’s disease and in normal subjects**

In two of the three patients with Cushing’s disease, the 08.00 hours control DOC and cortisol concentrations were elevated, but all other steroids were within normal limits. The circadian rhythms for steroids were abolished in the patients. After administration of dexamethasone in the three patients and the three normal subjects, the 08.00 hours aldosterone and 18-OHBP concentrations were less than the control recumbent values, but with assumption of upright posture both steroids showed either a return to the control upright level (1/6) or an exaggerated response (5/6). The postural increase in PRA did not change from control values after 2 days of dexamethasone. No significant changes occurred in potassium or PRA in this group.

**Discussion**

The exact mechanism of the delayed suppression of aldosterone by continued administration of ACTH remains an enigma. The sequential events are clear and well documented (Newton \& Laragh, 1968; Biglieri et al., 1969; Rauh et al., 1978). An initial rise in aldosterone excretion is followed by a return to control values and a further fall below control levels when ACTH is discontinued (Liddle et al., 1956; Biglieri et al., 1969). This pattern was first described from measurements of the 18-glucuronide metabolite of aldosterone. Even though the metabolic clearance rate of aldosterone and the shift towards the tetrahydrometabolite of aldosterone is increased during ACTH administration (Nowaczynski, Guthrie, Messeri, Genest, Kuchel, Honda \& Grose, 1977), the phenomenon is still observed with this metabolite used as an index of secretion (Rauh et al., 1978) and is now further documented convincingly by the concurrent changes of aldosterone in plasma (Rauh et al., 1978; Rost, Schambelan \& Biglieri, 1979). Altered metabolism cannot entirely explain the biphasic response. The decrement in aldosterone production observed is independent of salt intake, plasma potassium concentration (Newton \& Laragh, 1968; Biglieri et al., 1969) and PRA (Newton \& Laragh, 1968; Rauh et al., 1978) in normal subjects, and also occurs in patients with an aldosterone-producing tumour (Biglieri et al., 1969) or other types of hypertension (Newton \& Laragh, 1968; Rauh et al., 1978). Exceptions to the phenomenon are rare indeed: only patients with dexamethasone-suppressible hyperaldosteronism (Rauh et al., 1978) and, to date, one patient with congenital adrenal hyperplasia (non-salt-losing type) (Beitins, Bayard, Kowarski \& Migeon, 1972), maintain a high plasma concentration of aldosterone during ACTH administration. In the former group, plasma DOC concentration rose progressively in a manner similar to that observed in patients with other types of hypertension and in normal subjects (Rauh et al., 1978). Our results document further that plasma aldosterone concentrations have a biphasic response to 3 days of ACTH treatment (Rauh et al., 1978; Rost et al., 1979): after the initial rise, the concentrations returned to control values. No significant changes occurred in PRA or plasma potassium concentrations in the normal subjects or the patients while on a low sodium intake. In addition, our results confirm that aldosterone and 18-OHHP have a similar site of origin because of their virtually identical pattern of production during ACTH administration (Rost et al., 1979).

However, the observed patterns of the other steroids of the mineralocorticoid pathway indicate that the pathway was altered by an ACTH-induced intra-adrenal event: DOC concentration rose and continued to increase in a manner similar to that of cortisol; there was no biphasic response. The glucocorticoid pathway does not participate in the events that occur along the mineralocorticoid biosynthetic pathway: both corticosterone and 18-OHDOC showed a response similar to those of
aldosterone and 18-OHB but the decrease after initial stimulation was not as great, although moderately increased steroidogenesis was maintained. The highly significant correlation between 18-OHDOC and corticosterone, namely that a constant ratio of 18-OHDOC/corticosterone was maintained, provides strong support that the 11β- and 18-hydroxylating enzymes may be the same. Thus these three patterns identify and imply modification of the 11β- and 18-hydroxylating enzymes. Only the microsomal hydroxylating enzyme (21-hydroxylase) remains uninhibited by continued ACTH. The mineralocorticoid hormone DOC, and its glucocorticoid counterpart cortisol, rise progressively. In contrast, the mitochondrial enzymes, 11β- and 18-hydroxylase, are inhibited. Whereas corticosterone has a biphasic response and requires 11β-hydroxylation, 18-OHB and aldosterone require additional 18-hydroxylation and show a greater decrease in the biphasic curve. This can be interpreted as being in part the result of diminishing substrate, corticosterone (11β-hydroxylation), and the additional inhibition of 18-hydroxylation.

That the 11β-hydroxylating and 18-hydroxylating enzymes may be the same enzyme has been strongly suspected (Ulick, 1976; Björkhem & Karlmar, 1977). The reduced concentrations of 18-OHDOC found in patients with congenital adrenal hyperplasia (11β-hydroxylase-deficiency type) and after metyrapone administration support the presence of a single enzyme (Ulick, 1976). Additional support is provided by our finding of a strong correlation between 18-OHDOC and corticosterone during stimulation with ACTH.

The influence of endogenous concentrations of ACTH on 18-OHB and aldosterone was examined by measuring these steroids after overnight recumbency and after 4 h in the upright posture. In the three patients with Cushing’s disease (due to pituitary microadenoma resulting in increased endogenous levels of ACTH), DOC and cortisol concentrations were both elevated (2/3). Such elevations are more frequently seen in patients with Cushing’s disease than in those with adrenal adenoma and in the ectopic ACTH syndrome (Biglieri, Slaton, Schambelan & Kronfield, 1968; Schambelan, Slaton & Biglieri, 1971; Tan, Noth & Mulrow, 1976). When normal subjects and patients with Cushing’s disease were treated with dexamethasone, the recumbent 08.00 hours values of 18-OHB and aldosterone decreased, but the response to upright posture was as great or greater in all for the same postural change in plasma renin activity. These increased responses to posture can be interpreted as resulting from the removal of the blunting effect of ACTH.

These findings suggest that ACTH inhibits both the 11β- and 18-hydroxylating enzymes of the mineralocorticoid pathway. Such inhibition is not observed with enzymes involved in cortisol synthesis. The effect of ACTH in the mineralocorticoid hormone pathway is dependent on increased steroid production, particularly of the glucocorticoid hormones (cortisol) or the uninhibited DOC, which may be one of many ACTH-induced intra-adrenal events. This blunting effect is readily apparent when superphysiological amounts of ACTH are administered to normal persons and to patients with Cushing’s disease. Physiological concentrations of ACTH may limit the posturally stimulated renin increase in 18-OHB and aldosterone by this mechanism. A normal response for these steroids may, in part, be the resultant effect of the two factors, renin and ACTH, and their modulating effects on adrenal synthesis of mineralocorticoid hormone throughout the day.

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References


MURPHY, B.E.P. (1967) Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein binding radioassay. *Journal of Clinical Endocrinology and Metabolism*, 27, 975–990.


