Urinary Free and Serum 19-Nor-Deoxycorticosterone in Adrenal Regeneration Hypertension

Celso E. Gomez-Sanchez, M.D., Elise P. Gomez-Sanchez, D.V.M., Ph.D., Robert J. Upcavage, B.S., and Edwin B. Hall

SUMMARY Adrenal regeneration hypertension (ARH) is caused by increased secretion of mineralocorticoids. Deoxycorticosterone (DOC), which has been found in increased concentrations in the blood of rats with ARH after the second week, must play an important role in the pathogenesis of ARH. However, the increased sodium reabsorption early in the regeneration suggests that another steroid might also play a role in ARH. We previously isolated 19-nor-deoxycorticosterone (19-nor-DOC), a mineralocorticoid with two to five times the potency of DOC, from the urine of rats with regenerating adrenals. In this study, ARH was produced, and the urinary excretions of free DOC and 19-nor-DOC and serum 19-nor-DOC were measured on the 23rd day of regeneration, when the systolic blood pressure of the ARH rats was 172 ± 5 mm Hg (SEM, n = 12) in comparison to 129 ± 4 mm Hg for the controls. Urine excretion of 19-nor-DOC was increased from 0.9 ± 0.1 ng/day in controls to 2.3 ± 0.6 ng/day in ARH for DOC and from 5.0 ± 1.1 ng/day in controls to 7.9 ± 1.4 ng/day in ARH for 19-nor-DOC. Serum 19-nor-DOC was undetectable in both groups. These studies suggest that increased DOC might play an important role in ARH by serving as the initial substrate for the formation of a more powerful mineralocorticoid, 19-nor-DOC, as well as by its own mineralocorticoid activity. However, for 19-nor-DOC to be important it would have to be formed from a precursor at the target organ, since it does not seem to be a circulating steroid. (Hypertension 5 (supp I): I-32–I-34, 1983)

KEY WORDS • adrenal regeneration hypertension • deoxycorticosterone • 19-nor-deoxycorticosterone • hypertension • mineralocorticoids

Adrenal regeneration hypertension, first described by Skelton,1 is believed to be due to excessive secretion of a mineralocorticoid that occurs as a consequence of adrenal disruption and subsequent regeneration. Most known mineralocorticoids have been measured and found to be either normal or below normal.2 One exception is DOC, which investigators have described as elevated in the plasma,3,4 even though DOC production is low in the adrenal incubates from ARH animals at the same stage.4,5 The acceptance that DOC is the sole causative factor for ARH has not been uniform. Hall and Hall4 have proposed that the inconsistent finding of saline polydipsia argues against DOC being the cause of ARH. In addition, during the first week of ARH when DOC production and plasma concentrations are low,3 there is evidence of a salt-retaining factor of adrenal origin4,6 of unknown identity.

We have identified a powerful mineralocorticoid, 19-nor-deoxycorticosterone (19-nor-DOC), in the urine of rats with ARH and have recently developed a method to measure the urinary excretion of 19-nor-DOC in rats. We are reporting our results of the measurements of free 19-nor-DOC and DOC in the urine and 19-nor-DOC in the serum of rats with adrenal regeneration hypertension.

Materials and Methods

Male Sprague Dawley rats weighing 100–120 g were obtained from Holtzman Farms (Madison, Wisconsin) and housed in animal quarters at 23°C with a 12-hour light-dark cycle (light, 600–1800). After 1 week of acclimatization, two groups of 12 rats underwent right adrenalectomy and nephrectomy and, one group, left adrenal enucleation. The rats were maintained on standard lab chow and 1% sodium chloride drinking water. Indirect blood pressures3 and urine output were measured weekly. Urine from the 23rd day was used for the steroid measurements. This urine was collected in beakers containing 10 mg of sodium azide to minimize bacterial contamination. At the end of the experiment, rats were sacrificed by decapitation under quiescent conditions at 1700. Blood was collect-
ed, allowed to clot, and centrifuged, and the serum was stored at -60°C until assayed. This time of collection was elected because we have shown that it approximates the time of the peak of the adrenal circadian rhythm.12

Assay Methods
Free DOC and 19-nor-DOC were measured in the urine using a radioimmunoassay technique (Gomez-Sanchez CE et al., unpublished data, 1983). In short, to one-fifth of a 24-hour urine collection, 3000 dpm of HPLC purified (1,2-3H)-DOC and (1,2-3H) 19-nor-DOC were added for estimation of recoveries. The urine was extracted with dichloromethane. This extract was evaporated, redissolved in heptane:isopropanol, and purified by HPLC using a Lichrosorb diol column (5 μ, 0.4 × 25 cm), and eluted with a gradient of 5% to 10% isopropanol in heptane. The areas corresponding to DOC and 19-nor-DOC were used for radioimmunoassay determinations using relatively specific antibodies previously described.10-13 The average recovery of (1,2-3H) DOC and (1,2-3H) 19-nor-DOC from the whole procedure was 53% ± 4% and 50.7% ± 4.9% respectively. Intraassay variability was 9.8% and 10%, and interassay variability was 19% and 16.6%, for DOC and 19-nor-DOC respectively. Accuracy was measured by adding 5, 10, and 20 ng to 20 ml of urine of DOC and 19-nor-DOC and subjecting it to the procedure. The average recovery was 94% ± 4% (sd). The blanks were low and indistinguishable from zero. The sensitivity of the standard curve was 3 pg (2 so from the zero point). Serum 19-nor-DOC measured using the same radioimmunoassay. Serum corticosterone was measured by radioimmunoassay.14 Statistical evaluation was done by the Student t test for unpaired samples.

<table>
<thead>
<tr>
<th>TABLE 1. Fluid Intake and Systolic Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Urine output (ml/day)</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
</tr>
</tbody>
</table>

*Values are means ± SEM.
*p < 0.01.
TABLE 2. Steroid Measurements in Adrenal Regeneration Hypertension (ARH)

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Control</th>
<th>ARH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum corticosterone (μg/dl)</td>
<td>10.6 ± 1.3</td>
<td>8.2 ± 0.7</td>
</tr>
<tr>
<td>Urinary free DOC (ng/day)</td>
<td>0.9 ± 0.1</td>
<td>2.3 ± 0.6*</td>
</tr>
<tr>
<td>Urinary free 19-nor-DOC (ng/day)</td>
<td>5.0 ± 1.1</td>
<td>7.9 ± 1.4†</td>
</tr>
</tbody>
</table>

*p < 0.01.
†p < 0.05 < p < 0.06.

undetectable concentrations of 19-nor-DOC in the serum clearly shows that this is not a circulating steroid. In order for 19-nor-DOC to play a role in the pathogenesis of ARH, the conversion from a precursor (probably 19-oic-deoxycorticosterone) to 19-nor-DOC would have to occur in the target organ, the kidney. This would explain the presence of undetectable circulating levels of 19-nor-DOC, while clearly detectable levels of this steroid are found in the urine.

The mechanisms and relative quantitative contributions of both steroids to ARH remains to be elucidated. Dale et al.19 have recently shown that 19-nor-DOC excretions are elevated early in the development of hypertension in the spontaneously hypertensive rat. Further studies will be needed to establish the temporal relationship between 19-nor-DOC formation and ARH. It might be possible that the saline polydipsia and increased sodium reabsorption during the first few days of ARH could be explained by 19-nor-DOC production directly at the target organs.

Acknowledgments

The expert secretarial help of Ruby Choquette is gratefully acknowledged.

References

2. Gallant S, Brownie AC: Peripheral blood levels of corticosterone, 11-deoxycorticosterone and 18-hydroxy-11-deoxycorticosterone during the development of adrenal regeneration hypertension. Studies carried out at the high and low point of the circadian rhythm. Life Sci 24: 1097, 1979
Urinary free and serum 19-nor-deoxycorticosterone in adrenal regeneration hypertension.
C E Gomez-Sanchez, E P Gomez-Sanchez, R J Upcavage and E B Hall

Hypertension. 1983;5:I32
doi: 10.1161/01.HYP.5.2_Pt_2.I32

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/2_Pt_2/I32.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at: http://hyper.ahajournals.org//subscriptions/