The $C_{19}$-Mineralocorticoids in Hypertension

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SUMMARY

The excretion rates of the $C_{19}$-mineralocorticoids, 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol, were measured in subjects with low-renin essential hypertension and toxemia of pregnancy. $C_{19}$-mineralocorticoid excretion in low-renin essential hypertension ranged from 40-760 $\mu$g per day. No significant difference in 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol excretion was found between these subjects and normal controls. Subjects with toxemia of pregnancy excreted between 350 and 2500 $\mu$g per day of these steroids. No significant difference in 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol was found between toxemic and normal pregnancy. Thus, 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol probably do not play an important role in either low-renin essential hypertension or toxemia of pregnancy.

DURING previous studies of urinary mineralocorticoid activity in subjects with low-renin essential hypertension, we isolated and identified two new mineralocorticoids, 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol. These steroids had an antinatriuretic and kaliuretic action which was equal to DOC, and which was blocked by spironolactone, a mineralocorticoid antagonist. Because the excretion of these steroids appeared to be elevated in certain subjects with low-renin essential hypertension, we speculated that the excessive production of 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol was a cause of low-renin essential hypertension. However, further studies showed that the production of 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol was ACTH dependent. When we found that the blood pressure of the majority of low-renin subjects remained elevated despite the suppression of 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol excretion with glucocorticoid treatment, we concluded that these $C_{19}$-mineralocorticoids probably did not play an important role in the etiology of low-renin essential hypertension.

Nevertheless, we have continued to investigate these new mineralocorticoids. In particular, we have tested a large number of $C_{19}$-steroids for mineralocorticoid activity in order to study the relationship between $C_{19}$-mineralocorticoid activity and structure. Furthermore, we have measured $C_{19}$-mineralocorticoid excretion in a series of subjects with low-renin essential hypertension and with

References

toxemia of pregnancy in order to determine if the C_{19}-mineralocorticoids were markers for these diseases. Finally, we have studied the relationship between C_{19} mineralocorticoid excretion and bioassayable mineralocorticoid activity in urine extracts in order to find evidence for other new, yet unknown, mineralocorticoids, which might play a role in the etiology of low-renin essential hypertension.

Methods

MATERIALS

16α-Hydroxy-DHEA was obtained from Dr. D. N. Kirk of the Steroid Reference Collection in London; other steroids were purchased from Steraloids, Inc. The following abbreviations and trivial names are used: 16β-hydroxy-DHEA = 3β,16β-dihydroxy-androst-5-ene-17-one; 16α-hydroxy-DHEA = 3β,16α-dihydroxy-androst-5-ene-17-one; 16-oxo-androstenediol = 3β,17β-dihydroxy-androst-5-ene-16-one; etienic acid = 3β-hydroxy-androst-5-ene-17β-carboxylic acid; DHEA = 3β-hydroxy-androst-5-ene-17-one; 16-oxo-testosterone = 17β-hydroxy-androst-4-ene-3,16-dione; 16β-hydroxy-estrone = 3α,16β-dihydroxy-1,3,5(10)-estratriene-17-one; 16-oxo-estradiol = 3α,17β-dihydroxy-1,3,5(10)-estratriene-16-one; 16-oxo-testosterone = 2β,17β-dihydroxy-androst-4-ene-3-one.

SUBJECTS

Hypertensive patients were selected from the Vanderbilt University Hypertension Clinic. A patient was considered to have essential hypertension when, after complete evaluation, including renal arteriography and aldosterone measurements, all known causes of hypertension were excluded. Low-renin essential hypertension was diagnosed if a patient's peripheral plasma renin activity failed to exceed 1.67 ng/ml per hour following stimulation according to the method of Carey et al. and if the patient's blood pressure became normal during treatment with spironolactone. Preeclamptic patients were selected from the Vanderbilt University Obstetrics Clinic on the basis of blood pressures consistently above 140/90 mm Hg, peripheral edema and proteinuria. All of these subjects were in the third trimester of pregnancy. Vanderbilt University and DHEW rules for protection of human subjects were followed in this study. Informed consent was obtained from all patients.

MINERALOCORTICOID BIOASSAY

The method for the rat mineralocorticoid bioassay was based on the procedure of Kagawa and has been previously described.

GLUCOCORTICOID BIOASSAY

The method for a combined thymolytic and liver glycogen deposition assay was based on the procedure of Ringer et al. Male albino rats weighing 40-60 g were bilaterally adrenalectomized and maintained with a normal stock diet and saline drinking fluid. Following surgery, the rats received an ip injection of the test compounds in 0.5 ml of 10% ethanol daily for 3 days. The rats were fasted for 15 hours prior to and 7 hours after the last injection. Livers and thymus were then removed and weighed. The livers were digested with 30% KOH. The glycogen was precipitated with 95% ethanol and hydrolyzed with 1 M sulfuric acid. The resulting glucose was measured with an autoanalyzer.

ASSAYS BY GAS CHROMATOGRAPHY

16β-Hydroxy-DHEA and 16-oxo-androstenediol were measured using a gas chromatographic method similar to that we have previously described. Two important modifications of the assay were the substitution of ammonium sulfate for sodium chloride in the solvolysis step and chromatography on thin layer silica gel plates, instead of on paper, prior to injection into the gas chromatograph. The thin layer chromatography was developed in three systems: benzene; benzene-ethanol, 100:9; and benzene-acetone-water, 100:52:0.2. These modifications resulted in increased yield and recovery of the C_{19}-steroids. The coefficient of variation for this assay was 20%.

URINARY MINERALOCORTICOID ACTIVITY

Three-day collections of urine from human subjects were partitioned against dichloromethane in order to extract unconjugated steroids. The urine was then acidified to pH 1 with hydrochloric acid for 24 hours, after which it was extracted a second time with dichloromethane. The two dichloromethane fractions were combined, neutralized with sodium hydroxide and evaporated to dryness. The residue was partitioned between ethyl ether and water. The ether then was discarded, and the water partitioned against carbon tetrachloride, after which the water was discarded. The partitioned urine extracts were chromatographed on silica gel plates in a benzene-acetone-water, 100:52:0.2 system. Mineralocorticoid activity was found between corticosterone and DOC in this system. This chromatographic fraction did not contain any known mineralocorticoids other than 16β-hydroxy-DHEA and 16-oxo-androstenediol. This fraction was bioassayed and its mineralocorticoid activity compared to the amounts of 16β-hydroxy-DHEA and 16-oxo-androstenediol measured by the gas chromatograph.

Results

The mineralocorticoid potencies of the steroids that were tested in this study are listed in Table 1. Besides 16β-hydroxy-DHEA and 16-oxo-androstenediol, two other steroids were found to have sodium-retaining activity. These two new electrolyte-active steroids were 16-oxo-testosterone and etienic acid. 16-Oxo-testosterone had 72% the activity of aldosterone. Etienic acid had 1/3 the potency of aldosterone.

GLUCOCORTICOID EFFECT OF 16-OXO-ANDROSTENEDIOL

Pure glucocorticoids can lower the urinary Na-K ratio in adrenalectomized rats by increasing potassium excretion. In order to determine if part of the effect of the C_{19} mineralocorticoids on urinary electrolytes were a result of a glucocorticoid action, the glucocorticoid potency of 16-oxo-androstenediol was measured. Representative results
TABLE 1  Mineralocorticoid Potencies

<table>
<thead>
<tr>
<th></th>
<th>Relative potency*</th>
<th>95% confidence limits†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16β-Hydroxy-DHEA</td>
<td>1/40</td>
<td>1/26-1/60</td>
</tr>
<tr>
<td>16-Oxo-androstenediol</td>
<td>1/50</td>
<td>1/31-1/79</td>
</tr>
<tr>
<td>16-Oxo-testosterone</td>
<td>1/28</td>
<td>1/16-1/47</td>
</tr>
<tr>
<td>Etienic acid</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>Inactive steroids‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androst-5-ene-3β,16β-diol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androst-5-ene-3β,17β-diol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androst-5-ene-3β,16α,17β-triol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androst-5-ene-3β,16β,17β-triol</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,11β-Dihydroxy-5α-androstan-17-one</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>3β,16α-Dihydroxy-5α-androstan-17-one</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>16β,17β-Dihydroxy-androst-4-ene-3-one</td>
<td>1/71</td>
<td>1/32-1/157</td>
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<tr>
<td>3β,17α-Dihydroxy-androst-5-ene-16-one</td>
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</tr>
<tr>
<td>3β-Hydroxy-5α-androstan-16-one</td>
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<td>3β-Hydroxy-androst-5-ene-16-one</td>
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<td>1/32-1/157</td>
</tr>
<tr>
<td>16α-Hydroxy-DHEA</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>16β-Hydroxy-estrone</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>2β-Hydroxy-testosterone</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>16-Oxo-estradiol</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1/71</td>
<td>1/32-1/157</td>
</tr>
</tbody>
</table>

* Aldosterone = 1.
† Calculated according to the method of Pugsley.‡
‡ Inactive steroids had less than 1/500th the potency of aldosterone.

are recorded in Table 2. 16-Oxo-androstenediol had a negligible effect on the thymus-body weight ratio and on liver glycogen content. Thus, 16-oxo-androstenediol is not a glucocorticoid, and the electrolyte effects of this steroid cannot be attributed to glucocorticoid action.

**Urinary Excretion of the C19-Mineralocorticoids**

Etienic acid and 16-oxo-testosterone have not been identified in man. Urinary values of 16β-hydroxy-DHEA and 16-oxo-androstenediol in subjects with low-renin essential hypertension and in those with preeclampsia, each compared with appropriate control subjects, are shown in Figures 1 and 2. We found that 16β-OH-DHEA and 16-oxo-androstenediol tended to isomerize into each other. Thus, we have expressed the data as a sum of the excretion of these two isomers. Since both isomers have approximately equal biological activity, the combined excretion rate should be an accurate index of urinary C19-mineralocorticoid activity.

In this series, patients with low-renin essential hyperten-

**Table 2  Effects of 16β-Oxo-androstenediol and Cortisol**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose (mg)</th>
<th>Liver glycogen (mg/g)</th>
<th>Thymus wt (mg/100 g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5</td>
<td>5.3 ± 0.8</td>
<td>480 ± 40</td>
</tr>
<tr>
<td>16β-Oxo-androstenediol</td>
<td>7.5</td>
<td>6.3 ± 0.6</td>
<td>420 ± 40</td>
</tr>
<tr>
<td>Cortisol</td>
<td>1.5</td>
<td>27.5 ± 5</td>
<td>260 ± 20</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

- FIGURE 1  The sum of 16β-hydroxy-DHEA-sulfate and 16-oxo-androstenediol-sulfate excretion in 12 subjects with low-renin essential hypertension and 12 normal adult subjects.

- FIGURE 2  The sum of 16β-hydroxy-DHEA-sulfate and 16-oxo-androstenediol-sulfate excretion in 11 subjects with toxemia of pregnancy and 10 normal pregnant subjects in the third trimester.
Subjects with low-renin essential hypertension have certain features consistent with excessive mineralocorticoid activity. Besides the low plasma renin activity itself, these subjects show reductions in blood pressure when they are treated with aminogluthethimide (an inhibitor of steroid biosynthesis) and with spironolactone (an antagonist of mineralocorticoid action). Because the known mineralocorticoids are not elevated in the majority of low-renin essential hypertensive subjects, unknown mineralocorticoids were sought in the urine of such subjects. This search led to the discovery of two C$_{19}$ steroids, 16β-hydroxy-DHEA and 16-oxo-androstenediol, both of which have mineralocorticoid potency approximately equal to DOC in our bioassay.

Subsequent to our initial reports about the mineralocorticoid activity of 16β-hydroxy-DHEA and 16-oxo-androstenediol, two other laboratories published data about the mineralocorticoid activity of these steroids. Gomez-Sanchez et al. found that 16β-hydroxy-DHEA and 16-oxo-androstenediol had less than 0.1% the potency of aldosterone and 16-oxo-testosterone had 0.2% the potency of aldosterone when injected ip. In contrast to the Gomez-Sanchez results, Funder et al. found that 16β-hydroxy-DHEA had mineralocorticoid potency 1/40 aldosterone in rats which weighed 100 g. However, in younger or older rats, 16β-hydroxy-DHEA had no salt-retaining effect. The age-dependent effect of the C$_{19}$ mineralocorticoids has been confirmed in our own laboratory. It appears from this variety of results about C$_{19}$ mineralocorticoid activity that the potency of these steroids is dependent upon the particular rat and assay technique which is used.

In our initial measurements of 16β-hydroxy-DHEA excretion, we found elevated urinary levels of this steroid in subjects with low-renin essential hypertension compared either to subjects with normal-renin hypertension or to healthy adult subjects. Subsequent experience, however, has shown that there is much overlap in 16β-hydroxy-DHEA sulfate excretion between subjects with low-renin hypertension and normal subjects. In this study, we have reported the sum of the excretion of 16β-hydroxy-DHEA and 16-oxo-androstenediol and have found no difference between low-renin hypertensives and normal adult subjects. Similarly, Sekihara et al. have found that plasma levels of unconjugated 16β-hydroxy-DHEA are not elevated in subjects with low-renin essential hypertension. Thus, it does not appear that these C$_{19}$ mineralocorticoids are a useful marker for low-renin essential hypertension. The normal C$_{19}$ mineralocorticoid excretion and the failure of glucocorticoid treatment to lower blood pressure in the majority of low-renin essential hypertensive subjects indicate that C$_{19}$ mineralocorticoids do not play an important pathogenetic role in the majority of subjects with this type of disorder.

The disappearance of a significant difference in C$_{19}$ mineralocorticoid excretion in low-renin subjects compared to normal subjects between our earlier publication and this one is probably explained by the difficulty of measuring 16β-hydroxy-DHEA without contamination by 16-oxo-androstenediol. It is likely that the high values of 16β-hydroxy-DHEA excretion found in our earlier study for low-renin subjects represented contamination by 16-oxo-androstenediol. Apparently, we were more successful...
in purifying 16β-hydroxy-DHEA when we measured it in the urine of normal subjects than when we measured it in urine from subjects with low-renin hypertension. Using improved methods, we are now unable to demonstrate a difference in 16β-hydroxy-DHEA excretion between low-renin subjects and normal subjects.

Gant et al. found that the metabolic clearance rate of DHEA sulfate was low in subjects with preeclampsia compared to normal pregnant subjects. Our finding that 16β-hydroxy-DHEA and 16-oxo-androstenediol excretion was similar in preeclamptic subjects and normal subjects and the finding of Sekihara et al. that plasma levels of 16β-hydroxy-DHEA were the same in preeclampsia and normal pregnancy suggests that the metabolism of a 16β-hydroxy-DHEA and 16-oxo-androstenediol is not altered in toxemia of pregnancy.

Although 16β-hydroxy-DHEA and 16-oxo-androstenediol have not proved to be important in low-renin essential hypertension, we have continued to search for new salt-retaining substances in the urine of these subjects. Since there is more biological activity in our active chromatographic fraction than can be accounted for by the known mineralocorticoids including 16β-hydroxy-DHEA and 16-oxo-androstenediol, it appears that other unknown sodium-retaining substances are cochromatographing with the C19-mineralocorticoids. Neither these unknown steroids nor the hypertension of the majority of low-renin subjects is ACTH dependent. The steroids have been highly purified with high pressure liquid chromatography, but efforts to elucidate their structures and establish unequivocally their clinical significance have thus far been unsuccessful. Further studies are deemed warranted.

Acknowledgments

We thank Dr. D. N. Kirk of the Steroid Reference Collection, London, for supplying the 16β-hydroxy-DHEA.

References


Erratum

The following typographical errors appear in the article, "The Effect of Ouabain, Dinitrophenol, and Lithium on the Pacemaker Current in Sheep Cardiac Purkinje Fibers," by Drs. Ronald S. Aronson and Jeremiah M. Gelles [Circ. Res. 40: No. 5 (May), 517-523, 1977]: Under Discussion, p. 522, the symbol $I_n$ should be substituted for $I'_1$ in the last line of the first paragraph, for $K_1$ in the second paragraph, and for $I'_3$ in the ninth line of the third paragraph.