Unconjugated and Conjugated Urinary 19-Nor-deoxycorticosterone Glucosiduronate Elevated Levels in Essential Hypertension

GEORGE T. GRIFFING, THOMAS E. WILSON, AND JAMES C. MELBY

SUMMARY The mineralocorticoid 19-nor-deoxycorticosterone (19-nor-DOC) is present in the urine of rats and humans in unconjugated and conjugated forms. This study sought to compare levels of unconjugated and conjugated 19-nor-DOC glucosiduronate in essential hypertensive subjects. The essential hypertensive and normal subjects were admitted to a metabolic unit, and plasma and urine were collected at fixed intervals on a fixed-electrolyte intake (Na\(^+\), 128 mEq/day, K\(^+\), 80 mEq/day). The 19-nor-DOC was purified by chromatography and measured by radioimmunoassay. Unconjugated urinary 19-nor-DOC was elevated in essential hypertensive subjects (195 ± 21 [SE] ng/day; \(n = 21\)) compared with levels in normal subjects (118 ± 30 [SE] ng/day; \(n = 13\), \(p < 0.05\)). Two essential hypertensive subjects had very high levels (673, 729 ng/day), while levels in seven essential hypertensive subjects were below 118 ng/day. Conjugated 19-nor-DOC glucosiduronate also was elevated in essential hypertensive subjects (950 ± 88 [SE] ng/day; \(n = 8\)) compared with levels in normal subjects (680 ± 90 [SE] ng/day; \(n = 5\)). Seven of eight essential hypertensive subjects had levels greater than 680 ng/day. The unconjugated and conjugated urinary 19-nor-DOC glucosiduronate levels were positively correlated in both of these groups (\(\rho = 0.82\), \(p < 0.01\)). Other test results including plasma renin activity, plasma aldosterone levels, aldosterone secretion rates, and plasma and urine electrolyte levels were not different between groups. These results indicate that essential hypertensive subjects have increased 19-nor-DOC excretion, which is reflected by increases in both unconjugated and conjugated glucosiduronate forms. Some essential hypertensive subjects, however, had normal urinary 19-nor-DOC levels, which may reflect the heterogeneity of this disease.

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KEY WORDS  19-nor-deoxycorticosterone  essential hypertension  mineralocorticoid

THE mineralocorticoid 19-nor-deoxycorticosterone (19-nor-DOC) was synthesized more than 40 years ago,\(^1\) and Kagawa and Van Arman\(^2\) subsequently found the sodium-retaining activity of this hormone to be between two and five times that of deoxycorticosterone (DOC) itself. The biological significance of 19-nor-DOC was elucidated by the studies of Gomez-Sanchez et al.\(^3\) in rat adrenal regeneration hypertension. As 19-nor-DOC is a potent mineralocorticoid produced by both humans and rats,\(^4-7\) it would be of considerable interest to define the production and metabolism of this hormone in normal and hypertensive subjects. Like DOC, 19-nor-DOC may be synthesized and metabolized at its presumed site of action, the kidney. Biological synthesis of 19-nor-DOC involves decarboxylation of an adrenal 19-oxygenated DOC precursor, probably 19-oic-deoxycorticosterone.\(^8-9\) This conversion does not occur in the adrenal but at some peripheral location, perhaps analogous to the extra-adrenal formation of DOC from progesterone by renal 21-\(\alpha\)-hydroxylase.\(^10,11\)

The metabolism of DOC and 19-nor-DOC in humans has been compared by radiolabeled infusion studies with \([\text{H}]19\)-nor-DOC and \([\text{C}]\)DOC.\(^10\) Unlike DOC, in these studies very little 19-nor-DOC appeared in the urine as the sulfoconjugate; instead, the major metabolite was conjugated 19-nor-DOC glucosiduronate. The present study sought to measure urinary levels of both unconjugated and conjugated 19-nor-DOC glucosiduronate in essential hypertension. The results of this study suggest that elevated levels of both these forms of 19-nor-DOC occur in this disease.
URINARY 19-NOR-DOC IN ESSENTIAL HYPERTENSION/Griffing et al.

Materials and Methods
Written informed consent, approved by the Institutional Review Board, was obtained from all subjects. Thirteen normal subjects were used, including seven women, with a mean age of 25 ± 2 (se) years and mean weight of 166 ± 15 (se) lbs. There were 21 subjects with essential hypertension, 6 with primary aldosteronism and 1 with 17-α-hydroxylase deficiency. The essential hypertensive group, which included 10 women, had a mean age of 50 ± 4 (se) years and a mean weight of 180 ± 10 (se) lbs. The subjects had taken no concomitant medications, including estrogens, oral contraceptives, diuretics, or corticosteroids, for 4 weeks before the study. Some hypertensive subjects were maintained on small doses of prazosin, 2 to 6 mg/day, to control blood pressure. Subjects were admitted to a metabolic unit and fed a constant diet (Na+ 128 mEq/day and K + 80 mEq/day). Blood was drawn at 0800 hr (after 8 hours of overnight recumbency) and again at 1200 hr (after 4 hours of upright posture). Blood measurements included electrolyte levels, plasma renin activity, and plasma aldosterone levels and were conducted with standard assay methods.11 12 Twenty-four hour urine collections for measurement of electrolytes, creatinine, tetrahydroaldosterone, and aldosterone secretion rates were also performed according to previously reported techniques.13

To measure levels of 19-nor-DOC glucosiduronate, the urine was hydrolyzed with β-glucuronidase by the following method. The urine sample was adjusted to pH 4.0 with 2 M acetic acid and incubated at 37°C for 24 hours with 1/2 volume of β-glucuronidase (glucuronidase, Endo Laboratories Inc., Wilmington, DE), 1000 units/ml of buffer. The sample was cooled and extracted with 5 volumes of distilled CH2Cl2. The aqueous phase was discarded, and the sample was washed with half volume of 0.1 M sodium hydroxide and again with half volume of distilled water. The sample was reduced to dryness on a Rotavapor (Fisher Scientific, Pittsburgh, PA), resuspended in 2 to 3 ml of acetone, and dried under nitrogen gas.

Urine 19-nor-DOC concentration was measured after extraction, thin-layer chromatography, and radioimmunoassay. All urine extractions were performed in duplicate or triplicate in at least two of the three assays. To reduce interassay variation, all urinary 19-nor-DOC measurements from an individual were measured in a single radioimmunoassay.

The [H]19-nor-DOC (10 pg/sample) was added to the sample before extraction to correct for losses during purification. Hydrolyzed and unhydrolyzed urine samples were extracted and purified by thin-layer chromatography using a Celite plate impregnated with propylene glycol/acetone and developed with the solvent system toluene/hexane/propylene glycol (1:5:saturated). This chromatography system effectively separates 19-nor-DOC from DOC and other corticosteroids (Table 1). The radioimmunoassay for 19-nor-DOC has been previously described.5 Dr. Celso Gomez-Sanchez graciously provided the antibody and [H]19-nor-DOC.

### Table 1

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Migration distance (cm)</th>
<th>R&lt;sub&gt;DOC&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>11-Deoxy cortisol</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Cortisone</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Testosterone</td>
<td>2.3</td>
<td>0.35</td>
</tr>
<tr>
<td>19-Nor-DOC</td>
<td>4.9</td>
<td>0.75</td>
</tr>
<tr>
<td>DOC</td>
<td>6.5</td>
<td>1.00</td>
</tr>
<tr>
<td>DHEA</td>
<td>6.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>11.7</td>
<td>1.80</td>
</tr>
<tr>
<td>Progesterone</td>
<td>15.0</td>
<td>2.31</td>
</tr>
</tbody>
</table>

DHEA = dehydroepiandrosterone, DOC = deoxycorticosterone, 19-nor-DOC = 19-nor-deoxycorticosterone, R<sub>DOC</sub> = migration coefficient

The nonspecific binding without antibody was 2.5%, nearly identical to the nonspecific binding when excess 19-nor-DOC was added (800 pg, 2.3%; 2000 pg, 2.4%; 4000 pg, 2.2%; 10,000 pg, 2.2%). The specificity of the 19-nor-DOC antibody for other corticosteroids is shown in Table 2. The percent cross-reactivity between the 19-nor-DOC antibody was calculated from the ratio of the mass of 19-nor-DOC required to displace 50% of the labeled 19-nor-DOC to the mass of cross-reacting steroid required for 50% displacement, multiplied by 100. The DOC had the highest cross-reactivity (13.5%) and cortisol the lowest (<0.0001%). It is unlikely that DOC interfered with the present assay as previously reported levels of urinary free DOC (41 ± 19 [se] ng/day) are less than those of urinary free 19-nor-DOC (103 ± 54 ng/day) measured in this study. Furthermore, 19-nor-DOC is separated from DOC and other corticosteroids by the previously described thin-layer chromatography (see Table 1).

Within-assay precision, estimated by assaying seven samples from a urine pool in a single assay, was

### Table 2

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Cross-reactivity (wt/wt at 50% binding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-Nor-DOC</td>
<td>100 000%</td>
</tr>
<tr>
<td>DOC</td>
<td>13 500%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3 875%</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>0 194%</td>
</tr>
<tr>
<td>11-Deoxy cortisol</td>
<td>0 172%</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0 011%</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0 009%</td>
</tr>
<tr>
<td>DHEA</td>
<td>0 008%</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0 0001%</td>
</tr>
</tbody>
</table>

DHEA = dehydroepiandrosterone, DOC = deoxycorticosterone, 19-nor-DOC = 19-nor-deoxycorticosterone.
6.07 ± 0.63 (SD) ng (121 pg/tube), which gave an intra-assay coefficient of variation of 10.4%. Between-assay precision, estimated by assaying samples from the same urine pool in 10 different assays, was 5.93 ± 0.59 (1 SD) ng (119 pg/tube), which gave an interassay coefficient of variation of 9.9%.

Two buffer samples and two Celite plate blank samples were run in each assay. The plate blank was nearly always unmeasurable, and the Celite plate blank was 12 ± 11 (SD) pg (n = 8). The plate blanks taken from plates with DOC standards were not increased compared with plate blanks without standards. The minimal detectable dose of 19-nor-DOC was 34 pg (defined as the plate blank value + 2 SD). All samples in this study had between 100 and 400 pg of 19-nor-DOC per tube.

The mean recovery of [3H]19-nor-DOC following extraction and thin-layer chromatography was 67 ± 12% (SD). The recovery of unlabeled 19-nor-DOC measured by radioimmunoassay following extraction and thin-layer chromatography at three dose levels between 8000 and 1600 pg added to assay buffer was 104 ± 14% (SD). The recovery of unlabeled 19-nor-DOC from three urine dilutions of 1:2:3 volumes measured by radioimmunoassay following extraction and thin-layer chromatography was greater than 90%.

Comparison data are reported as means ± SE. Baseline data appeared to be normally distributed, but too few subjects were available for goodness-of-fit tests. A complete block design (two-way) analysis of variance and a Dunnett’s t test for multiple comparisons were used.14 Student’s t tests and rank sum tests were used for pair-wise comparisons. Linear regression and correlation were calculated using the method of least mean squares and Spearman’s “rho.” Significance is reported as a rejection of the null hypothesis at a probability of p < 0.05.

**Results**

Results of the measurement of urinary unconjugated (free) 19-nor-DOC are shown in Figures 1 and 2. Unconjugated 19-nor-DOC levels were elevated in essential hypertensive subjects (950 ± 88 ng/day; n = 21) compared with normal subjects (118 ± 30 ng/day, n = 13, p < 0.05). Unconjugated 19-nor-DOC levels also were elevated in six hypertensive subjects with primary aldosteronism (1238 ± 570 ng/day) and in a patient with 17-α-hydroxylase deficiency (3800 ng/day). Two essential hypertensive subjects had very high levels of unconjugated 19-nor-DOC (673, 729 ng/day), while levels in seven essential hypertensive subjects were below 118 ng/day. Conjugated 19-nor-DOC glucosiduronate levels also were elevated in the essential hypertensive subjects (950 ± 88 ng/day; n = 8) compared with levels in the normal subjects (680 ± 90 ng/day; n = 5, p < 0.05). Seven of eight subjects with essential hypertension had levels greater than 680 ng/day. The urinary unconjugated and conjugated 19-nor-DOC glucosiduronate levels were positively correlated in both of these groups (rho = 0.82, p < 0.01; Figure 3). The plasma and urinary electrolyte levels, supine and upright plasma renin activity and plasma aldosterone levels, and urinary tetrahydroidosterone and aldosterone secretion rates were not significantly different between groups (Table 3).

**Discussion**

Our results indicate that unconjugated and conjugated 19-nor-DOC glucosiduronate levels are ele-
vated in essential hypertensive subjects compared with levels found in normal subjects. Although the pathoge-
netic role of 19-nor-DOC cannot be determined in this study, other studies have found that 19-nor-DOC ad-
ministration will produce sodium retention and hyper-
tension in rats\(^\text{16,17}\) and that 19-nor-DOC excretion cor-
relates with blood pressure in low renin essential hypertension and 17-α-hydroxylase deficiency.\(^\text{18,19}\) The unconjugated 19-nor-DOC levels found in this study are consistent with marked biological activity.

**Figure 2.** Unconjugated and conjugated urinary 19-nor-DOC glucosiduronate levels in normal subjects (Norm, \(n = 13\) and 5 respectively) and essential hypertensive subjects (HTN, \(n = 21\) and 8 respectively). *\(p < 0.05\)

**Figure 3.** Correlation of unconjugated and conjugated 19-nor-DOC glucosiduronate in normal subjects (\(n = 5\)) and essential hypertensive subjects (\(n = 5\), rho = 0.82, \(p < 0.01\)).
Compared with aldosterone, 19-nor-DOC has 140% the mineralocorticoid receptor affinity in rat renal cytosol and 19-nor-DOC is equipotent in stimulating the mineralocorticoid receptor affinity in rat renal cytosol. Extrapolating these in vitro measurements of relative mineralocorticoid activity with the in vivo levels of unconjugated urinary 19-nor-DOC (118 ± 30 ng/day) and urinary aldosterone (350 ± 125 ng/day), the mineralocorticoid activity of 19-nor-DOC relative to aldosterone was approximately 40% [(100 ng/day × 140%)/350 ng/day]. This estimate of 19-nor-DOC bioactivity, however, is speculative as only in vitro and cross-species estimates of mineralocorticoid potency are available.

The mineralocorticoid 19-nor-DOC may be among a newly recognized class of hormones that undergo peripheral biosynthesis (or modification) and metabolism at the target organ. For example, DOC, dihydrotestosterone, estrogens, 1,25-hydroxvcholecalciferol, and triiodothyronine are formed in extraglandular tissues by the actions of 21-α-hydroxylase, aromatase, 1-α-hydroxylase, and monodeiodinase respectively. The reasons for the extraglandular biosynthesis and activation of these hormones are unknown, but it can be speculated that this is biochemically efficient because it produces high concentrations of active hormone at the target tissue site. Furthermore, theoretically there are multiple control points that allow a greater regulation of hormone production. This proposal is entirely speculative for 19-nor-DOC, however, because its regulation is not entirely known. Adrenocorticotropic hormone appears to be an important activator of 19-nor-DOC production, while sodium intake and the renin-angiotensin system have comparatively lesser effects.

The metabolism of 19-nor-DOC is also under active investigation. Casey et al. infused [3H]19-nor-DOC and [14C]DOC into human subjects and found a distinct difference in their urinary metabolites. Unlike DOC, the majority of [3H]19-nor-DOC was conjugated with glucuronoside (Figure 4), and little urinary [3H]19-nor-DOC was sulfoconjugated or unconjugated. These investigators also found no interconversion of [3H]19-nor-DOC and [14C]DOC. The production rate of 19-nor-DOC, calculated from the specific activity of urinary 19-nor-DOC glucosiduronate, was approximately 10 to 16 μg/day. These observations are consistent with the idea that 19-nor-DOC is formed in the kidney by conversion of a 19-oxygenated derivative of DOC, rather than DOC itself.

In summary, this study provides evidence that elevated levels of unconjugated and conjugated 19-nor-DOC glucosiduronate occur in essential hypertension. These findings suggest that 19-nor-DOC production may be elevated in essential hypertension. This was true in some, but not all, hypertensive subjects, which probably reflects the heterogeneity of this disease.

Acknowledgments

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