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Deoxycorticosterone Acetate-Induced Renin Suppression in the Absence of Antidiuretic Hormone (40948)

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Abstract. Sprague–Dawley rats and Brattleboro rats homozygous and heterozygous for hereditary diabetes insipidus were treated with deoxycorticosterone acetate (DOCA; 100 mg/kg) for periods of up to 2 weeks and the activities of renin in plasma (PRA) and kidney tissue (RRA) were measured. PRA decreased dramatically in all groups within 4 days of DOCA treatment, suggesting that the presence of antidiuretic hormone (ADH) is not necessary for DOCA-induced renin suppression. After 11 and 14 days of treatment, PRA was suppressed less in the homozygous diabetes insipidus rats than in the other two groups, suggesting that the lack of ADH alters the response of PRA to DOCA treatment in the chronic state. RRA was not changed by DOCA treatment in any group, thus demonstrating that the initial response of the renin–angiotensin system to DOCA treatment is a suppression of secretion. These results suggest that the initial response of the renin–angiotensin system to DOCA is not dependent on the presence of ADH.

Treatment of dogs (1) and rats (2, 3) with deoxycorticosterone acetate (DOCA) and diets high in sodium is known to deplete both the plasma and the renal activity of renin. Although the mechanism by which this treatment regime suppresses renin is not known, it is doubtful that the increase in arterial pressure normally associated with this treatment is of critical importance. In rats, the development of increased blood pressure may require as long as 3 weeks of treatment (2), while renin suppression in the plasma is evident within days after initiation of DOCA (4). In addition, we have recently observed that in two-kidney, one-clip Goldblatt dogs, renin depletion following DOCA administration is equivalent in both the clipped and the untouched kidneys, indicating that the pressure differential caused by the clamp had no effect on the renal renin response to DOCA (1). Möhring *et al.* (5, 6) have described increases in the plasma concentration of antidiuretic hormone (ADH) in rats made hypertensive with DOCA/sodium treatment. Since ADH is known to suppress the secretion of renin when infused acutely (7, 8), we have examined the possibility that elevated plasma levels of ADH may contribute to renin suppression in DOCA-treated rats.

Methods. Male and female Sprague–Dawley rats (190–265 g) and rats (140–331 g) of the Brattleboro strain that were either heterozygous (Di/di) or homozygous (di/di) for hereditary diabetes insipidus were used in these studies. The Brattleboro rats were bred in the Animal Research Facility at the University of Michigan and were differentiated after weaning according to their water intake. Five- to six-week-old rats were placed in individual cages. If they drank 80 ml of water or more per day for 3 consecutive days they were considered homozygous.

Rats were anesthetized with ether and weighed. Silastic rubber strips impregnated with DOCA (Sigma Chemical Co.) which were fashioned after the technique of Terris *et al.* (9) were implanted subcutaneously near the dorsal right rib cage and the wounds were closed with clips. The dose of DOCA in the implants was approximately 100 mg/kg body wt. After recovery, the rats were allowed free access to normal rat chow and tap water. Deoxycorticosterone (DOC) levels in three control rats averaged 34.9 ng/dl. Two rats were killed 4 days after implant and two after 15 days. Their DOC

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levels averaged 2010 and 2060 ng/dl, respectively.

After periods of 3 or 4, 7, 11, and 14 days, rats were killed by decapitation and blood was collected into chilled centrifuge tubes using EDTA as an anticoagulant. The blood was centrifuged and plasma was removed and frozen (−20°) for later analysis of plasma renin activity (PRA). The left kidney was removed, cleared of perirenal tissue, decapsulated, and frozen (−20°) for later analysis of renal renin activity (RRA). Unimplanted rats of the Sprague–Dawley, heterozygous, and homozygous groups served as controls.

Plasma samples were assayed for PRA using the timed generation of angiotensin I after the technique of Haber *et al.* (10) using a commercial radioimmunoassay kit (New England Nuclear). Kidneys were homogenized in ice-cold distilled water and the homogenate was centrifuged. RRA was determined on the supernatant of the homogenate using the radioimmunoassay for generation of angiotensin I from substrate derived from 72-hr nephrectomized dogs as previously described (11). PRA was expressed as ng AI generated/ml plasma × hr incubation or as a percentage of the average value derived from unimplanted rats. RRA was expressed as ng AI generated/mg kidney × hr incubation or as a percentage of the average value derived from unimplanted rats. DOC levels were measured by radioimmunoassay (12).

Differences between the absolute values of RRA and PRA in the three groups or between percentage remaining RRA and PRA in the three groups were assessed by one-way analysis of variance. If significant differences were found using this analysis, the group(s) responsible for the significance were determined by the Newman–Keuls test. Significance was taken to be a P value of less than 0.05.

Results. The control values for both PRA and RRA were significantly higher in the homozygous diabetes insipidus rats than in both their heterozygous littermates and Sprague–Dawley rats. As shown in Table 1, PRA was about 90% higher in the homozygous rats than the heterozygous Brattleboro and the Sprague–Dawley rats. RRA was about 60% higher in the homozygous than the heterozygous rats while it was about 115% higher than in the Sprague–Dawley strain. Although the RRA of the heterozygous rats was higher than Sprague–Dawley, this difference was not significant.

In spite of the control differences in PRA between the three groups of rats, treatment with DOCA resulted in a dramatic and rapid depression of PRA within 4 days in all groups. In Fig. 1 it can be seen that in 3 days, PRA of Sprague–Dawley rats had fallen to 56% of control and the PRA of Brattleboro rats at 4 days had fallen to 40 and 23% of control in the homozygous and heterozygous rats, respectively. For the remaining 10 or 11 days, PRA dropped more slowly in all groups, reaching final values of 8.7, 9.1, and 33.7% of control for Sprague–Dawley, heterozygous, and homozygous diabetes insipidus rats, respectively. The percentage remaining PRA of the homozygous diabetes insipidus rats was significantly higher than the other two groups at 11 and 14 days after the beginning of DOCA treatment. The PRA of the heterozygous rats was depressed below that of Sprague–Dawley rats at 7 days of

TABLE I. CONTROL VALUES OF RENIN ACTIVITY IN PLASMA AND RENAL TISSUE OF CONTROL RATS AND RATS HETERO- AND HOMOZYGOUS FOR DIABETES INSIPIDUS

	Plasma renin activity (ng AI/ ml × hr)	Renal renin activity (ng AI/ mg × hr)
Control Sprague–Dawley	10.3 ± 1.5 (9)	41.6 ± 2.9 (9)
Heterozygous (Di/di)	9.9 ± 0.7 (9)	57.4 ± 3.3 (9)
Homozygous (di/di)	18.7 ± 1.9 (6)*	90.1 ± 10.7 (8)*

Note. Values given are means ± 1 SEM. The numbers in parentheses are the number of animals in each group. A single asterisk (*) denotes significantly different from control Sprague–Dawley and heterozygous (Di/di) diabetes insipidus rats, $P < 0.01$.

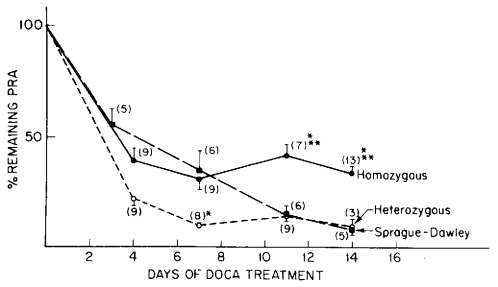

FIG. 1. Percentage remaining plasma renin activity (PRA) as a function of days on treatment with deoxycorticosterone acetate (DOCA; 100 mg/kg). Represented are means ± SEM. Numbers in parentheses are number of rats at each time period. Single asterisk (*) denotes significantly different from control Sprague–Dawley rats, $P < 0.01$. Double asterisk (**) denotes significantly different from heterozygous (Di/di) diabetes insipidius rats, $P < 0.01$.

treatment, but this difference disappeared with continued treatment length.

The effect of DOCA on RRA was less dramatic than its effect on PRA. RRA was not changed by DOCA in any of the groups studied for any length of the experiments. The percentage remaining RRA as a function of days of treatment is shown in Fig. 2. In Sprague–Dawley rats, there was an initial elevation in RRA 3 days after implantation. Although the reason for this elevation is not known, it may be related to surgical trauma. No elevation in RRA was seen at 4 days in the Brattleboro rats, and there were no significant differences between groups

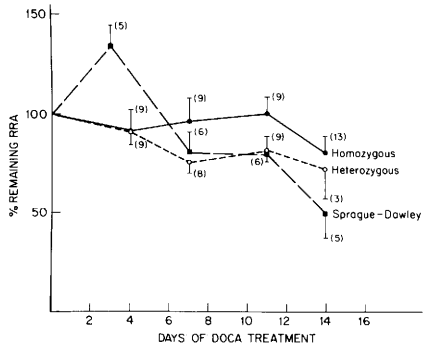

FIG. 2. Percentage remaining renal renin activity (RRA) as a function of days on treatment with deoxycorticosterone acetate (DOCA; 100 mg/kg). Represented are means ± SEM. Numbers in parentheses are number of rats at each time period.

during the remaining course of the experiment.

Discussion. The suppression of renin by DOCA does not appear to be related to elevations in blood pressure usually associated with this treatment. Normal rats treated with DOCA may require 3 weeks to develop increases in blood pressure even when given the additional stresses of uninephrectomy and a high-sodium diet (2). Although we did not measure blood pressure in the present experiments, the short period of DOCA treatment (2 weeks) and the fact that the rats were given a normal sodium diet mitigate against a role of blood pressure in suppressing PRA. Moreover, the PRA was reduced an average of about 60% in only 4 days.

Since Möhring *et al.* (5, 6) have demonstrated that plasma ADH is elevated in rats given DOCA and fed a high-sodium diet, it is possible that ADH may alter the activity of the renin–angiotensin system during DOCA treatment. Although we did not measure ADH in the present study, it has been previously demonstrated that ADH is absent in homozygous diabetes insipidus rats and decreased in rats heterozygous for the trait (13). In the present study, both PRA and RRA were significantly higher in the nontreated homozygous diabetes insipidus rats when compared to both Sprague–Dawley and heterozygous diabetes insipidus rats (Table I), thus supporting a normal role for ADH in suppressing renin activity. Whether this role for ADH is direct or indirect (e.g., through reduced body fluid volumes) cannot be discerned from these results. However, treatment with DOCA resulted in a rapid suppression of PRA in all groups. Thus, at least in the early stage of DOCA treatment, suppression of PRA is independent of ADH. At 11 and 14 days PRA decreased more in rats possessing ADH than in the homozygous rats. This could be due to the inhibitory effect of ADH on renin release. However, it is also possible that the difference in the response of homozygous rats and the other two groups was, as in the case with the initial values, not directly dependent upon ADH. Rather, it could be due to some secondary factor related to its absence. The fact that

there were no significant differences between the heterozygous rats and Sprague–Dawley rats may reflect the fact that there is sufficient ADH present in the heterozygous Brattleboro rats to obscure any differences between these groups.

Although there were small differences between the groups of rats with respect to the suppression of PRA, there were no significant differences with respect to RRA suppression. These data suggest that the primary response to DOCA treatment in all groups is a suppression of renin secretion. The decrease of renal renin content may require time periods longer than those of the present study if the additional stress of a high-sodium diet is not imposed.

Even though the suppression of PRA was different in the groups studied, it is apparent from Fig. 1 that some factor other than ADH is required to explain the majority of the suppression of PRA following DOCA treatment. This is evident from the fact that the homozygous rats, lacking ADH, showed a steep and prompt fall in PRA on DOCA treatment. It is also unknown, from the results of the present studies, whether the initial differences seen between the homozygous diabetes insipidus rats and the other two groups are due directly to ADH or may be explained by a secondary difference in the homozygous group resulting from the lack of ADH. The present results, however, do suggest that the lack of ADH alters to a small extent the long-term (11 and 14 day) response of PRA to DOCA treatment. Whether the majority of DOCA-induced renin suppression is due to alterations in sodium metabolism (14), a direct renal effect of DOCA, or some other factor remains to be determined.

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