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MECHANISMS ESTABLISHING THE MINERALOCORTICOID HORMONE PATTERNS IN THE 17α-HYDROXYLASE DEFICIENCY SYNDROME

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SUMMARY

The sequential events leading to the final steroid pattern expressed in the untreated patient with 17α-hydroxylase deficiency syndrome (170HDS) is examined. Cessation of suppressive treatment of adrenocorticotropin (ACTH) in four patients with 170HDS resulted in a reemergence of increased amounts of all the major mineralocorticoid hormones: deoxycorticosterone, corticosterone, 18-hydroxycorticosterone (180HB), and aldosterone. An ACTH-dependent rhythm was established for all mineralocorticoid hormones in the presence of a fixed renin system. A mechanism for suppression of aldosterone and elevation of 180HB in patients with 170HDS is proposed. Expansion of extracellular fluid by all mineralocorticoid hormones suppresses the renin system leading to reduction of aldosterone and 180HB. Further suppression is envisaged through intra-adrenal retardation of 18-hydroxylation, but the final expression of elevated 180HB and reduced aldosterone levels is the combined result of the retained sensitivity of 180HB to ACTH and the impedence of K in the conversion of 180HB to aldosterone.

INTRODUCTION

The pattern of the steroids in the mineralocorticoid hormone biosynthetic pathway has been remarkably consistent in patients with the 17α-hydroxylase deficiency syndrome form of congenital adrenal hyperplasia. Production of deoxycorticosterone (DOC), corticosterone (B), and 18-hydroxycorticosterone (180HB) is increased, but aldosterone secretion is subnormal and the renin system is suppressed. Treatment with glucocorticoid hormones corrects the hypertension and hypokalemia by reducing the levels of DOC and B to normal values and restores the renin and aldosterone system in almost all cases. Recent reviews of reported cases amply confirm these findings [1, 2], but treatment often must be prolonged to effect these changes [2, 3]. This report examines the sequential events that effect both the abnormal steroid patterns in patients with 170HDS and the correction of these events by suppression of adrenocorticotropin (ACTH) with glucocorticoid hormones.

MATERIALS AND METHODS

Patients 1 [4], 3, and 4 [5] of this report were reported previously in detail. Patient 2 was a 43-year-old genotypic male (46XY) with a 20-year history of hypertension (up to 180/120 mm Hg). A laparotomy in 1952 revealed no uterus; small ovid structures reported as "testes" and the clitoris were removed and a vagina was constructed. No axillary hair was present and pubic hair was sparse. During an earlier study after suspension of glucocorticoid therapy for two weeks, serum electrolyte values were Na 144.0, K 3.0, CO_2 32.0, and Cl 100.0 mEq/l. and plasma steroid levels were elevated (Table 1); plasma cortisol was not detected. Three years ago, withdrawal of therapy for a longer period of time revealed the absence of 17-hydroxycorticosteroids and a urinary level of the 18-glucuronide of aldosterone of 4.0 μg/24 h (normal range 4–17 μg/24 h), but urinary levels of tetrahydrodeoxycorticosterone and tetrahydrocorticosterone were 165.7 μg/24 h (normal range 15–50 μg/24 h) and 964 μg/24 h (normal range 100–300 μg/24 h), respectively. Plasma renin concentration was 0.1 ng/ml/h (mean normal values are 1.2 ± 0.2 in the recumbent position and 3.0 ± 0.6 in the upright position).

In an attempt to study patients in the untreated state, glucocorticoid therapy was discontinued for two weeks in patients 1 and 2 and one week in patients 3 and 4. All studies of patients 1 and 2 were performed in the Clinical Study Center at San Francisco General Hospital Medical Center while on a fixed electrolyte diet: 122 mEq Na/day and 60–80 mEq K/day. Blood and urine samples from patients 3 and

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4 were obtained between 0800 and 1000 hours after ambulation while on an unselected diet and after fasting.

Circadian rhythm after cessation of therapy for 2 weeks was established in patients 1 and 2 by steroid measurements in blood samples that were drawn every 4 hours beginning with an overnight recumbent sample at 0800 hour. Normal postural activity was permitted throughout the day until 2200 hour.

After completion of the circadian study, dexamethasone, 0.50 mg, was administered orally at 2200 hour daily for 1 month in patients 1 and 2. Blood samples for steroid measurements were obtained on days 1 and 30.

In a separate study of patient 1 during a subsequent admission to the Centre, dexamethasone was discontinued for 2 weeks and then a single dose of 1.0 mg of dexamethasone was administered at 0800 hour and blood samples for steroid measurements were obtained at 1500 hour and 0800 hour the next morning.

Plasma steroids were measured by the radioimmunoassay techniques developed and/or performed in our laboratory: DOC [6], B [7], 18OHB [8], and aldosterone (PAC) [9].

RESULTS

Plasma steroids after suspension of glucocorticoid treatment

All of the steroids of the major mineralocorticoid pathway were elevated in all four patients (Table 1). Plasma aldosterone concentration was unusually increased above normal values in all patients, whereas initial pretreatment production of aldosterone was virtually absent.

Circadian rhythm of plasma steroids after cessation of therapy for two weeks

Mean plasma values for all steroids were elevated in the presence of a normal plasma renin concentration and moderate hypokalemia in both patients (1 and 2). The circadian rhythm for all steroids followed an ACTH rhythm with a peak between 0400 and 0800 hours and a nadir between 2000 and 0400 hours in both patients. This rhythm also existed for PAC, whereas plasma renin concentrations remained fixed at normal levels with little variation throughout each 24-hour period [10].

Table 1. Plasma mineralocorticoid hormone levels in partially treated patients with 17-hydroxylase deficiency syndrome*

Patient No.	DOC (ng/dl)	B (ng/dl)	18OHB (ng/dl)	Aldosterone (ng/dl)
1	429.6	14500	358	51.6
2	328.8	15100	386	19.2
3	239.5	23000		79.0
4	19.6	800		45.6
Normal range	4–16	100–310	9–30	4–14

* DOC = deoxycorticosterone; B = corticosterone; 18OHB = 18-hydroxycorticosterone.

Circadian rhythm of plasma steroids after treatment with dexamethasone

On day 1 after administration of 0.50 mg dexamethasone at 2200 hour, mean plasma levels of DOC, B, and 18OHB fell dramatically in both patients (1 and 2), whereas PAC fell to subnormal values (Table 2). Plasma renin concentration fell slightly but was still within the normal range and remained relatively fixed throughout day 1. Serum K levels increased. This dose of dexamethasone did not restore all steroid levels to normal values in patient 2 except for PAC during the 24-h period. The normal circadian rhythm for all steroids was obliterated in both patients on day 1.

On day 30 after daily administration of 0.50 mg dexamethasone at 2200 hour, plasma levels of all steroids were within the normal range in patient 1 including the previously subnormal PAC (Table 2) although mean plasma renin concentration remained the same with a fixed circadian rhythm. Similarly, the circadian rhythm for steroids remained obliterated without any variation throughout day 30. Hypertension was corrected.

Table 2. Mean 24-h plasma steroid levels before and after daily administration of 0.50 mg dexamethasone on days 1 and 30 at 2200 hours in two patients with 17-hydroxylase deficiency syndrome*

Patient No.	Day of treatment	DOC (ng/dl)	B (ng/dl)	18OHB (ng/dl)	Aldo (ng/dl)	PRC (ng/ml/h)	K (mEq/l.)
1	Control	291.0	8100	332.0	28.6	6.2	3.0
	Day 1	6.8	95	10.0	1.3	4.0	4.3
	Day 30	9.4	230	--	13.1	4.0	4.0
2	Control	250.0	12200	338.0	14.4	4.0	3.6
	Day 1	34.1	821	27.2	10.0	2.6	4.6
	Day 30	29.0	1600		4.9	2.2	4.4

* DOC = deoxycorticosterone; B = corticosterone; 18OHB = 18-hydroxycorticosterone; Aldo = aldosterone; PRC = plasma renin concentration; K = potassium.

In contrast, 0.50 mg dexamethasone daily for 30 days did not normalize DOC and B in patient 2 even though it corrected the hypokalemia (Table 2). Hypertension was not corrected by day 30 in this patient (but blood pressure eventually became normalized with increased glucocorticoid therapy). The circadian rhythm of all steroids was also abolished by dexamethasone therapy in patient 2.

After administration of 1.0 mg dexamethasone at 0800 hour following a 2-week suspension of therapy, DOC, B, and 180HB fell rapidly and profoundly in patient 1 and PAC was undetectable. By 0800 hour the next day, the return of the increased ACTH drive was apparent.

DISCUSSION

One of the consistent abnormalities in steroid production in 170HDS is the virtual absence of or subnormal production of aldosterone. Two reports have suggested that there may be an additional defect in late aldosterone biosynthesis. This hypothesis is based on increased 180HB production [3, 11] and continued suppression of aldosterone production during glucocorticoid treatment [2, 3]. There are three mechanisms whereby aldosterone can be suppressed in this disorder. First, the mineralocorticoid hormones, principally DOC, effect Na retention and volume expansion and suppress the renin system [4, 12]. Second, ACTH plays an intra-adrenal modulating role on the oxidases in the zona glomerulosa, possibly through steroid inhibition [13]. Third, an associated deficiency exists in late aldosterone synthesis at methyl oxidase type I (18-hydrogenation) or methyl oxidase type II (18-dehydrogenation) or some other events occur that impede the final production of aldosterone. New patients reported recently by deLange *et al.*[2] had a limited and delayed return of aldosterone production after adequate glucocorticoid therapy. Before one can infer a block in late aldosterone synthesis, prolonged treatment is required. This is not unlike the state of zona glomerulosa suppression after the removal of an aldosterone-producing adenoma in which adrenal refractoriness to the rising levels of renin can exist for up to 2 years [14, 15].

The normal major pathways of steroid biosynthesis and primary regulators are shown in Figure 1. After brief cessation of glucocorticoid therapy, the elevated levels of DOC, B, and 180HB were quickly reestablished (Fig. 2). The finding of secondary hyperaldosteronism is of great interest. The circadian rhythm for the steroids of the mineralocorticoid pathway have an ACTH-dependent rhythm [10]. This occurs in the presence of normal but fixed plasma renin concentration. Thus, aldosterone is modulated throughout a 24-h period by the secretion of ACTH. One may assume that the earliest event in the generation of steroid abnormalities is a general hypersecretion of all the hormones in the mineralocorticoid pathway of the zona glomerulosa and fasciculata as depicted

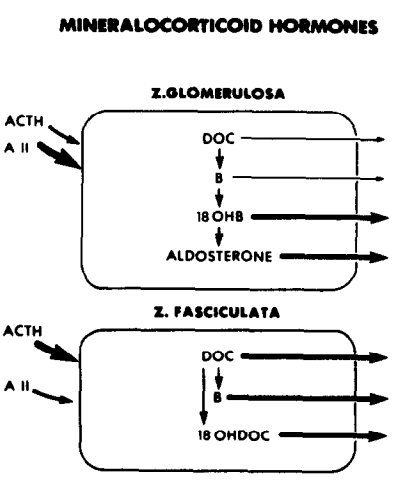

Fig. 1. Early events in steroidogenesis when adrenocorticotropin suppression is withdrawn in patients with the 17-hydroxylase deficiency syndrome.

in Fig. 1. The initial drive of increased amounts of ACTH is to increase the production of DOC, B, 180HB, and aldosterone. With an excessive amount of mineralocorticoid hormones, especially aldosterone and DOC, volume expansion and fixation and then suppression of the renin mechanism are likely to occur. This is one of the earlier phases whereby aldosterone is suppressed (Fig. 2).

The second important fact is related to "turn off" of aldosterone during continued administration of ACTH in man. During ACTH administration, a transient increase in aldosterone production occurs, but production returns to control or slightly lower than control levels and finally to definitely subcontrol levels on cessation of ACTH [13]. This unique effect of ACTH both in normal subjects on low salt diets and, particularly, in patients with an aldosterone-producing adenoma further establishes that this is an important mechanism, and potential second phase, for turning off aldosterone *and* that it can occur in the virtual absence of the renin system [13]. It may take up to 7 days after cessation of ACTH treatment for the zona glomerulosa steroids to begin to respond to normal stimuli. The second phase in the suppres-

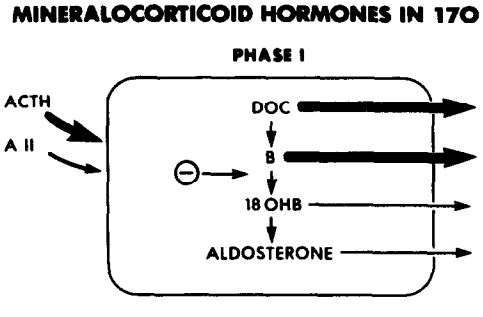

Fig. 2. Phase I in which increased amounts of adrenocorticotropin (ACTH) lead to excessive deoxycorticosterone and corticosterone production. ⊖ Represents a retardation of final synthesis of mineralocorticoid hormone by ACTH.

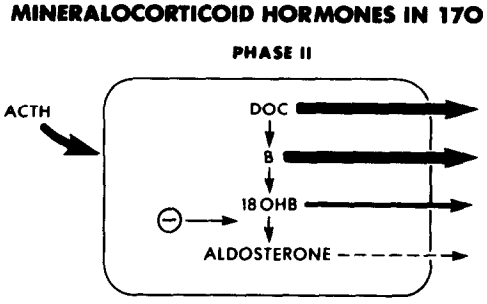

Fig. 3. Phase II represents the proposed final pattern of mineralocorticoid production: a combination of renin suppression, adrenocorticotropin (ACTH) intra-adrenal effect, and possible potassium effect in the final synthesis of aldosterone ⊖.

sion of aldosterone production may then be an additional intra-adrenal effect of ACTH (Fig. 3), as well as the continued gradual suppression of the renin system that occurs. At this point, both 18OHB and aldosterone production begin to diminish from their initial high production rates [16].

The final phase in the development of the entire steroid pattern is that while aldosterone production is suppressed, 18OHB seems to preserve its high secretion rate or increases to a higher level. The urinary metabolite of 18OHB has been measured and found to be increased in two patients with 17OHDS [3, 11]. Thus, with the addition of our two cases, 18OHB was high in all four of the patients so studied. The question of the interpretation of the 18OHB levels in this

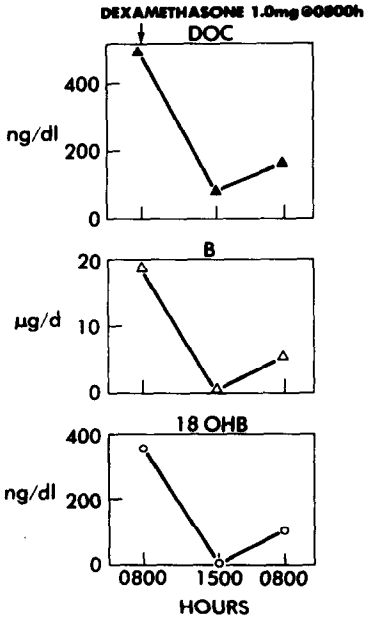

Fig. 4. The effect of the administration of 1.0 mg dexamethasone at 0800 hours in plasma deoxycorticosterone, corticosterone, and 18-hydroxycorticosterone in untreated patients with 17-hydroxylase deficiency syndrome. Aldosterone levels were undetectable.

disease rests to a great extent on the site of origin of 18OHB. In a recent publication Ulich[11] suggested that in patients with 17OHDS the major source of 18OHB is the zona fasciculata on the basis of the ready suppressibility of 18OHB with glucocorticoid hormones. However, recent observations support a common site of origin for both aldosterone and 18OHB. First, infusions of angiotensin II or angiotensin III increased only 18OHB and aldosterone in a similar manner [17]: the correlation between the log dose-response curve to angiotensin III and the levels of 18OHB and aldosterone was extremely high. Second, chronic ACTH treatment in man produced the same transient increase in 18OHB as in aldosterone [17]; in fact, the ratio of the two throughout a 3-day treatment period remained remarkably the same and correlation between the two was highly significant. There was no correlation between these steroids and plasma cortisol during ACTH treatment. Thus, there is strong evidence for a common site of origin in the zona glomerulosa for both 18OHB and aldosterone. Clinically, this was also manifested in a recent study in which 18OHB was uniquely elevated in patients with an aldosterone-producing adenoma (Biglieri, unpublished observations) [17].

If both 18OHB and aldosterone have a common origin in the zona glomerulosa, how does one explain the discrepancy between 18OHB and aldosterone in the untreated state of 17OHDS? In a study of patients with primary aldosteronism, there was a significant negative correlation between changes in K concentration and the 18OHB: aldosterone ratio (Biglieri, unpublished observations). Baumann and Muller[18] suggested that K depletion plays a role in late aldosterone synthesis; they observed that there is less conversion of tritiated B and 18OHB to aldosterone in capsular adrenal glands of the rat than in K-repleted rats. Potassium depletion per se reduces aldosterone production. In patients with primary aldosteronism, reduction in serum K concentration increased the 18OHB:aldosterone ratio (Biglieri, unpublished observations). Thus, a final phase in the determination of the steroid patterns observed may well be the crucial role of K depletion in establishing a functional impairment in the final dehydrogenation of 18OHB. The sustained influence of normal amounts of ACTH and K depletion may thus be sufficient to establish a level of 18OHB in the range observed. This is the range of 18OHB that often occurs after infusion of small doses of ACTH and in the absence of a functioning renin system (Biglieri, unpublished observations). Adrenocorticotropin under these conditions may be a major determinant of 18OHB levels in patients with 17OHDS. A marked sensitivity of 18OHB, DOC and B was demonstrated when a small dose of dexamethasone restored serum concentrations of these steroids to normal levels and virtually eliminated 18OHB (Fig. 4). The total dependence of plasma aldosterone on the circadian rhythm of ACTH is additional evidence that ACTH plays a dominant role

in the early development of this disorder of the mineralocorticoid pathway. The renin system apparently is fixed at different levels by the gradually increasing amounts of mineralocorticoid hormone. During treatment with dexamethasone, volume depletion, reduction of ACTH levels, and gradual restoration of serum K concentration occur. The renin system gradually begins to function. The frequent initial fall of aldosterone that occurs after a single dose of dexamethasone results solely from suppression of ACTH [19]. The return to a more normal level of aldosterone production results from reestablishment of the dominance of the renin system as the prime regulator. Whether or not the fixed renin system resumes a normal circadian rhythm with response to the upright posture could not be determined from these studies. It did not return to a normal physiologic rhythm after a month of therapy at which time a balance seemed to have been struck between the renin system and the secretion of ACTH.

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ADDENDUM

The unpublished observations referred to in the Discussion are now in press: Biglieri E. G., Schambelan M.: The significance of elevated levels of plasma 18-hydroxycorticosterone in patients with primary aldosteronism. *J. clin. Endocr. Metab.* In press.