References


The C19-Mineralocorticoids in Hypertension

JORDAN A. SENNETT, LARRY R. YARBRO, PAUL E. SLATON, JOHN W. HOLLIFIELD, AND GRANT W. LIDDLE

SUMMARY The excretion rates of the C19-mineralocorticoids, 16β-hydroxy-DHEA and 16-oxo-androstenediol, were measured in subjects with low-renin essential hypertension and toxemia of pregnancy. C19-mineralocorticoid excretion in low-renin essential hypertension ranged from 40-760 μg per day. No significant difference in 16β-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 μg per day of these steroids. There was no significant difference between toxemic and normal pregnancy. Thus, 16β-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,1 we isolated and identified two new mineralocorticoids, 16β-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 16β-hydroxy-DHEA and 16-oxo-androstenediol was a cause of low-renin essential hypertension. However, further studies showed that the production of 16β-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 16β-hydroxy-DHEA and 16-oxo-androstenediol excretion with glucocorticoid treatment, we concluded that these C19-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 C19-steroids for mineralocorticoid activity in order to study the relationship between C19-mineralocorticoid activity and structure. Furthermore, we have measured C19-mineralocorticoid excretion in a series of subjects with low-renin essential hypertension and with...
toxemia of pregnancy in order to determine if the C_{19}-
mineralocorticoids were markers for these diseases. Fi-
nally, 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

16β-Hydroxy-DHEA was obtained from Dr. D. N.
Kirk of the Steroid Reference Collection in London; other
steroids were purchased from Steraloids, Inc. The follow-
ing abbreviations and trivial names are used: 16β-hy-
droxy-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-dihy-
droxy-androst-5-ene-16-one; etienic acid = 3β-hy-
droxy-androst-5-ene-17β-carboxylic acid; DHEA = 3β-hy-
droxy-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; 2β-hydroxy-testosterone = 2β,17β-dihydroxy-
androst-4-ene-3-one.

SUBJECTS

Hypertensive patients were selected from the Vander-
bilt University Hypertension Clinic. A patient was consid-
ered 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 accord-
ing to the method of Carey et al.,3 and if the patient's
blood pressure became normal during treatment with spi-
rone. Preeclamptic patients were selected from the Vander-
bilt University Obstetrics Clinic on the basis of
blood pressures consistently above 140/90 mm Hg, pe-
ripheral 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 Kagawa4 and has been previ-
ously described.1

GLUCOCORTICOID BIOASSAY

The method for a combined thymolytic and liver glycogen
deposition assay was based on the procedure of Kingerl et al.5
Male albino rats weighing 40-60 g were bilaterally adrener-
catomized and maintained with a nor-
mal 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 thyri were then removed and
weighed. The livers were digested with 30% KOH. The
glycogen was precipitated with 95% ethanol and hydro-
dyzed 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.1 Two important modi-
fications of the assay were the substitution of ammonium
sulfate for sodium chloride in the solvolysis step and chro-
matography 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 sys-
tems: benzene; benzene ethanol, 100:52:0.2. These modifi-
cations 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 ex-
tract 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, neutral-
ized with sodium hydroxide and evaporated to dryness.
The residue was partitioned between ethyl ether and wa-
ter. The ether then was discarded, and the water parti-
tioned against carbon tetrachloride, after which the water
was discarded. The partitioned urine extracts were chro-
matographed 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 mea-
sured 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 V/3
the activity of aldosterone. Etienic acid had V/4 the po-
tency of aldosterone.

GLUCOCORTICOID EFFECT OF
16-OXO-ANDROSTENEDIOL

Pure glucocorticoids can lower the urinary Na-K ratio in
adrenalectomized rats by increasing potassium excretion.7
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>Active steroids</th>
<th>Relative potency</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<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>1/25</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></td>
<td></td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,11β-Dihydroxy-5α-androstan-17-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,16α-Dihydroxy-5α-androstan-17-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16β,17β-Dihydroxy-androst-4-ene-3-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,17α-Dihydroxy-androst-5-ene-16-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β-Hydroxy-5α-androstan-16-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β-Hydroxy-androst-5-ene-16-one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16α-Hydroxy-DHEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16β-Hydroxy-estrone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2β-Hydroxy-testosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-Oxo-estradiol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></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 preclampsia, 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 hypertension excreted a total of between 40 and 760 μg per day of 16β-hydroxy-DHEA sulfate and 16-oxo-androstenediol sulfate. C19-mineralocorticoid excretion was not significantly different in subjects with low-renin essential hypertension compared to normal controls.

The pregnant women excreted between 350 and 2500 μg of these steroids per day in the third trimester, which is elevated compared to nonpregnant women. There was no significant difference between toxemic and normal pregnancy.

RELATION BETWEEN C19-MINERALOCORTICOID EXCRETION AND BIOASSAYABLE MINERALOCORTICOID ACTIVITY

In order to seek evidence for other new, yet unknown mineralocorticoids, the total bioassayable mineralocorticoid activity in the corticosterone to DOC chromatographic fraction was compared to the amounts of 16β-hydroxy-DHEA and 16-oxo-androstenediol that were
measured in that fraction, both expressed in terms of aldosterone equivalents. These comparisons were performed with urine obtained from nine subjects with low-renin essential hypertension during control periods and during treatment with dexamethasone 0.5 mg every 12 hours. Results for three representative subjects are shown in Figure 3. Five subjects were similar to subject 1. Only a portion of total mineralocorticoid activity could be accounted for by C_19-mineralocorticoid content. Dexamethasone suppressed C_19-mineralocorticoid excretion and also tended to decrease but did not abolish total mineralocorticoid activity. Two subjects were similar to subject 3. These subjects excreted only small amounts of the C_19-mineralocorticoids and yet had substantial bioassayable mineralocorticoid activity. Dexamethasone had little effect on total mineralocorticoid activity. Subject 2 was one of two subjects in whom all of the bioassayable activity could be attributed to the C_19-mineralocorticoids. These subjects showed complete suppression of biological activity by dexamethasone. Interestingly, of the nine subjects who were studied, only these two experienced normalization of their blood pressures during treatment with dexamethasone. Subjects who continued to have substantial mineralocorticoid activity in their urine while on dexamethasone did not normalize their blood pressures with this treatment.

Thus, one or more unknown mineralocorticoids, which are not suppressible with dexamethasone, appear to cochromatograph with 16β-hydroxy-DHEA and 16-oxo-androstenediol. These unknown factors have been found in a majority of low-renin essential hypertensive patients. They were not found in two subjects whose blood pressures normalized on dexamethasone.

**Discussion**

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 aminoglutethimide (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.12 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.11 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 chromographic 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 $I_\infty$ should be substituted for $I_T$ in the last line of the first paragraph, for $K_3$ in the second line of the second paragraph, and for $I_2$ in the ninth line of the third paragraph.
The C19-mineralocorticoids in hypertension.
J A Sennett, L R Yarbro, P E Slaton, J W Hollifield and G W Liddle

Circ Res. 1977;41:260-264
doi: 10.1161/01.RES.41.2.260

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/41/2/260

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/