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Effect of Deoxycorticosterone Acetate and 16β-Hydroxy-Dehydroepiandrosterone on Blood Pressure and Plasma Renin Activity of Rats[1] (39364)

S. AYACHI, C. E. HALL, O. B. HOLLAND, AND C. GOMEZ-SANCHEZ

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550; and Department of Internal Medicine, University of Texas Health Science Center, Dallas, Texas 75235

The blood pressure response of patients with low renin essential hypertension to drugs which interfere either with the synthesis of adrenocortical hormones, or with the action of these hormones on peripheral target organs, suggests that hypersecretion of an as yet unidentified mineralocorticoid is causative (1). Recently, it has been found that patients having that disorder excrete excessive quantities of a steroid, 16β-hydroxy-dehydroepiandrosterone (16β-OH-DHEA), unlike patients with either nonhypertensive disorders or other forms of hypertension. Studies on the urinary Na/K ratio of adrenalectomized rats indicated that the steroid possessed one-fortieth the mineralocorticoid activity of aldosterone, suggesting that this steroid might be responsible for low renin essential hypertension (1).

In a previous study we reported that neither 1 nor 2 mg/day of 16β-OH-DHEA caused hypertension in rats sensitized to mineralocorticoid hypertension by mononephrectomy and a high salt intake (2). Under this regimen, however, plasma renin activity (PRA) was maximally depressed even in salt-loaded control rats, doubtless as a result of the salt excess (3), thus making it impossible to determine whether the steroid had mineralocorticoid effects on this function.

The present experiment was designed to compare PRA, blood pressure, and organ weight changes in kidney intact rats on a normal salt intake with equipotent mineralocorticoid dosages of deoxycorticosterone acetate (DOCA) and 16β-OH-DHEA.

Materials and methods. Female SPD rats were obtained from ARS Sprague-Dawley Company. They were divided into groups as indicated in Table I. Animals of groups 1, 2, and 3 received, respectively, 3.125 mg of DOCA in sesame oil, 5 mg of 16β-OH-DHEA diacetate in oil, and oil vehicle only by subcutaneous injection once daily. DOCA treatment causes hypertension in rats having both kidneys and on a normal diet (4). However, we have found that regardless of the dosage employed, treatment must be continued for at least 3 weeks in order to obtain a significant incidence of the disorder (unpublished observations). Therefore, the dosage of 16β-OH-DHEA was determined as the maximal daily dosage that could be given for 3 weeks, with the quantity available. The dosage of DOCA was then set at 0.625 of that amount, a quantity greatly in excess of that required to cause hypertension (4), since the steroids have been assigned mineralocorticoid activities, respectively, one-fortieth and one-twenty-fifth that of aldosterone. The animals were individually caged in temperature-controlled quarters and given Purina Laboratory Chow and tap water *ad libitum*.

The systolic pressure of unanesthetized animals was taken on the twenty-third day, using a PE-300 Programmed Electrosphygmomanometer and appropriate modules attached to a DMP-4B Physiograph (Narco Biosystems, Inc., Houston, Texas). Values above 150 mm Hg were regarded as hypertensive.

16β-OH-DHEA was synthesized according to the method of Aoki *et al.* (5). The steroid, obtained as the diacetate, proved to have physical properties identical to an authentic sample obtained from Steraloids and to yield on hydrolysis a compound that was indistinguishable from a sample of 16β-OH-DHEA provided by Dr. Kirk from the Steroid Reference Collection (London).

At the end of each week the rats were individually placed in metabolism cages for 24 hr for urine collection. During this pe-

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TABLE I. PERTINENT FINDINGS IN RATS TREATED WITH DOCA OR 16β-OH-DHEA.

	DOCA	16β-OH-DHEA	Controls
Number of animals	6	7	6
Body wt (g)			
Initial	101 ± 1[a]	102 ± 2	100 ± 2
Final	169 ± 7	183 ± 1	183 ± 3
Urine volume (ml/24 hr)			
Week 1	10.7 ± 2.1[b]	5.4 ± 0.9	4.3 ± 0.8
Week 2	7.9 ± 1.2	5.0 ± 0.4	5.8 ± 0.6
Week 3	8.8 ± 0.6	6.1 ± 1.2	7.4 ± 0.7
Systolic pressure (mm Hg)	157 ± 10[b]	120 ± 3	128 ± 5
Heart rate (/min)	345 ± 15	399 ± 5	368 ± 22
PRA	0.08 ± 0.04[b]	1.5 ± 0.3	1.8 ± 0.2
Organ wt (mg/100 g body wt)			
Heart[c]	343 ± 9[b]	265 ± 6[b]	289 ± 6
Kidneys	900 ± 23[b]	744 ± 22	762 ± 27
Thymus	128 ± 20	119 ± 9[b]	143 ± 5
Adrenals	24.6 ± 1.1	25.0 ± 0.9	27.8 ± 1.1

[a] Mean ± SEM.
[b] Different from controls ($P < 0.05$).
[c] Ventricles only.

riod water, but not food, was available.

The animals were killed by rapid decapitation on the twenty-fourth day, and trunk blood was collected within 5 sec into chilled, heparinized flasks. It was then transferred into plastic vials and frozen at −20°, where it remained until plasma renin activity (PRA) was measured by the method of Pettinger *et al.* (6) and expressed as nanograms of angiotensin generated per milliliter of plasma per hour of incubation.

Results. Measurement of 24-hr urine output during the experiment revealed that DOCA-treated rats tended to excrete larger urine volumes than controls, whereas rats given 16β-OH-DHEA did not (Table I).

By the twenty-third day, DOCA-treated rats had become hypertensive, average systolic pressure of the group being 157 ± 10 mm Hg. This contrasted with 128 ± 5 in controls and 120 ± 3 in those given 16β-OH-DHEA. The heart rate varied inversely with the blood pressure, the respective values being 345 ± 15, 368 ± 22, and 399 ± 5 beats/min (Table I).

Plasma renin activity of control rats ranged 1.1–2.7 (av. 1.8 ± 0.2) ng/ml/hr. This compared with 0.0–0.3 (av. 0.08 ± 0.04) in DOCA-treated rats and 0.7–3.1 (av. 1.5 ± 0.3) ng/ml/hr in rats given 16β-OH-DHEA (Table I).

Organ weights revealed a significant enlargement of hearts and kidneys in DOCA-treated rats whereas these organs in rats given 16β-OH-DHEA were slightly smaller than in controls. There were no significant differences between thymus or adrenal weights in any of the groups (Table I).

Discussion. Despite the fact that 3.125 mg of DOCA should have equivalent mineralocorticoid potency to 5 mg of 16β-OH-DHEA, assuming that the respective levels of activity were one-twenty-fifth and one-fortieth that of aldosterone as reported, the former caused hypertension, enlargement of hearts and kidneys, and a reduction of plasma renin activity to undetectable levels, whereas the latter had no such effects.

Our previous studies have shown that 16β-OH-DHEA had no detectable effects at either 1 or 2 mg/day on blood pressure or organ weight of salt-loaded mononephrectomized rats. Funder *et al.* (7) have reported that it has negligible affinity for mineralocorticoid binding sites in the rat kidney. Both Edelman (personal communication) and Higgins (personal communication) have found it to have no effect on sodium transport in the isolated toad bladder. Finally, we have been unable to detect significant mineralocorticoid activity in this steroid in two different rat bioassay systems (<0.1 that of aldosterone).

Taken together, these findings would suggest that 16β-OH-DHEA is essentially biologically inert as a mineralocorticoid. If so, there is little reason to assume that it could directly have anything to do with the genesis

of low renin essential hypertension. It could, of course, be a metabolite of some other steroid, presumably of adrenal origin, which has blood pressure elevating properties.

Alternatively, although possessing insignificant mineralocorticoid properties of its own, 16β-OH-DHEA might potentiate the activity of other steroids; such behavior has recently been described for 16α, 18 dihydroxy-deoxycorticosterone, a metabolite of 18 hydroxy-deoxycorticosterone. The metabolite, at a dosage of 10 μg, did not promote sodium retention in adrenalectomized rats, although the same dosage greatly enhanced the sodium retention resulting from the administration of 50 μg of aldosterone. Interestingly, the adrenal glands from patients with low renin essential hypertension converted the precursor into the metabolite under *in vitro* conditions from four to five times more effectively than did control adrenals (8). This raises the possibility that the disease might be caused by steroids which potentiate the activity of mineralocorticoids, rather than playing a direct causative role.

Summary. Intact female Sprague–Dawley rats were given daily injections of either 3.125 mg of deoxycorticosterone or 5.0 mg of 16β-hydroxy-dehydroepiandrosterone, calculated from the activities reported for each to be equivalent mineralocorticoid dosages. The former caused hypertension, cardiorenal enlargement, increased urine output and depressed PRA. Treatment with 16β-OH-DHEA had no such effect, raising questions regarding its classification as a mineralocorticoid.

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