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SUPPRESSION AND STIMULATION OF MINERALOCORTICOID HORMONES (MCH) IN THE SIMPLE VIRILIZING FORM OF CONGENITAL ADRENAL HYPERPLASIA (CAH) EVALUATED BY THE QUANTITATION IN ADRENAL VENOUS BLOOD

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SUMMARY

Four untreated female patients with the nonsalt-losing form of congenital virilizing adrenal hyperplasia (21-hydroxylase deficiency) (21-OHD) maintained on a daily sodium intake of 120 m-equiv were studied by bilateral adrenal vein catheterization. Simultaneous right and left adrenal and peripheral blood samples were collected for determination of cortisol (F), progesterone (P), 17-hydroxyprogesterone (17-OHP), aldosterone (Aldo), and deoxycorticosterone (DOC). All patients were studied during sequential ACTH suppression (30 min after intravenous administration of 4 mg of dexamethasone) and stimulation (5 min after intravenous administration of 250 μg β-ACTH [cosyntropin]). Basal peripheral concentrations of Aldo, DOC, P and 17-OHP were increased, whereas F concentrations were in the lower limit of the normal range. Dexamethasone suppressed adrenal secretion in all subjects. Subsequent adrenal stimulation by ACTH increased P, 17-OHP and DOC, whereas F returned to only control levels. DOC responses to ACTH in the adrenal vein effluents correlated significantly with Aldo responses but not with the 17-OHP increments, suggesting that the adrenal responses of Aldo and DOC to ACTH are events that probably occur in the same zone.

INTRODUCTION

Congenital adrenal hyperplasia due to the 21-hydroxylase deficiency (21-OHD) is an inherited error of steroid metabolism in which the enzymatic defect induces compensatory hypersecretion of ACTH in an attempt to restore cortisol (F) production to normal levels resulting in increased secretion of adrenal adrogens and clinical virilism [1]. Patients with this disease exhibit a salt-losing tendency that is related to accumulation of F precursors progesterone (P) and 17-hydroxyprogesterone (17-OHP) that have some natriuretic properties. This natriuretic challenge can be overcome by increased production of aldosterone in the simple virilizing forms of the disease [2], whereas salt-losers exhibit a reduced capacity to increase aldosterone production [3].

Two different theories have been proposed to explain the existence of these variants. One is the existence of only one enzyme defect in both the glomerulosa and the fasciculata zones, with salt-wasting occurring as a function of the degree of deficiency [4]. A second suggests two enzymatic defects. For the salt losers, the 21-OHD would occur in both the glomerulosa and fasciculata zones, whereas in the simple virilizing form the defect would occur predominantly in the zona fasciculata, sparing the zona glomerulosa [5, 6].

In the study reported herein, we examined the dynamics of aldosterone (Aldo) and deoxycorticosterone (DOC) secretion in patients with the simple nonsalt-losing form of 21-OHD by simultaneously measuring these steroids in bilateral adrenal and peripheral venous blood.

MATERIALS AND METHODS

Four previously untreated female patients with the nonsalt-losing form of 21-OHD, 9–28 yr of age, were studied at the Diabetes and Adrenal Unit of the Hospital das Clínicas of São Paulo, Brazil. The studies were approved by the committee on human research, and informed consent was obtained from the patients or their parents and the control subjects.

All patients ingested low sodium diets of < 10 m-equiv supplemented by NaCl tablets to

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Table 1. Laboratory and radiologic data for 4 patients with 21-hydroxylation deficiency

Patient no.	Age (yr)	Urinary† 17-OHCS (mg/m² per 24 h)	Urinary‡ 17-KS (mg/24 h)	Plasma renin activity§ Na intake (m-equiv/day) <10 Supine (8 a.m.)	<10 4 h-Standing (noon)	120 Supine (8 a.m.)	120 4 h-Standing (noon)	Venographic findings‖ RAV	LAV
1	9	2.1	20.1	8.2	10.2	4.8	8.5	+ + +	+ +
2	14	1.8	26.8	10.5	13.0	1.6	9.4	NL	+ + +
3	28	3.1	52.6	1.5	6.0	0.5	1.2	+ + + +	+ + + +
4	12	1.6	123.2	9.2	10.6	8.0	15.9	+ +	+ + +

* 17-OHCS = 17-hydroxycorticoids; 17-KS = 17-ketosteroids; RAV = right adrenal vein; LAV = left adrenal vein.

† Normal values for children above 5 yr and adults, 2.4–5.0 mg/m² per 24 h.

‡ Normal values for adults: males 7–23 mg/24 h (mean, 15 mg/24 h), females 5.8–17 mg/24 h (mean, 10.2 mg/24 h).

§ Normal values: on 120 m-equiv Na intake/day, supine, 0.53 ± 0.17 (1 SD) ng/ml per h, 4 h-standing (noon) 1.30 ± 0.80 ng/ml per h; on less than 10 m-equiv Na intake/day, supine 1.52 ± 1.06 ng/ml per h, 4 h-standing (noon) 4.21 ± 1.76 ng/ml per h.

‖ + to + + + + = normal (+) to degree of enlargement of adrenal gland.

120 m-equiv sodium per day and 80–100 m-equiv potassium per day for at least five days before test procedures were performed. Adherence to the diets was assessed by measuring sodium and potassium levels in 24-h collections of urine; serum sodium and potassium concentrations were within the normal range throughout the study.

All subjects fasted overnight before blood samples were obtained. Percutaneous venous catheterization via the femoral vein was performed in the four patients; the catheters were placed in both adrenal veins between 8:00 a.m. and 9:30 a.m. Adrenal venograms were obtained to confirm correct catheter placement. This was followed by simultaneous venous blood sampling from the right and left adrenal effluents and from a peripheral vein. To study the dynamics of the adrenal steroids, all patients received a bolus intravenous injection of dexamethasone, 4.0 mg. Blood was obtained from both adrenal veins and a peripheral vein 30 min later. ACTH (cosyntropin, 250 μg) was then immediately injected into a pheripheral vein, and the sampling procedure was repeated 5 min later. DOC, Aldo, P, 17-OHP and F were measured in peripheral and adrenal venous samples obtained during suppression and stimulation tests. All steroids were measured by specific radioimmunoassays after separation in 5 × 1 cm Sephadex LH-20 columns [1, 6]. Urinary 17-hydroxysteroids were measured by the method of Porter and Silber [7] and 17-ketosteroids by the method of Drekter *et al.*[8]. Plasma renin activity (PRA) was measured after overnight recumbency and after 4 h of upright posture and was determined by radioimmunoassay of angiotensin I with assay material obtained from New England Nuclear (Boston, MA).

RESULTS

Urinary levels of 17-hydroxycorticosteroids and ketosteroids, PRA values, and venographic findings are summarized in Table 1. All patients were nonsalt losers during intake of 120 m-equiv sodium per day and conserved sodium within five days on a low salt intake. Patient 4 manifested a mild salt-losing tendency only during prolonged reduction of sodium intake to <10 m-equiv/day. Patients 1, 2 and 4 had increased levels of PRA in both recumbent and upright postures during both low and normal sodium intakes regardless of posture. The degree of elevation seemed to correlate with the degree of 21-OHD evaluated by clinical and laboratory criteria. Patient 3, who had 21-OHD, PRA was within the normal range.

Plasma concentrations of Aldo, DOC, P, 17-OHP and F in peripheral veins and adrenal venous effluents are summarized in Table 2 and Fig. 1. Mean values for the right and left adrenal glands were similar. In the peripheral veins, basal concentrations of Aldo, DOC, P and 17-OHP were higher in the patients than in the normal subjects [6] and decreased after dexamethasone and increased after ACTH administration. In both the right and left adrenal veins, the concentrations of all steroids also decreased 30 min after dexamethasone. Suppression of adrenal secretion was considered complete in 3 patients (Nos 1, 2, and 3) because the concentrations of steroids collected from the adrenal venous effluents did not differ from the respective simultaneous peripheral concentrations. Patient 4 exhibited partial suppression indicated by persistent gradients of concentrations of steroids between the adrenal venous effluents and the peripheral samples. ACTH stimulated adrenal secretion of all steroids, except F, in all patients to levels greater than control levels in both the adrenal effluents and peripheral blood. In response to ACTH, F increased only to a level similar to the nonsuppressed control level in the adrenal effluents.

In the 3 patients who had total adrenal suppression with dexamethasone (Nos 1, 2, and 3), we estimated the adrenal responsiveness to ACTH by the absolute increments in concentrations of the several steroids in the adrenal effluents compared with values in the suppressed state ($\Delta = Peak_{ACTH} - Nadir_{Dex}$) (Figs 2 and 3). Using both adrenal vein values for each patient, there was a significant and positive correlation

Table 2. Levels of mineralocorticoid (aldosterone and deoxycorticosterone) and cortisol, and its precursors in peripheral and adrenal venous blood in 4 patients with congenital adrenal hyperplasia (21-hydroxylase deficiency) on 120 m-equiv sodium intake*

	Peripheral vein					Right adrenal vein					Left adrenal vein				
Subjects	Aldo (n/100 ml)	DOC (ng/100 ml)	P (ng/ml)	17-OHP (ng/ml)	F (μg/dl)	Aldo (ng/100 ml)	DOC (ng/100 ml)	P (ng/ml)	17-OHP (ng/ml)	F (μg/dl)	Aldo (ng/100 ml)	DOC (ng/100 ml)	P (ng/ml)	17-OHP (ng/ml)	F (μg/dl)
Patients															
Control	30.0	33.1	2.6	152	5.0	139	123.4	35.5	4102	15.8	209	154	38.5	3780	17.5
(1) Dex	10.0	9.2	1.4	88	4.4	8.0	10.2	1.5	94	7.4	11.6	10.9	1.2	97	9.0
ACTH	29.9	54.6	2.9	117	5.4	348	233.4	75.2	4263	16.5	269	371	70.2	5570	22.5
Control	27.0	50.0	6.0	36	3.3	70.8	162	22	559	11.9	138	336	23	629	12.7
(2) Dex	11.0	29.1	4.0	29	2.6	6.5	42	5	39	4.8	11.9	32.2	5	43	2.3
ACTH	38.4	110.2	16.0	155	3.2	364	386	116	3814	10.8	556	636	134	4144	11.0
Control	33.5	33.2	9.7	90	5.1	197	203	18.8	162	26.6	81.0	172	15.8	178	26.7
(3) Dex	18.7	14.2	4.9	53	4.9	25.2	14.5	5.4	53	4.5	18.1	15.7	4.6	60	9.5
ACTH	42.0	43.9	14.7	123	5.4	1418	884	814.9	6546	22.0	1214	898	635	5351	23.9
Control	37.0	70.9	5.7	155	4.2	80.0	164	37.3	1082	7.7	93.0	180	36.2	1037	8.0
(4) Dex	22.5	36.5	3.9	84	3.8	57.6	82	10.8	517	7.4	60.0	93	15.3	429	6.9
ACTH	30.5	120.0	9.5	117	5.9	231	483	107.4	2732	10.2	220	435	118.6	2479	10.5
Mean ± SEM															
Control	31.9 ± 2.2	46.8 ± 9.0	6.0 ± 1.4	108.2 ± 28	4.4 ± 0.41	127.7 ± 29.3	163.1 ± 16.2	28.5 ± 4.7	1476.25 ± 895	15.5 ± 4.0	130.2 ± 29.0	210.5 ± 42.2	27.9 ± 5.1	1406 ± 810.5	16.22 ± 4.0
Dex	15.5 ± 3.0	22.2 ± 6.3	3.5 ± 0.7	63.5 ± 13.9	3.9 ± 0.5	24.3 ± 11.9	37.2 ± 16.5	5.7 ± 1.9	175.7 ± 114.3	6.0 ± 0.8	25.4 ± 11.6	37.9 ± 18.9	6.5 ± 3.0	157.2 ± 91.3	6.9 ± 1.6
ACTH	35.5 ± 3.0	82.2 ± 19.2	10.8 ± 3.0	143.0 ± 14.1	4.97 ± 0.6	590.25 ± 277	496.6 ± 138.9	278.3 ± 178.9	4332.0 ± 803.9	14.9 ± 2.3	564.7 ± 228.7	585 ± 118.6	293.6 ± 132.7	4386 ± 708.7	16.97 ± 3.6
Normal subjects															
Aldo	4.2–8.5 ng/dl														
DOC	6.5–12.2 ng/dl														
P	0.2–0.9 ng/ml†														
17-OHP	0.2–0.8 ng/ml†														
F	5.0–18.0 μg/dl														

* Aldo = aldosterone; Dex = dexamethasone; DOC = deoxycorticosterone; F = cortisol; P = progesterone; 17-OHP = 17-hydroxy-progesterone.
† Mid-follicular phase.

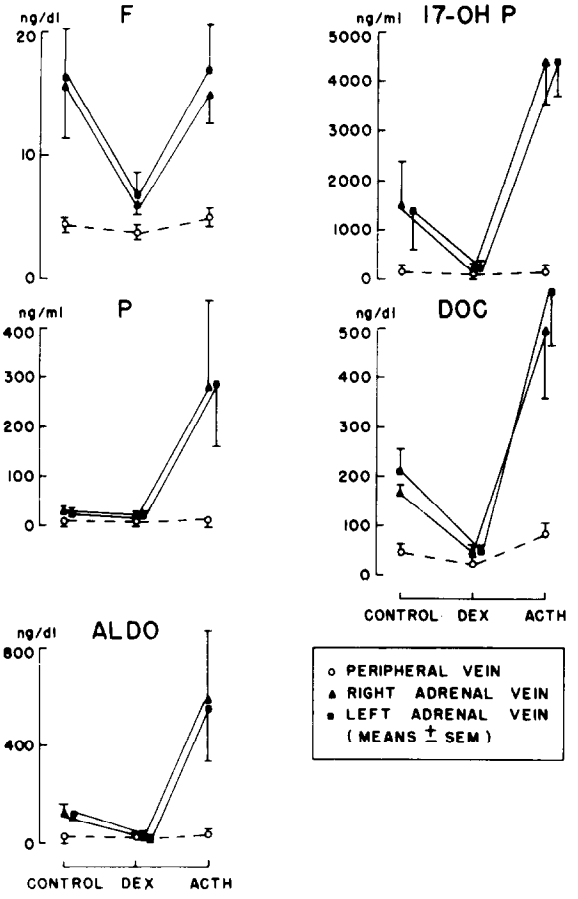

Fig. 1. Cortisol (F), progesterone (P), 17-hydroxyprogesterone (17-OHP) and mineralocorticoids (aldosterone [Aldo] and deoxycorticosterone [DOC]) in peripheral and adrenal venous blood in 4 patients with 21-hydroxylase deficiency.

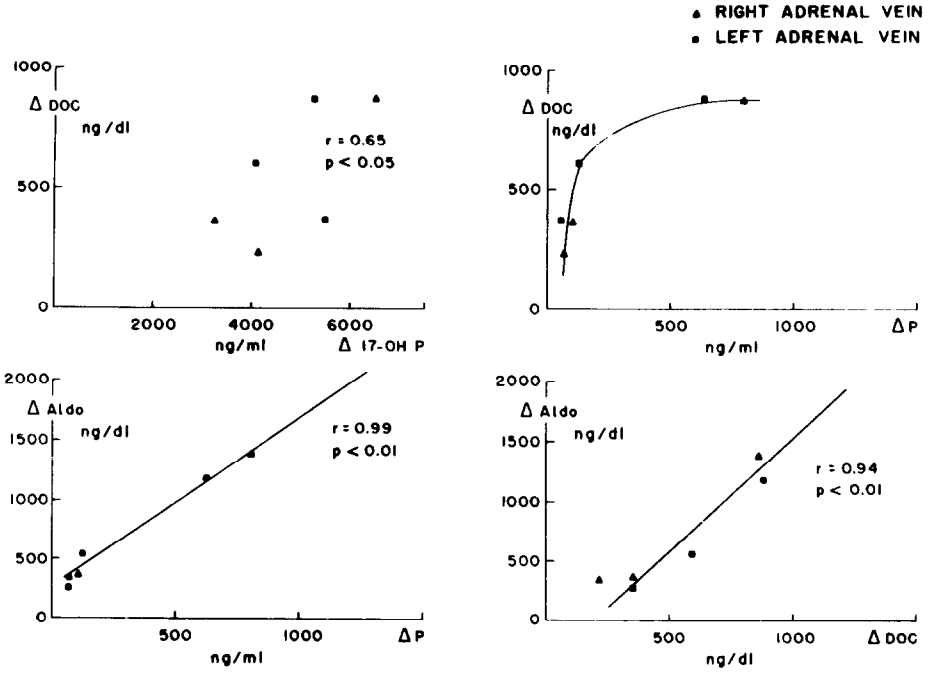

Fig. 2. Relationship between adrenal steroid precursors and mineralocorticoid increments (Δ) after intravenous administration of ACTH in the adrenal vein effluents during studies in 3 patients with total adrenal suppression with dexamethasone (Nos 1, 2 and 3)—$\Delta = \text{Peak}_{ACH} - \text{Nadir}_{Dex}$.

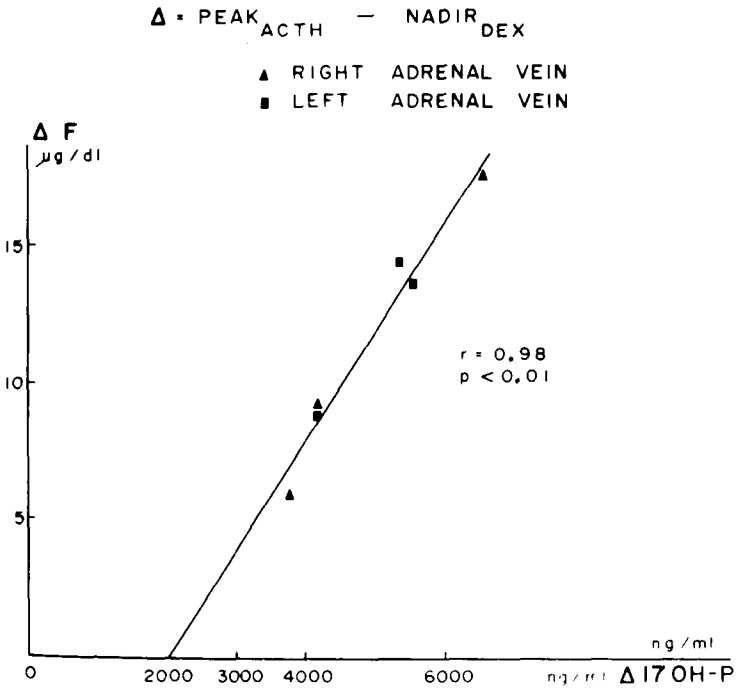

Fig. 3. Relationship between cortisol (F) and 17-hydroxyprogesterone (17-OHP) increments (Δ) after intravenous administration of ACTH in the adrenal vein effluents in 3 patients with total adrenal suppression with dexamethasone (Nos 1, 2, and 3).

between ΔDOC and ΔAldo ($r = 0.94$, $P < 0.01$, ΔAldo = $-212.44 + 1.63\,\Delta$DOC), ΔP and ΔAldo ($r = 0.99$, $P < 0.01$, ΔAldo = $266.63 + 1.42\,\Delta$P). Although Δ17-OHP and ΔF correlated significantly ($r = 0.98$, $P < 0.01$, ΔF = $-8.08 + 0.004\,\Delta$17-OHP), Δ17-OHP did not correlate with ΔDOC ($r = 0.65$, $P > 0.05$). Finally, the correlation between ΔP and ΔDOC was exponential, adjusted to the function ΔDOC = $891.115\ (1 - e^{-0.0061\,\Delta P})$ by nonlinear least squares statistics.

DISCUSSION

Data presented in this report confirm previous references to the presence of increased circulating levels of Aldo in patients with the simple virilizing form of congenital adrenal hyperplasia (21-OHD) [2, 6]. Our data also showed that endogenous ACTH represents a component in the maintenance of this state of aldosterone hypersecretion, as indicated by the reduction of its peripheral level and complete cessation of adrenal secretion in response to administration of dexamethasone in three of the patients. This, of course, does not imply that other stimulators of Aldo secretion, such as angiotensin II, are not involved in the control of Aldo secretion in this disorder. In fact, the frequent, but not always, finding of elevated levels of PRA in patients with 21-OHD that increased further with upright posture suggests that angiotensin II is also involved in the maintenance of hyperaldosteronism [6, 9, 10] despite the fact that Aldo and its precursors, DOC and 18-hydroxycorticosterone (18-OHB), cannot be stimulated further by the increase in renin effected by upright posture [6].

Increased peripheral concentrations of Aldo are an indication that the adrenal gland can secrete this steroid. The Aldo response to ACTH, indicated by the increments to above control levels in the adrenal venous effluents, confirm that the 17-desoxy pathway in the zona glomerulosa is not only intact but can also be further stimulated to levels above control levels with pharmacologic amounts of ACTH. However, this responsiveness is not necessarily normal [6].

On the other hand, F concentrations in peripheral blood were always in or below the lower limits of the normal range, indicating a reduced secretory capacity of the adrenal glands to maintain normal glucocorticoid hormone secretion. This assumption is confirmed by the return of F levels in the adrenal venous effluents in response to ACTH levels similar to control levels. In contrast, subjects without glucocorticoid disorders studied by Spark *et al.*[11] had, after stimulation, a manifold increase above control levels in F concentrations in the venous adrenal effluents. In our patients, the elevated peripheral levels of 17-OHP, together with the dissociated responses of F and 17-OHP in the adrenal venous effluents in response to ACTH, indicate that the 17-hydroxy pathway of the zona fasciculata in our patients is impaired due to the 21-OHD and suggest that F production is at maximal capacity.

The zonal origin of DOC in our studies deserves further comment. This steroid is a precursor in the synthesis of mineralocorticoids in both the glomerulosa and fasciculata zones. The peripheral control levels of the steroids in the mineralocorticoid pathways in the patients with 21-OHD show two distinct patterns that give clues to the zonal origins of these hormones. In the presence of low and fixed plasma F concentrations, the plasma mineralocorticoid hormone levels were either extremely high (DOC, 18-OHB and Aldo) or within normal limits (corticosterone [B] and 18-OHDOC) [6]. In addition, assuming 17-OHP and Aldo are markers of the zona fasciculata and zona glomerulosa, respectively, the DOC responses to ACTH in the adrenal vein effluents correlated significantly with Aldo responses but not with the 17-OHP increments. This may be considered as further evidence that the adrenal responses of both Aldo and DOC to ACTH are parallel events, probably occurring in the same zone in patients with 21-OHD. On the other hand, as expected, there was a significant correlation between F and 17-OHP responses in the adrenal vein effluents, indicating that the F response to ACTH only started when the Δ17-OHP was >2000 ng/ml, a measure of the degree of 21-hydroxylation block in the zona fasciculata. The nonlinear correlation between ΔP and ΔDOC in the adrenal effluents is difficult to interpret but may reflect the bizonal distribution of P associated with differently affected steroid pathways in 21-OHD and/or a reflection of the different hydroxylating enzyme kinetics [16].

Increased renin levels could, in part, be the cause of the high 18-OHB and Aldo levels [6]. However, it has been suggested that the lack of an intact glucocorticoid pathway provides a sustained stimulating effect of ACTH on the entire mineralocorticoid pathway of the zona glomerulosa, rather than the normalization of Aldo and 18-OHB, as occurs on continued administration of ACTH regardless of renin activation [17–19]. A reasonable explanation for hypersecretion of all mineralocorticoids of the zona glomerulosa pathway is that both ACTH and renin are involved in the activation of the entire pathway.

In conclusion, the simple 21-OHD syndrome is characterized by diminished and/or fixed levels of 21-hydroxylated steroids of the glucocorticoid pathway and increased steroid levels proximal to the deficiency site in the zona fasciculata, i.e. P and 17-OHP. The deficiency of 21-hydroxylation in the zona fasciculata involves the mineralocorticoids produced by that zone because B and 18-OHDOC are within normal limits, fixed and unresponsive. However, 21-hydroxylation of the mineralocorticoid hormone pathway in the zona glomerulosa is involved to a lesser degree, with limited elevation of DOC, 18-OHB and Aldo that is maintained by the increased ACTH and Renin.

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