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ADRENAL STEROIDOGENESIS IN "LOW RENIN" OR HYPORENINEMIC HYPERTENSION*

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SUMMARY

Suppressed plasma renin activity (PRA) and volume dependent hypertension have been associated with adrenocortical structural abnormalities, excessive deoxycorticosterone, 18-hydroxydeoxycorticosterone (18-OH-DOC) hypersecretion or non-suppressible aldosterone secretion and may be observed in patients thought to have "essential" hypertension. Dale and Melby (1973) encountered an additional alteration in steroid metabolism in two such patients while studying steroidogenesis *in vitro* by their sectioned adrenals. Conversion of labeled 18-OH-DOC to a new structure 16α,18-dihydroxydeoxycorticosterone was demonstrated to be greatly accelerated by the adrenal tissue in these patients as compared to normal adrenal tissue (70–80% vs 15% conversion). Hypersecretion of 16α,18-dihydroxydeoxycorticosterone occurred in each. This steroid exerted no effect on sodium metabolism in adrenalectomized rats and in the toad bladder assay, but markedly enhanced activity of subthreshold doses of aldosterone in reducing sodium excretion in urine of adrenalectomized rats. We have concluded that excessive 16α,18-dihydroxydeoxycorticosterone secretion may be important in the genesis of suppressed renin in some patients with hypertension because of the unique activity of this steroid which appears to function as a cooperative or positive allosteric effector of aldosterone.

INTRODUCTION

Low renin "essential" hypertension

Numerous studies beginning in 1967, with Küchel *et al.*[1] and Helmer and Judson[2], have identified a subgroup of patients who seemingly have essential hypertension but who exhibit suppressed or hyporesponsive plasma renin activity (PRA) when challenged by any maneuver which will acutely produce substantially negative sodium balance. This subpopulation of patients represents approximately 20–25% of the hypertensive population at large. Interestingly, the only other situation in which untreated patients with hypertension have suppressed PRA is in the mineralocorticoid-hypertensive syndrome [3]. In Table 1 are listed the features in low renin "essential" hypertension which are common to the mineralocorticoid-hypertensive syndrome. The demonstration of increased exchangeable sodium, extracellular fluid and plasma volumes and also the hypotensive effects of sodium deprivation and diuretic therapy in both disorders suggests that increased sodium and water retention is an immediate cause of volume-dependent hypertension observed. Furthermore, compelling evidence has been obtained which supports the idea that adrenal mineralocorticoid excess has a primary role in the genesis of low-renin "essential" hypertension, since Woods *et al.*[4] were able to reduce the blood pressures of patients with low PRA by administering aminoglutethimide, an inhibitor of steroidogenesis at an early step. On the other hand, aminoglutethimide was ineffective in reducing the blood pressures of hypertensive patients with normally responsive PRA. Gunnells *et al*[5] observed obvious structural abnormalities of the adrenals in low renin patients undergoing adrenalectomy. Grim *et al*[6] reported that all of their low renin patients undergoing bilateral total adrenalectomy became normotensive.

It is tempting to conclude that the subpopulation of patients with low PRA are afflicted with the mineralocorticoid-hypertensive syndrome and that the mineralocorticoid remains to be characterized. Though Crane *et al.*[7] found that low renin patients had lower Na:K ratios in saliva, little alteration in potassium balance has been described. Total body potassium in low renin "essential" hypertension is not different from that found in hypertension with normally responsive PRA [8]. It would seem necessary to postulate that any new structure must have a proportionately lesser effect on potassium metabolism.

The mineralocorticoid-hypertensive syndrome

A concept of the mineralocorticoid-hypertensive syndrome has gradually evolved since Conn first described the manifestations of excessive aldosterone secretion in man, in 1955 [11]. The essential features of the mineralocorticoid-hypertensive syndrome include suppressed plasma renin activity (PRA), hypervolemic or volume-dependent hypertension, adrenocortical structural abnormalities and excessive mineralocorticoid secretion. These structures appear in Fig. 1. It is well established that repeated injections of aldosterone and deoxycorticosterone into animals

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Table 1. Features of low renin hypertension resembling the mineralocorticoid-hypertensive syndrome

Finding	Reference
PRA unresponsive to sodium depletion	Küchel *et al.*[1]; Helmer and Judson[2]
ASR unresponsive to sodium depletion	Helmer and Judson[2]
Plasma aldosterone unsuppressible by sodium	Grim *et al.*[6]
Increased blood volume	Helmer and Judson[2]
Increased exchangeable sodium	Woods *et al.*[4]
Increased plasma volume	Tarazi *et al.*[9]
Increased extracellular fluid volume	Jose *et al.*[3]
Low salivary Na:K ratio	Crane *et al.*[7]
Normotensive with adrenal inhibitor	Woods *et al*[4]
Characteristic response to spironolactone	Spark and Melby[10]
Structural adrenal abnormalities	Gunnells *et al.*[5]
Normotensive after adrenalectomy	Grim *et al.*[6]

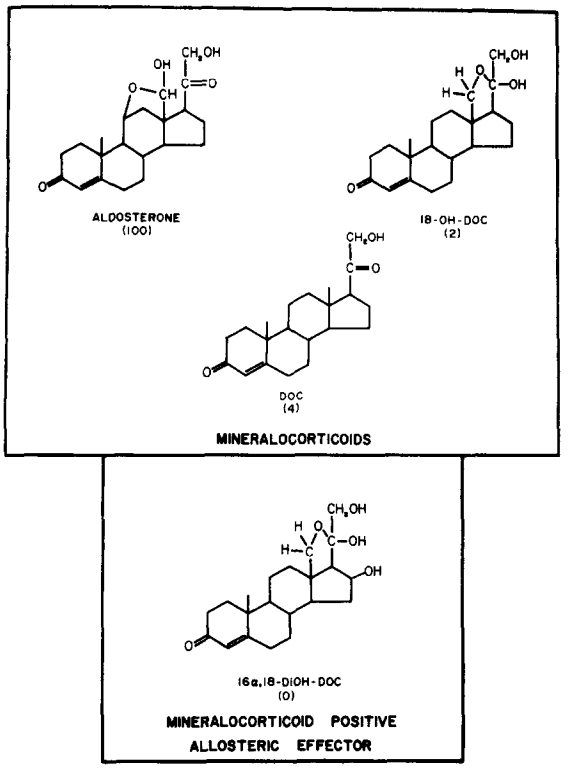

Fig. 1. Structural formulae of the three known mineralocorticoids-aldosterone, deoxycorticosterone (DOC) and 18-hydroxydeoxycorticosterone (18-OH-DOC). The numbers in parentheses indicate relative sodium retaining activity. 16α,18-dihydroxydeoxycorticosterone (16α,18-Di-OH-DOC) may potentiate mineralocorticoid activity.

and man can produce hypertension [12–14], and it has been recently shown that injections of 18-OH-DOC into rats for three weeks have induced hypertension [15]. The mineralocorticoid-hypertensive syndrome resembles hyporeninemic essential hypertension in several important respects. The most important common characteristic is that of volume-dependent, sustained hypertension. Patients with the mineralocorticoid-hypertensive syndrome or with hyporeninemic essential hypertension respond equally well to diuretic therapy alone, and dramatically to mineralocorticoid antagonists such as spironolactone [10]. The known mineralocorticoid-hypertensive syndromes are listed in Table 2.

16α,18-Dihydroxydeoxycorticosterone

We had previously reported that 18-OH-DOC secretory excess was found to be the sole steroid abnormality in between 5 and 10% of hypertensive patients with suppressed plasma renin activity [24, 25]. This proportion holds for a larger group of patients (Fig. 2). Because 18-OH-DOC is a relatively weak mineralocorticoid in comparison to aldosterone, we wondered whether 18-OH-DOC functioned as a biological marker in these patients and whether it may not have been an end product of steroidogenesis in the human but rather a precursor of a more active mineralocorticoid. The analogy that was immediately suggested was the precursor relationship of 18-hydroxycorticosterone (18-OH-B) to aldosterone. 18-OH-B's conversion to aldosterone by the action of an 18-hydroxy steroid dehydrogenase in the glomerulosa would be analogous to the conversion of 18-OH-DOC to a hypothetical 11-deoxy aldosterone in the zona fasciculata, as seen in Fig. 3. Human adrenal incubation studies with radioactive 18-OH-DOC by Grekin *et al.*[27] demonstrated efficient conversion to 18-hydroxycorticosterone and less efficient conversion to aldosterone. Radiochromatographic scans of paper chromatograms demonstrated significant conversion to a substance more polar than 18-OH-B. This material was subsequently identified by Dale and Melby[28] to be 16α,18-dihydroxy-11-deoxycorticosterone.

Preliminary work by Dr. John Funder suggested increased binding of labeled aldosterone to its rat kidney cytosol receptor *in vitro* in the presence of 16α,18-dihydroxydeoxycorticosterone. To evaluate the biological action of 16α,18-dihydroxydeoxycorticosterone *in vivo*, we studied the effect of this compound on enhancing the sodium retaining ability of subthreshold doses of aldosterone in the rat; and the conversion of labeled 18-hydroxydeoxycorticosterone to this compound by adrenal tissue from patients with low PRA essential hypertension.

EXPERIMENTAL

Adrenal tissue and incubations

Adrenal glands of hypertensive patients with suppressed PRA were obtained and incubated with [1, 2-

Table 2. The mineralocorticoid-hypertensive syndrome

	Reference
A. Aldosteronism	
Primary—solitary adenoma	Conn[11]
Idiopathic—bilateral nodular hyperplasia	Katz[16]
Glucocorticoid. Remediable	Sutherland *et al*[17]
Congenital	Moran *et al.*[18]
B. Deoxycorticosterone (DOC) excess	
Idiopathic—nodular hyperplasia (?)	Kahn *et al.*[19]
	Brown et al.[20]
17α-Hydroxylase deficiency	Biglieri *et al*[21]
11β-Hydroxylase deficiency	Eberlein and Bongiovanni[22]
Ectopic ACTH Cushing's syndrome	Biglieri *et al.*[23]
C. 18-Hydroxydeoxycorticosterone (18-OH-DOC) excess	
Idiopathic	Melby *et al.*[24]
Solitary adenoma	Melby *et al.*[25]
17α-Hydroxylase deficiency	Melby *et al.*[25]
Ectopic ACTH Cushing's syndrome	Melby *et al.*[24]
D. 16α,18-Dihydroxydeoxycorticosterone excess (?)	Dale and Melby[26]

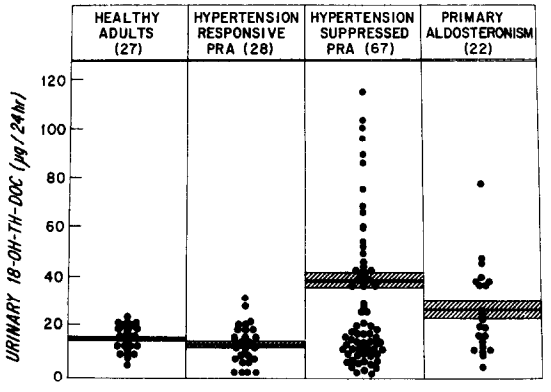

Fig. 2. Urinary 18-OH-TH-DOC excretion in healthy subjects compared with hypertensive patients.

^{3}H]-18-OH-DOC. The patients were hypokalemic, hyporeninemic and hypertensive. Aldosterone secretion was low (40 to 22·5 μg/24 h) and urinary tetrahydrodeoxycorticosterone was normal (22 to 10 μg/24 h). 18-OH-DOC secretion rates were normal as was urinary 18-OH-TH-DOC. Spironolactone treatment reduced blood pressure and corrected serum electrolyte alterations.

"Normal" adrenal tissue, obtained from women undergoing adrenalectomy for breast cancer, was incubated with [1, 2-^{3}H]-18-OH-DOC for control studies. Standard 18-OH-DOC (1 mg/g adrenal slices) was added to some incubations for the preparation of sufficient quantities of 16α,18-dihydroxydeoxycorticosterone for use in the sodium retention assay. One gram of adrenal slices was incubated for 3 h in 10 ml Krebs–Ringer bicarbonate buffer containing 200 mg% glucose in an 95/5-O_2/CO_2 atmosphere. One μCi of [1, 2-^{3}H]-18-OH-DOC (S.A. = 12–15 μCi/μg), prepared as previously described [5] was added for each gram of adrenal slices. After adding [^{14}C]-16α,18-dihydroxydeoxycorticosterone to correct for procedural losses, the slices and media were processed and steroids isolated [28].

Sodium retention bioassay

Male Sprague–Dawley rats weighing between 180–200 g were adrenalectomized between 9:00–11:00 a.m. Animals were given tap water to drink and food was removed. Twenty-four hours later, the rats were injected subcutaneously with test substances dissolved in 0·2 ml of 5% aqueous ethanol. Immediately afterwards, the urethra was ligated and a total of 2·5 ml of saline was injected subcutaneously into the hind leg areas. After 3 h, the animals were sacrificed and their bladders removed, rinsed and the contents diluted to 10 ml with deionized water in a 10 ml graduate. Sodium content was analyzed on a flame photometer and expressed in m-equiv × 100.

RESULTS

Adrenal incubations

The conversion of [1, 2-^{3}H]-18-OH-DOC to 16α,18-dihydroxydeoxycorticosterone by normal adrenal slices and adrenal slices from the low renin essential hypertensive patients is shown in Fig. 4. Normal adrenal tissue converted a mean of 15% of the added labeled 18-OH-DOC to the 16α metabolite while conversion of 64% was observed in patients, as seen in Fig. 4. Hypersecretion into the medium was observed to have occurred in patients' incubations relative to normal adrenal incubations, as estimated by U.V. absorption on thin layer plates and paper chromatograms.

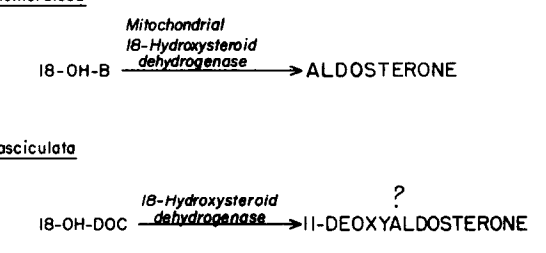

Fig. 3. Zonal adrenocortical steroidogenic analogy. 11-Deoxyaldosterone is a theoretical end product.

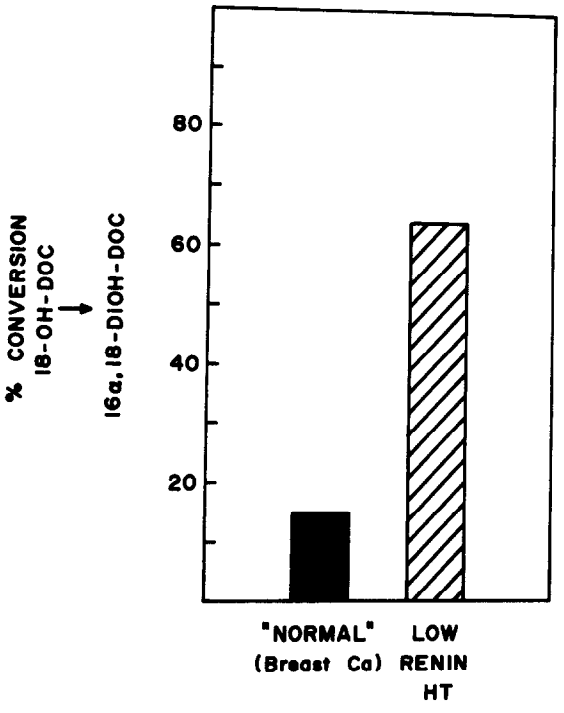

Fig. 4. Per cent conversion of labeled 18-OH-DOC to 16α,18-Di-OH-DOC by "normal" adrenal cortex and adrenal tissue from patients with low renin hypertension.

Sodium retention assay

Experiment 1. (a) When 16α,18-dihydroxydeoxycorticosterone was tested for sodium retaining activity, at a dose of 10 μg per rat, no significant difference was observed from that of adrenalectomized control animals. These results are illustrated in Fig. 5. Mean urinary sodium excretion for control animals was 17·7 ± 2·9 m-equiv × 100 and for test animals 15·9 ± 1·3 m-equiv × 100.

(b) Also, in Fig. 5, is shown the comparison of 50 ng injections of aldosterone with and without 10 μg of 16α,18-dihydroxydeoxycorticosterone on sodium excretion. Mean urinary sodium for control rats was 30·6 ± 6·8 m-equiv × 100. Fifty nanograms of aldosterone injected alone reduced sodium excretion to 22·3 ± 2·2 m-equiv × 100 and was not significantly different from control values. Simultaneous injection of 50 ng of aldosterone and 10 μg 16α,18-dihydroxydeoxycorticosterone reduced the mean sodium excretion to 11·8 ± 3·3 m-equiv × 100 which was significantly different ($P < 0.01$) from aldosterone injected by itself.

Experiment 2. The effect of 16α,18-dihydroxydeoxycorticosterone on the sodium retaining ability of a larger dose of aldosterone was examined. These results are depicted in Fig. 6. Sodium excretion in animals injected with 100 ng of aldosterone was 60% (9·2 ± 1·7 m-equiv × 100) that of adrenalectomized controls (15·2 ± 4·2 m-equiv × 100). Aldosterone (100 ng) in the presence of 10 μg of 16α,18-dihydroxydeoxycorticosterone decreased urinary sodium excretion even further to a mean value of 6·7 ± 1·5 m-equiv × 100.

Experiment 3. The effect of potassium exclusion in the diet was examined. Adrenalectomized animals were placed on a sodium and potassium deficient diet and given 1% saline to drink for 2 days. The 3rd day, the saline was replaced by tap water as

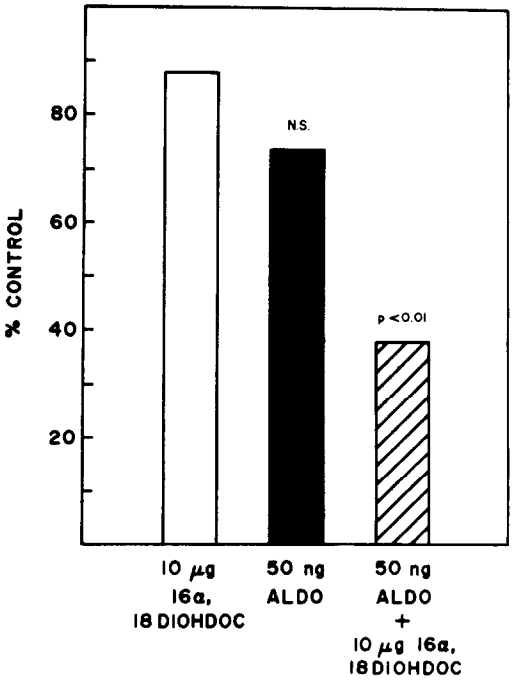

Fig. 5. (a) Effect of 16α,18-Di-OH-DOC alone on urinary sodium excretion in adrenalectomized rat. (b) Effect of aldosterone (50 ng) and aldosterone plus 16α,18-Di-OH-DOC (10 μg) on urinary sodium excretion in the adrenalectomized rat.

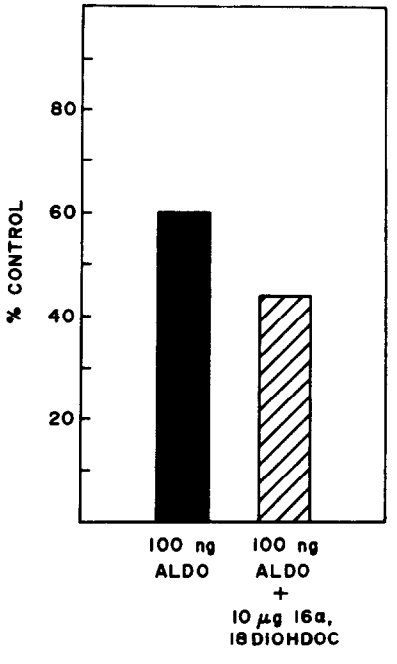

Fig. 6. Effect of aldosterone (100 ng) alone and aldosterone plus 16α,18-Di-OH-DOC (10 μg) on sodium excretion in adrenalectomized rat.

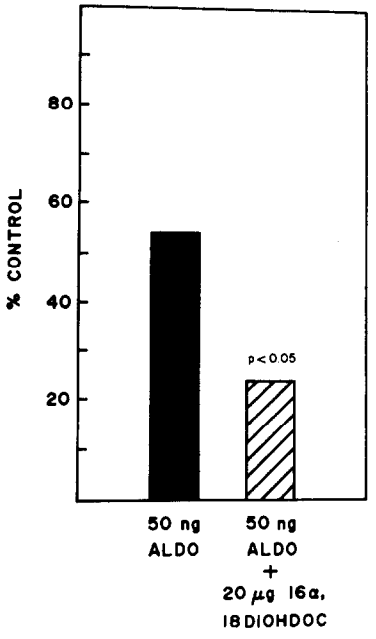

Fig. 7. Effect of aldosterone (50 ng) and aldosterone + 16α,18-dihydroxydeoxycorticosterone (50 ng + 20 μg) injected one hour prior to urethra ligation on sodium excretion in adrenalectomized rat.

drinking fluid. At 9:00 a.m. of the 4th day, test substances were injected and after 1 h the urethra was ligated and saline injected as described above.

The results of this experiment are illustrated in Fig. 7. Injection of 50 ng of aldosterone led to significant sodium retention when compared to control animals (9·4 ± 1·1 m-equiv × 100 vs 18·3 ± 3·3 m-equiv × 100, respectively). Twenty micrograms of 16α, 18-dihydroxydeoxycorticosterone plus 50 ng of aldosterone injected resulted in a mean sodium excretion of 4·8 ± 1·4 m-equiv × 100; a level 50% of that of aldosterone injected alone ($P < 0·05$).

DISCUSSION

The findings of excessive conversion of labeled 18-OH-DOC to 16α,18-dihydroxydeoxycorticosterone by sectioned adrenals from patients with low renin "essential" hypertension, and the steroids' unique property of enhancing aldosterone's biological activity, suggest the possibility that even normal concentrations of plasma aldosterone in such patients may be "inappropriate". Swaneck *et al*[29] have shown enhancement of labeled aldosterone binding to rat kidney nuclear and cytosol receptor proteins when rats were injected with estradiol simultaneously. They attributed this enhancing effect of estradiol to stabilization of the active site of the receptor.

This report describes the enhancing effect of 16α,18-dihydroxydeoxycorticosterone on the sodium retaining properties of subthreshold doses of aldosterone in the rat. No effect on potassium excretion at the dose levels tested was observed. Previous experience with the Kagawa assay has indicated that aldosterone increases urinary potassium, only when doses exceed 250 ng. With smaller doses of aldosterone, potassium excretion is quite variable, thus no definite trend was observed. Urinary sodium:potassium ratios were lower in animals given 16α,18-dihydroxydeoxycorticosterone plus aldosterone than in animals given aldosterone alone, however.

The mechanism by which 16α,18-dihydroxydeoxycorticosterone enhances aldosterone activity has not been characterized. The presence of 16α,18-dihydroxydeoxycorticosterone could produce conformational changes in inactive aldosterone cytosol receptors resulting in formation of receptors capable of binding aldosterone. Removal of active receptors by complexing with aldosterone should favor formation of more active receptors. The existence of higher concentrations of aldosterone receptors in target tissues responsible for sodium conservation [18] seems to support the concept that formation of an aldosterone–receptor complex is prerequisite for expression of aldosterone's metabolic effect. The net effect of 16α,18-dihydroxydeoxycorticosterone would be to increase the physiologic action of the existing concentration of aldosterone.

The finding of increased conversion of 18-OH-DOC to the 16α compound in the hypertensive patients with suppressed PRA and normal or known mineralocorticoid levels fits the hypothesis above. Excessive secretion of 16α,18-dihydroxydeoxycorticosterone might induce prolonged positive sodium balance in patients with low PRA essential hypertension.

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