Absence of renin suppression by deoxycorticosterone acetate in rats

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GOODWIN, FRANK J., ABBIE I. KNOWLTON, AND JOHN H. LARAGH. Absence of renin suppression by deoxycorticosterone acetate in rats. Am. J. Physiol. 216(6): 1476-1480. 1969.—The influence of deoxycorticosterone acetate (DCA) on renin release was studied in rats. DCA-treated and normal control groups of animals were maintained on either sodium-free or sodium-rich diets for 6 weeks. In the normal rat, sodium deprivation induced a significant increase in peripheral plasma renin activity. Concurrent administration of DCA did not influence this response. A high-sodium diet alone failed to suppress peripheral plasma renin activity: in this respect the rat appears to differ strikingly from man. The combination of this diet and DCA reduced plasma renin activity to almost undetectable levels: this suppression appeared to be caused not as a result of increased levels of the hormone per se, but rather as an indirect consequence of mineralocorticoid-induced changes in sodium metabolism. It is concluded that in the absence of available dietary sodium, DCA exerts no influence upon renin release from the juxtaglomerular apparatus.

suppression of plasma renin activity; mineralocorticoid; renin and sodium balance

This study was undertaken to investigate further the mechanism by which mineralocorticoid hormones influence the control of renin secretion. In 1956 (17, 18) the administration of large doses of deoxycorticosterone acetate (DCA) was shown to reduce the renal content of pressor substance, presumably renin, to undetectable levels. More recently it has been demonstrated that patients with aldosterone-secreting tumors have very low or zero levels of circulating renin activity (7) and little or no rise in plasma renin activity occurs in response to dietary sodium restriction. Furthermore, the use of plasma renin activity has been advocated as a diagnostic aid in distinguishing between cases of primary and secondary hyperaldosteronism (6). The interrelationship between renin and aldosterone is of great interest in view of the uncertainty concerning the nature of the stimuli regulating renin release. Previous studies (13, 17) have attempted to distinguish between a direct inhibitory feedback mechanism of mineralocorticoid upon the juxtaglomerular apparatus and an indirect mechanism whereby renin is in some way suppressed by the accompanying sodium retention. The present experiment was designed to elucidate further this problem, by separating the direct and sodium-retaining properties of DCA and observing their effects upon renin release in the rat.

METHODS

Thirty-eight female Sprague-Dawley albino rats, each weighing approximately 150 g, were randomly divided into four groups. Two groups of twelve rats each were treated by subcutaneous implantation of DCA pellets; two groups of seven rats each served as controls and received no DCA. One treated and one control group received a sodium-free diet; one treated and one control group received a high-sodium diet. The groups were therefore labeled group a, DCA, Na free; group b, DCA, high Na; group c, control, Na free; and group d, control, high Na. Prior to the experiment the animals had been maintained on a Purina rat chow containing 0.42 % of sodium and had free access to tap water. Systolic blood pressure was measured once weekly throughout the experiment by the micromanometer tail method (12), the animals remaining conscious and at rest; body weight was measured at the same time.

Protocol

Day 0. Under light ether anesthesia, 1 ml of blood was drawn from the jugular vein through a small skin incision into a polyethylene syringe fitted with a no. 22-gauge needle, the lumen of which was filled with 0.006 M EDTA in isotonic saline. The syringes were centrifuged immediately at 4 C, the plasma separated and stored in polyethylene vials at -20 C until required for the determination of plasma renin activity. Two 25-mg pellets of DCA were then implanted subcutaneously into each rat in groups a and b. All rats were then given a sodium-free diet (19) (General Biochemicals); groups...
a and c received distilled water to drink, groups b and d 0.9% saline; this regimen was continued until the end of the experiment, 45 days later. In a preliminary study, mean daily urinary sodium excretion rates ± SE in microequivalents per 24 hr per gram body weight of groups of rats maintained for 2 weeks on a) this diet plus distilled water, b) the same diet plus 0.9% saline, and c) Purina chow plus tap water, were a) 0.06 ± 0.00 (N = 5), b) 15.20 ± 1.7 (N = 6), and c) 5.69 ± 0.65 (N = 6), respectively. In the present experiment, therefore, the sodium intake of groups b and d while maintained on the sodium-free diet plus isotonic saline was several times greater than that before day 0, when the animals received Purina chow plus tap water.

Day 17. Each rat was bled a second time.

Day 15. Under light ether anesthesia as before, the rats were bled rapidly from the aortic bifurcation into empty syringes: each blood sample was divided into two parts, the first of which was anticoagulated with EDTA for the determination of plasma renin activity, the second with lithium heparin for the measurement of plasma electrolyte concentrations. These samples were centrifuged and stored as before. The adrenal glands, kidneys, and heart were removed from each rat, cleaned and weighed, the weights being expressed as a percentage of the animal’s body weight.

**Measurement of Plasma Renin Activity**

The method employed in this experiment is a simplified modification of a previous approach (14) in which renin substrate is added in excess to the incubation mixture. However, in the present method the need for dialysis of the plasma samples was obviated, since boiled unincubated samples exhibited no detectable pressor activity.

To 0.1 ml of plasma collected in EDTA were added 0.55 ml of isotonic saline, 0.1 ml of 0.001 M picrylmercuric acetate, 0.1 ml of a solution containing 0.2 M maleic acid and 0.02 M EDTA, 0.15 ml of purified hog renin substrate preparation, and one drop of diisopropyl fluorophosphate (DFP) which had been diluted 20 times in isopropyl alcohol to further inhibit angiotensinase activity. The mixture was incubated at pH 6.5 for 24 hr at 37°C and the reaction stopped by placing the tubes on ice. The pH was then lowered to 5.5 with 0.25 N HCl and the tubes placed in a boiling water bath for 10 min. After centrifugation the supernatant was frozen at −20°C until assay in pentobarbitone-anesthetized ganglion-blocked rats. The pressor response to the unknown samples was compared with that to a standard preparation of angiotensin II amide (Hypertensin-Ciba) and plasma renin activity expressed as nanograms of angiotensin generated per milliliter of plasma per hour of incubation. The final concentration of renin substrate in the incubation mixture was equivalent to approximately 1,800 ng angiotensin/ml, as determined by incubation with an excess of rat renin. Renin activity is therefore measured under conditions in which substrate concentration can be shown not to be rate limiting, as it may be in vivo in the rat. Measured and expressed in this way, renin activity more closely approaches the true circulating renin concentration (3).

For the present studies the optimal pH for the reaction between rat renin and hog substrate was found to be 6.5. Zero-order kinetics and complete inhibition of angiotensinase during the incubation was assured by demonstration of a linear rate of angiotensin production and by studies demonstrating the recovery of 90–110% angiotensin added to the incubation mixture. The lower limit of sensitivity of the method was 0.5 ng angiotensin/ml per hr of incubation.

**Plasma Electrolytes**

Plasma sodium and potassium concentrations were measured by flame photometry, using an internal lithium standard.

**Analysis of Results**

All the data in this experiment, i.e., body and organ weights, plasma renin activity, electrolyte concentrations and blood pressure were evaluated by the method of analysis of variance. The analysis of the measurements of plasma renin activity was performed after logarithmic transformation, in view of the marked dissimilarity between the variances of the groups on high- and low-sodium diets. In comparing the means of observations between selected pairs of groups of animals, the significance of the differences was evaluated by the methods recommended by Scheffe (24) and Dunn (10). Statistical significance was defined as P < 0.05.

**RESULTS**

**Body Weight**

On day 0 there was no significant difference in mean body weight of the four groups of rats. The mean weight gain of each group during the 45 days of the experiment is shown in Table 1. There was no significant difference in weight gain between the two groups deprived of dietary sodium nor between those given saline to drink. The difference in weight gain between the two DCA treated groups was highly significant (P < 0.01) as also was the difference between the control groups (P < 0.01). Therefore the administration of DCA had no influence on growth rate, but sodium deprivation markedly slowed and later halted growth regardless of the presence or absence of DCA. Rats given saline to drink appeared to grow normally.

**Blood Pressure**

The mean systolic blood pressures in each group are shown in Table 2. During the first 2 weeks there were no
TABLE 1. Effect of DCA on body weight gain, adrenal, heart, and kidney weights, and plasma sodium and potassium concentrations in rats maintained on sodium-free and high-sodium diets

<table>
<thead>
<tr>
<th>Group</th>
<th>DCA, Na Free</th>
<th>DCA, High Na</th>
<th>Control, Na Free</th>
<th>Control, High Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mean weight gain, g</td>
<td>52.8 ± 3.6</td>
<td>152.7 ± 5.8</td>
<td>58.0 ± 3.0</td>
<td>170.9 ± 0.5</td>
</tr>
<tr>
<td>Mean adrenal weight, % body wt</td>
<td>1.06 ± 0.07</td>
<td>0.80 ± 0.04</td>
<td>1.94 ± 0.12</td>
<td>0.90 ± 0.08</td>
</tr>
<tr>
<td>Mean heart weight, % body wt</td>
<td>3.08 ± 0.07</td>
<td>3.61 ± 0.09</td>
<td>3.36 ± 0.16</td>
<td>3.00 ± 0.03</td>
</tr>
<tr>
<td>Mean kidney weight, % body wt</td>
<td>7.07 ± 0.02</td>
<td>9.22 ± 0.09</td>
<td>7.37 ± 0.16</td>
<td>6.34 ± 0.03</td>
</tr>
<tr>
<td>Mean plasma sodium concn, mEq/liter</td>
<td>129 ± 0.5</td>
<td>135.5 ± 0.6</td>
<td>130.0 ± 0.6</td>
<td>133.0 ± 1.0</td>
</tr>
<tr>
<td>Mean plasma potassium concn, mEq/liter</td>
<td>5.3 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>6.1 ± 0.4</td>
<td>5.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± se.

TABLE 2. Effect of DCA on blood pressure measured at intervals throughout the experiment in rats maintained on sodium-free and high-sodium diets

<table>
<thead>
<tr>
<th>Group</th>
<th>DCA, Na Free, N = 12</th>
<th>DCA, High Na, N = 12</th>
<th>Control, Na Free, N = 7</th>
<th>Control, High Na, N = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>135 ± 2.9</td>
<td>138 ± 4.3</td>
<td>132 ± 3.7</td>
<td>133 ± 5.8</td>
</tr>
<tr>
<td>Day 14</td>
<td>132 ± 2.2</td>
<td>129 ± 2.2</td>
<td>130 ± 2.5</td>
<td>127 ± 4.5</td>
</tr>
<tr>
<td>Day 27</td>
<td>136 ± 2.5</td>
<td>164 ± 4.9</td>
<td>136 ± 3.5</td>
<td>115 ± 3.9</td>
</tr>
<tr>
<td>Day 41</td>
<td>137 ± 2.5</td>
<td>189 ± 6.4</td>
<td>139 ± 2.4</td>
<td>125 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± se in millimeters Hg.

significant differences in blood pressure between the four groups, but thereafter the DCA-treated rats given saline to drink developed hypertension and by day 27 the mean systolic blood pressure in this group was 164 ± 4.9 se mm Hg, significantly (P < 0.01) elevated above that on day 0. On day 41 the mean systolic blood pressure in this group had risen further to 189 ± 6.4 se mm Hg; between the other three groups there were still no significant differences in blood pressures, which remained normal.

Renin

Plasma renin activity was measured in each rat on days 0, 17, 31, and 45 (Table 3 and Fig. 1). On day 0, the animals having been maintained on a diet containing 0.42 % sodium, mean plasma renin activity of all 38 rats was 4.4 ± 0.6 ng/ml per hr (mean ± se). The mean plasma renin activities of individual groups are shown in the table. Although on day 0 there were minor differences in mean plasma renin activity between the four groups, these were not statistically significant. On day 17, both the DCA-treated and control groups on the sodium-free diet showed a marked rise in mean plasma renin activity, compared with the groups which drank saline. In both former groups mean plasma renin activity continued to rise until day 45, when the experiment was terminated. The control, high-Na group showed no significant change in mean plasma renin activity throughout the experiment, but mean plasma renin activity in the DCA, high-Na group showed a significant downward trend, 7 out of the 12 rats having no demonstrable plasma renin activity on day 45; the trends in plasma renin activity of these two groups throughout the experiment were significantly different (P < 0.01). There was, however, no significant difference between the trends of mean plasma renin activity throughout the experiment in the two groups on a sodium-free diet. The trends of the two groups on the sodium-free diet were highly significantly different from those of the groups which drank saline (P < 0.01).

Organ Weights

In view of the difference in growth rate between the rats on the two diets, organ weights have been expressed as a percentage of body weight multiplied for convenience by a factor of 100 for the adrenal glands, and by a factor of 10 for the heart and combined kidneys. The results are shown in Table 1. Neither the adrenal hypertrophy which occurred in animals deprived of sodium, nor the atrophy occurring in those which drank saline, were significantly influenced by DCA. However, the
differences in mean adrenal weight between the groups on different diets were highly significant ($P < 0.01$). In only the DCA, high-Na group did significant cardiac hypertrophy occur compared with the other groups ($P < 0.01$), between which there were no significant differences in heart weight. The hypertensive DCA group with cardiac hypertrophy also showed a marked increase in kidney weight compared with the other groups ($P < 0.01$), between which there were no significant differences.

**Plasma Electrolytes**

Plasma concentrations of sodium and potassium were measured in each animal on day 45 (Table 1). The mean plasma sodium concentration of the DCA, high-Na group was significantly higher ($P < 0.01$) than that of the DCA-treated group deprived of sodium; the difference between the two control groups was not significant. Plasma potassium concentration was markedly and significantly lowered in the DCA, high-Na group compared with that in the other three groups ($P < 0.01$), between which there were no significant differences in potassium concentrations.

**DISCUSSION**

In 1941, Carnes et al. (4) demonstrated that prolonged administration of DCA in large doses to rats produced adrenocortical atrophy, with nearly complete disappearance of the heavy osmiophilic deposit in the peripheral zone of the gland. This was confirmed in 1947 by Greep and Deane (15) who suggested that DCA might maintain the normal equilibrium of sodium, depletion of which stimulated the zona glomerulosa to secrete mineralocorticoid. This was strongly supported by Knowlton et al. (22) who showed that the adrenal atrophy and glomerulosal depletion seen after the administration of DCA to rats on a normal sodium diet was prevented in rats maintained on a diet low in sodium. This was early evidence against a direct feedback action of DCA on the adrenal cortex and supported the view that the adrenocortical suppression caused by DCA was in some way related to its ability to retain sodium. In 1949 Dumaille (9) showed that DCA prevented or reversed the abnormal increase in renal juxtaglomerular cell granularity which otherwise occurred in adrenalectomized rats and monkeys. Subsequently Harrold and Harrold (20) showed in rats that the granularity was dependent upon dietary sodium intake; the accumulation of granules which occurred in animals maintained for 5–6 weeks on a low-salt diet was unaffected by the concurrent administration of DCA; degranulation occurred in rats given 2% saline to drink and was accentuated if DCA were given also. Similar experiments performed on rats demonstrated that overdosage with either DCA (17, 18) or aldosterone (16) caused renin to disappear from the kidneys, but that the effect could be prevented by dietary sodium restriction. Using the isovolemic cross-circulation technique, Rondell (23) showed that pressor substances, i.e., renin, were absent from the plasma of rats pretreated with DCA.

In this experiment peripheral plasma renin activity was measured in DCA-treated and control rats maintained on diets deficient or high in sodium. It was shown that large doses of DCA in no way prevented the marked increase in circulating renin activity which followed sodium deprivation, making unlikely the possibility that DCA has a direct inhibitory effect upon renin secretion. The fact that plasma renin activity continued to rise until the experiment was terminated may have been due to the influence of continued slightly negative sodium balance throughout the study owing to the extreme severity of sodium deprivation. Metabolic balance studies were not carried out in these experiments but it is unlikely that during the 6 week period of study there was any appreciable difference in sodium conservation between the two groups on the sodium-free diet; weight gain was identical in both groups, and we have previously shown that after 2 weeks on this diet the urine of normal rats is virtually devoid of sodium. Not only did DCA fail to suppress plasma renin activity in sodium-deprived animals, but it also failed to elicit the usual hypokalemia, renal hypertrophy, hypertension, and cardiac hypertrophy observed when sodium is provided in the diet.

The failure of a high-sodium diet to depress plasma renin activity in untreated animals in this experiment is in agreement with another study (11) in which the renin content of the kidneys of normal rats given a high-sodium diet for 2 weeks was also unaffected. In this respect the rat differs strikingly from man, in whom a high-sodium diet causes plasma renin activity to fall to a low level (2). In the present study, however, the combination of a high-sodium diet and DCA caused marked depression or even disappearance of plasma renin activity. Although direct evidence of sodium retention from balance studies was not obtained, and no significant difference in weight gain between the two groups on a high-sodium diet was demonstrated, the dependence of the effect of DCA in suppressing plasma renin activity upon available dietary sodium is striking. The fact that DCA suppressed plasma renin activity when dietary sodium was available, but was ineffective in doing so when it was not, is strong indirect evidence that renin suppression was in some way sodium dependent and not due to a direct inhibitory effect of DCA itself. Evidence that DCA enhanced sodium retention in the group given a high-sodium diet is the demonstration (21) that the carcasses of adrenalectomized rats made hypertensive with DCA and salt loading contained more sodium than did intact controls fed a similar diet. It is conceivable that the hypertension itself suppressed renin release; however, although not achieving statistical significance, probably owing to the low levels of plasma renin activity being measured in this group and to the small number of animals studied, the data suggest that plasma renin activity had already fallen by day 17, at which time the blood pressure had not yet risen significantly. At this time, 7 out of the 12 rats in this group already had no detectable plasma renin activity.

Geelhoed and Vander (13) in a somewhat similar study
in dogs, showed that when steroid-induced changes in sodium balance were prevented by dietary sodium restriction, aldosterone given either acutely or for 5 days produced no change in plasma renin activity. Other workers (3, 8) have shown that infusions of aldosterone fail to influence renin release acutely. These, as well as our own more prolonged experiments confirm the absence of a direct action of mineralocorticoid hormones on renin release.

In the present study renin activity was measured in the presence of exogenous renin substrate, suggesting that the true circulating renin concentration was probably suppressed by the effect of DCA and salt. The design of this approach rules out the possibility of a DCA-induced suppression of substrate concentration as a factor in reducing renin activity. A previous report (5) also suggests that DCA and sodium do not markedly influence circulating renin substrate levels. The possibility still remains that the suppression of renin by the combined administration of DCA and salt might have resulted from the suppression of an activator or the stimulation of an inhibitor of renin (25).

In view of these findings it would be anticipated that it should be possible to raise plasma renin activity in cases of primary aldosteronism by vigorous sodium depletion. Conn et al. (7) failed to demonstrate a rise in plasma renin activity in three patients with undetectable levels after severe restriction of sodium for as long as 16 days. However, it has been shown more recently (1) that in some cases of primary aldosteronism, plasma renin activity does rise during sodium depletion; in four patients given 10 or 20 mEq of sodium daily for 5–8 days, plasma renin concentration rose and in one case was comparable to that seen in two normal subjects under similar dietary restriction. These observations, which are in accord with the results of this experiment, show that even in the presence of high levels of circulating aldosterone, plasma renin activity may be freely manipulated by means of changes in sodium balance, although in many cases of primary aldosteronism, possibly because of prolonged suppression of the juxtaglomerular cells, such changes may have to be greater than those producing similar alterations in plasma renin activity in normal subjects.

We are greatly indebted to Dr. Agnes P. Berger and Mrs. Livia R. Turgeon of the Division of Biostatistics, School of Public Health and Administrative Medicine, Columbia University, for their advice on the statistical analysis of the data. The authors also thank Dr. Augustus Gibson of the Schering Co., Bloomfield, New Jersey, for supplying the DGA pellets and Dr. A. B. Gould who generously supplied the hog renin substrate.

This work was supported by Public Health Service Grants HE-01275 and HE-05741 from the National Institutes of Health.

F. J. Goodwin was supported, in part, by a Lilly International Fellowship.

Received for publication 28 October 1968.

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