Relationship of 19-Nor-Deoxycorticosterone to Other Mineralocorticoids in Low-Renin Hypertension

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SUMMARY A number of mineralocorticoids have been proposed as etiologic factors in low-renin hypertension. In this study, urinary free 19-nor-deoxycorticosterone (UF 19-nor-DOC) was compared to other mineralocorticoids — aldosterone, deoxycorticosterone (DOC), and 18-OH-DOC, in 11 low-renin hypertensive patients on a controlled diet in a metabolic unit. Results demonstrated that both UF 19-nor-DOC and tetrahydro-DOC (TH-DOC) excretion were elevated (2086 ± 926, nl = 339-579 ng/day, and 18 ± 7, nl = 5–15 mcg/day, respectively), and positively correlated (r = 0.95). Neither 18-OH-DOC nor aldosterone secretion rates were elevated, and neither of these hormones correlated with UF 19-nor-DOC, with exception of the supine plasma aldosterone (SPA) (r = 0.86). In conclusion, both UF 19-nor-DOC and TH-DOC were increased and positively correlated in the present series of hypertensives. This association is possibly indicative of a precursor-product relationship between DOC and 19-nor-DOC. 19-Nor-DOC, furthermore, correlated with supine plasma aldosterone (SPA), which could, in part, reflect their shared adrenocorticotropic hormone (ACTH) dependence.

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KEY WORDS • mineralocorticoids • aldosterone • deoxycorticosterone • urinary-free 19-nor-deoxycorticosterone • low-renin hypertension • renin • tetrahydro-deoxycorticosterone • adrenocorticotropic hormone (ACTH)

APPROXIMATELY 20% to 30% of patients with hypertension have suppressed renin,1,2 and these patients selectively respond to mineralocorticoid antagonists.3,4 This suggests that an adrenal corticosteroid may be responsible for their hypertension, and a number of mineralocorticoids have been advocated to produce this syndrome.5,6 Aldosterone is well known to be a rare but important cause of low-renin hypertension. Also, 11-deoxycorticosterone (DOC) and 18-OH-DOC are mineralocorticoids that are sometimes associated with hypertension,7-10 but not always.11-14 Both DOC and 18-OH-DOC are weak mineralocorticoids, however, and the increased levels seen in hypertensive patients described in most studies are rarely sufficient to cause hypertension.15,16 These mineralocorticoids are unique, though, since they are predominantly synthesized in the zona fasciculata and regulated to a greater degree by adrenocorticotropic hormone (ACTH) than either dietary sodium or angiotensin-II (All).17,18 It is possible that increased secretion of either DOC or 18-OH-DOC, while not responsible for the hypertension, may be a marker for another more potent mineralocorticoid directly responsible for low-renin hypertension.

19-Nor-DOC is one such candidate, since it is a potent mineralocorticoid in vitro19-21 and in vivo.22-24 19-Nor-DOC is a naturally occurring corticosteroid and is associated not only with experimental animal hypertension,25,26 but also human hypertension.27 This study undertook the evaluation of various mineralocorticoids including aldosterone, DOC, and 18-OH-DOC to 19-nor-DOC in a group of low-renin hypertensive patients. Results of this study suggest that 19-nor-DOC is associated with DOC excretion but not 18-OH-DOC or aldosterone in low-renin hypertensives.

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Materials and Methods

Subjects
This study is part of an ongoing study of mineralocorticoids in human hypertension. Informed consent was obtained from all subjects prior to the study in accordance with the institutional review board. There were 11 patients, six men who ranged in age from 31 to 57 years and in weight from 101 to 302 lbs. All patients had low-renin hypertension as defined by a plasma renin activity (PRA) < 1.3 ng Al/ml/hr after 4 hours of upright posture and after administration of furosemide, 40 mg orally. Two patients had primary aldosteronism, one patient had bilateral adrenal hyperplasia diagnosed by adrenal venous sampling and another patient had an aldosterone-producing adenoma diagnosed at operation. Mean blood pressure was calculated as the diastolic (phase 5) plus one-third of the pulse pressure. The mean blood pressure of this group was 124 ± 6 mm Hg (range 106-160 mm Hg). Upon admission to the metabolic unit, the patients had a comprehensive history taken, physical examination, and screening laboratory work to exclude secondary causes of hypertension. Plasma potassium was low in only the two patients with primary aldosterone and the aggregate mean value was 3.7 ± 0.2 (range 2.2-4.1). While in the metabolic unit, patients had 24-hour urine collections and 0800 hour supine, and 1100 hour upright, blood determinations for the various corticosteroids in this study.

Corticosteroids
PRA was measured as the generation of Al at a pH of 7.4 as previously described. The plasma aldosterone was measured by an aldosterone-γ-lactone radioimmun assay with an intra- and interassay coefficient of variation of 4.9% and 8.2%, respectively. The aldosterone secretion was measured following the administration of 3H-aldosterone and quantitation of urinary H-tetrahydroaldosterone by radioimmunoassay following derivatization and chromatography as previously described. The 18-OH-DOC secretion rates, 18-OH-tetrahydro-DOC, plasma DOC and plasma 18-OH-DOC were measured by methods previously described. The intra- and interassay coefficients of variation were 8% and 9%, respectively, and the lower limit of detection for this method is 0.2 ng/dl. The intra- and interassay coefficients of variation were 9.1% and 10.3%, respectively. Tetrahydro-DOC was measured by a modified Porter-Silber reaction following B-glucuronidase hydrolysis, methylene chloride extraction, and thin-layer chromatography by a method previously described. The recovery of labeled 3H-TH-DOC averaged 60% and normal values range from 5 to 20 mcg/day.

Measurement of 19-Nor-DOC

Materials
All solvents were of high-pressure liquid chromatography (HPLC) grade (Fisher Scientific Company, Medford, Massachusetts), with the exception of ethyl acetate, which was reagent grade quality. Standard 19-nor-DOC was a generous gift from Dr. Marcel Gut of the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts. The 1,2-3H-deoxycorticosterone (SA = 46 Cl:mmol) was obtained from New England Nuclear Corporation (Boston, Massachusetts) and was purified before use by HPLC on a Zorbax C-18 column (Du Pont) Model 850 liquid chromatographic system equipped with an ultraviolet detection sensor and a chart recorder. Radioactivity was monitored using a Packard Model 3200 liquid scintillation spectrophotometer after addition of instagel counting solution. Counting efficiency was 40%. Standard deoxy corticosterone was obtained from Steraloids (Wilton, New Hampshire).

Isolation of 19-Nor-DOC
Urine, equivalent to one-half or more of the entire 24-hour collection, was extracted twice with equal volumes of ethyl acetate after the addition of 100,000 dpm of 1,2-3H-DOC. The ethyl acetate extracted was washed successively with 1/10 volume of 0.1 N sodium hydroxide in 1/10 volume water. The organic layer was reduced to dryness in vacuo and the residue chromatographed on a 375 μ silica gel GF 254 (Brinkman Instruments) thin-layer plate using as the mobile phase chloroform/ethanol (97/3 v/v). DOC standard was applied at each edge of the plate. After development, the standard was located by ultraviolet light, and an area of the sample 0.5 cm above and 1.5 cm below the standard was eluted by suspending the silica gel in 5 ml water and extracting with 50 ml of methylene chloride. 19-Nor-DOC migrates slightly slower than DOC in this system (Rf = 0.95). The area corresponding to 19-nor-DOC was eluted as above and subjected to HPLC using 65% methanol/water as the eluting solvent. The fraction from 20 to 34 minutes was collected and reduced to dryness in vacuo. Retention time of 19-nor-DOC = 26.3 minutes, and DOC = 29 minutes.

Derivative Formation
The tritiated DOC was counted to monitor losses through the first HPLC. The 19-nor-DOC residue was acetylated overnight in acetic anhydride: pyridine (2:1) after addition of a known quantity of DOC (0.5 μg), which served as an internal standard. The acetylated material was chromatographed on silica gel using chloroform:acetone as the mobile phase. The residue was dissolved in 50 μl of methanol and subjected to HPLC using 70% methanol:water as the eluting solvent. Quantitation of 19-nor-DOC-Ac was achieved by comparison of the peak height with that of the added DOC-Ac internal standard. The limit of detection was estimated to be 50 ng/sample with radioactive recoveries averaging 40% to 50%. Values are expressed as average ng excreted per subject per day.

Urine collected from rats adrenalectomized 48 hours prior to collection was used to assess recovery data and blank values of the procedure. No ultraviolet (UV) absorbing peak migrating with 19-nor-DOC-Ac was discernible from background. Recovery of 19-nor-
19-NOR-DEOXYCORTICOSTERONE AND OTHER MINERALOCORTICOIDS/Griffing et al.

Table 1. Plasma Studies in Low Renin Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
<th>Normal range</th>
<th>r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (ng Al/ml/hr)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Supine, 0800 hours</td>
<td>0.5 ± 0.15</td>
<td>(0.1–1.6)</td>
<td>(0.6–1.2)</td>
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<tr>
<td>Upright, 1100 hours</td>
<td>0.7 ± 0.11</td>
<td>(0.2–1.3)</td>
<td>(1.3–2.2)</td>
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<tr>
<td>Post-furosemide</td>
<td>0.7 ± 0.15</td>
<td>(0.4–1.1)</td>
<td>(1.3–2.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine, 0800 hours</td>
<td>9 ± 3</td>
<td>(3–33)</td>
<td>(4–11)</td>
<td>0.86</td>
</tr>
<tr>
<td>Upright, 1100 hours</td>
<td>21 ± 5</td>
<td>(7–44)</td>
<td>(10–33)</td>
<td>0.68</td>
</tr>
<tr>
<td>Post-furosemide</td>
<td>21 ± 4</td>
<td>(11–41)</td>
<td>(30–50)</td>
<td>0.63</td>
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<tr>
<td>Plasma DOC (ng/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 hours</td>
<td>6 ± 1</td>
<td>(5–8)</td>
<td>(5–20)</td>
<td>0.34</td>
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<tr>
<td>1100 hours</td>
<td>3 ± 0.3</td>
<td>(2–3)</td>
<td>(5–20)</td>
<td>0.72</td>
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<tr>
<td>Plasma 18-OH-DOC (ng/dl)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0800 hours</td>
<td>7 ± 1</td>
<td>(5–12)</td>
<td>(3–10)</td>
<td>0.02</td>
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<tr>
<td>1100 hours</td>
<td>3 ± 1</td>
<td>(2–3)</td>
<td>(3–10)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*Correlation with UF 19-nor-DOC.

Results of this study are seen on table 1. Mean PRA was suppressed in upright and postfurosemide maneuvers, and PRA did not correlate with UF 19-nor-DOC. The corresponding plasma aldosterone (PA) determinations were also in the normal range and the supine PA was significantly correlated with UF 19-nor-DOC, as seen in figure 1. The 0800- and 1100-hour plasma DOC levels were normal, and the 1100-hour DOC correlated with UF 19-nor-DOC (r = 0.72). The plasma 18-OH-DOC was normal and did not correlate with 19-nor-DOC at 0800 or 1100 hours.

Results of secretion rates and urinary metabolite determinations are seen in table 2. The mean urinary 19-nor-DOC was distinctly elevated compared to a

DOC (1 μg) added to adrenalectomized rat urine was 88% to 93.5%. Reproducibility of the procedure from acetylation through quantitation was assessed by peak height of the added internal standard. The mean peak height measured was 202.9 ± 13.6 (sd) mm (n = 23), with a coefficient of variation of 6.6%. No difference was observed between peak height of the internal standard when analyses were made on the same day or on different days.

Statistical Analysis

Statistical analysis was performed on data obtained throughout the study and results are reported as mean ± standard error (SEM) for the study group and mean ± 1 standard deviations for the controls. Tests for significance include the paired and nonpaired Student's t test, the Mann-Whitney test, Pearson's coefficient of correlation and linear regression using the method of least mean squares. Significance is reported as a rejection of the null hypothesis at a probability of p < 0.05.

Results

Results of this study are seen on table 1. Mean PRA was suppressed in upright and postfurosemide maneuvers, and PRA did not correlate with UF 19-nor-DOC. The corresponding plasma aldosterone (PA) determinations were also in the normal range and the supine PA was significantly correlated with UF 19-nor-DOC, as seen in figure 1. The 0800- and 1100-hour plasma DOC levels were normal, and the 1100-hour DOC correlated with UF 19-nor-DOC (r = 0.72). The plasma 18-OH-DOC was normal and did not correlate with 19-nor-DOC at 0800 or 1100 hours.

Results of secretion rates and urinary metabolite determinations are seen in table 2. The mean urinary 19-nor-DOC was distinctly elevated compared to a

![Graph showing relationship of supine, 0800-hour plasma aldosterone to UF 19-nor-DOC in patients with low-renin hypertension (r = 0.86).](image-url)
normotensive population (2086 ± 926 vs 459 ± 120 ng/day). The range of urinary 19-nor-DOC values (227 to 11,137 ng/day) was extremely wide, however, with four patients having normal levels. One patient with primary aldosteronism had a normal level (587 ng/day). Urinary tetrahydro-DOC was also elevated (18 ± 0.95).

The aldosterone secretion rate was normal and did not correlate with UF 19-nor-DOC. The aldosterone secretion rate was also normal (95 ± 10 vs 100 ± 50 mcg/day) and did not correlate with UF 19-nor-DOC.

Discussion

The results of this study demonstrate a positive correlation between UF 19-nor-DOC and both tetrahydro-DOC excretion and 0800-hour supine plasma aldosterone (SPA) in 11 low-renin hypertensives. The fact that 19-nor DOC was not elevated in all of the patients in this study is consistent with the concept that low-renin hypertension is a heterogeneous syndrome with multiple etiologies. Although in the present study it is impossible to establish that 19-nor-DOC was responsible for hypertension in these patients, it is possible to extrapolate the biologic activity of the UF 19-nor-DOC with UF aldosterone in other studies. The urinary free measurement of both of these compounds should, theoretically, provide an index of biologic activity since it is the free, unbound fraction that is available for binding to mineralocorticoid receptors. The level of urinary free aldosterone in normotensive subjects is 350 ± 150 ng/day, and in primary aldosteronism is 950 ± 590 ng/day. Since the mineralocorticoid receptor affinity of 19-nor-DOC is comparable to that of aldosterone, the levels of UF 19-nor-DOC measured in the present study are likely to be biologically significant.

Tetrahydro-DOC (TH-DOC) excretion was elevated in this study, and this is consistent with previous reports that DOC is increased in some but not all low-renin hypertensives. DOC, unlike 19-nor-DOC, is a relatively weak mineralocorticoid and is seldom elevated to biologically significant levels. The increased TH-DOC in the present study is by itself unlikely to be physiologically significant, but increased DOC could be a marker for altered adrenocortical steroid synthesis. Furthermore, subtle increases in DOC can be demonstrated by ACTH-induced DOC responses which are selectively increased in low-renin hypertension.

It is not completely surprising that tetrahydro-DOC excretion should be positively correlated with UF 19-nor-DOC, since it is believed that DOC is a precursor in the synthesis of 19-nor-DOC. It is postulated at present that DOC undergoes oxidation at the C-19 position forming the sequential intermediates: 19-OH-DOC, 19-oxy-DOC, and 19-oic-DOC. One of these intermediates (probably 19-oic-DOC) is then secreted by the adrenal gland and is converted by extraadrenal, nonendocrine tissues to 19-nor-DOC.

Although 18-OH-DOC has been demonstrated to be increased in some hypertensive patients, the mean values were not elevated in the present study. Furthermore, there was a lack of correlation between 19-nor-DOC and 18-OH-DOC, suggesting that the 18-hydroxylation and 19-oxidative pathways are independent of one another and possibly regulated by different mechanisms. Even though both 18-OH-DOC and 19-nor-DOC are activated by ACTH, several investigators have demonstrated that dietary sodium intake and All stimulation significantly influenced 18-OH-DOC but we have not been able to demonstrate a sodium-dependent regulation for 19-nor-DOC. These observations suggest that 18-OH-DOC and 19-nor-DOC secretion may be regulated by different factors, and this could account for the lack of association in the present study.

The aldosterone secretion rate was normal and not reduced in the present study despite a low PRA and increased UF 19-nor-DOC. Although the mechanism for maintaining normal aldosterone secretion in this syndrome is unknown, it may be related to either adrenal All hypersensitivity, or possibly non-All secretagogues related to ACTH. ACTH itself may have a role in the correlation between SPA and UF 19-nor-DOC since both are strongly associated with ACTH secretion. ACTH excess, however, is unlikely to account for the elevated UF 19-nor-DOC since none of the patients had either clinical hypercortisolism or elevated urinary free cortisol or metabolites (five patients).

In conclusion, low-renin hypertensive patients in the present study demonstrated increased UF 19-nor-DOC and TH-DOC levels which were positively correlated, but 18-OH-DOC and aldosterone secretion were normal and not correlated with UF 19-nor-DOC. The concomitant increase and correlation of UF 19-nor-DOC and TH-DOC suggest a shared biosynthetic pathway which may be augmented in some patients with low-renin hypertension.
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References

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