Deoxycorticosterone Hypertension in the Intact Weanling Rat Without Salt Loading

Deoxycorticosterone (DOC) hypertension in the rat is generally induced in rats at an age of approximately 3 months. Both uninephrectomy and a high sodium diet are necessary, however, to induce DOC hypertension. Considering the inability of the developing kidney to adequately excrete a sodium load, we studied the possibility that DOC alone might induce hypertension when treatment is initiated in rats at the age of 21 days. The contribution of volume expansion as a factor mediating the pressor response to DOC was assessed in rats given a high sodium diet instead of DOC. Systolic blood pressure increased in DOC-treated rats within 3 weeks. Although systolic blood pressure also increased in rats on a high sodium diet, the increase was transient and of a lesser magnitude than that observed in DOC-treated rats. The rise in blood pressure in both groups of rats was associated with suppression of plasma renin activity and aldosterone concentration. Furthermore, extracellular fluid volume was similarly increased in DOC-treated rats and rats given a high sodium diet. Consistent with these data, DOC-treated rats showed an exaggerated natriuretic response to acute saline loading as compared with a vehicle-treated control group. Discontinuation of DOC treatment after 5 weeks led to normalization of all variables studied including blood pressure. Yet, when DOC was continued for 8 weeks, stopping treatment did not lower blood pressure despite normalization of the renin-angiotensin-aldosterone system and the natriuretic response to saline loading. In contrast, discontinuation of the high sodium diet after 8 weeks normalized blood pressure. These data show induction of DOC hypertension in the developing rat without reduction of renal mass or high sodium intake. Furthermore, the data suggest that volume expansion might participate in the initiation but not in the maintenance of hypertension in this experimental model.

Deoxycorticosterone (DOC)-salt hypertension is a widely studied model of mineralocorticoid-induced low renin hypertension associated with transient extracellular fluid volume expansion.1,2 This model of hypertension is generally induced in 3-month-old uninephrectomized rats fed a high sodium diet. Both uninephrectomy and chronic sodium loading exacerbate steroid-induced fluid volume expansion and, thus, help produce sustained hypertension.

Although increased susceptibility to DOC-salt hypertension3 and to chronic hypertonic sodium loading per se4 have been demonstrated in the prepubertal rodent, the induction of irreversible mineralocorticoid hypertension in the absence of chronic sodium loading and reduction of renal mass has not been previously described in the rat. Considering the relative inability of the kidneys to adequately excrete a salt load during the neonatal and weanling periods of development,5,6 administration of DOC at this age might result in a greater degree of volume expansion than that observed in the mature rat.

Several studies have demonstrated the injurious effects of increased dietary sodium intake in prepubertal rat.5,6 The administration of hypertonic saline to weanling rats, for example, results in the development of hypertension, whereas under the same conditions, blood pressure does not rise in the mature rat.6 It has been postulated that the lack of a
circulating natriuretic hormone in the prepubertal rat might partially explain both the reduced ability of developing kidneys to excrete sodium as well as the hypertensinogenic effects of chronic saline loading observed in weanling rats. Based on such observations, it might be anticipated that any manipulation leading to extracellular fluid volume expansion might produce greater effects in the weanling than the mature rat. In the present study, we examined the possibility that long-term administration of DOC alone to rats from the age of 21 days might induce hypertension. We also evaluated long-term changes in blood pressure when DOC treatment was discontinued in separate groups of rats. The importance of fluid volume expansion in producing changes in blood pressure was evaluated through chronic is tonic saline loading in rats of the same age.

Methods
A total of 85 normal Wistar rats were selected from litters limited in size at birth to 6–8 rats. All rats were housed in a room with an ambient temperature of 26°C and a 12-hour light/dark cycle. At an age of 21–23 days, the rats were divided at random into three age- and sex-matched groups. Thirty rats were given DOC either in biweekly subcutaneous injections (15 rats; 35 mg/kg body wt/wk in oil) or through pelleted DOC implants (15 rats) (Innovative Research of America, Toledo, Ohio) providing a comparable but constant dose. Another 30 rats were used as control groups and received either biweekly subcutaneous oil injections (15 rats) or placebo pellets (15 rats). The remaining 25 rats were placed on a high salt diet (0.9% saline, replacing drinking water), but these rats were not given DOC. Except for the high salt group, rats were maintained on a standard chow diet (0.46% sodium) and tap water ad libitum. When the rats were 35 days old, systolic blood pressure (SBP) was measured by tail-cuff plethysmography. At this age, the use of smaller restraining cages and tail cuffs is necessary to achieve normal and reproducible SBP measurements. Indeed, the earliest age at which we were able to make SBP measurements in these rats was at 28 days. SBP was expressed as the mean value of 10 consecutive measurements. Half of the rats were then transferred to small rodent metabolism cages, and after a 48-hour period of adaptation, three consecutive 24-hour urine samples were collected under oil for the determination of urine flow rate and urinary concentrations of sodium and potassium. Food and water intake as well as body weight were also measured daily. The intake and urinary excretion of sodium and potassium were then calculated per 100 grams body weight. Afterwards, a similar 3-day balance study was performed in the remaining rats from each group. SBP was then measured weekly throughout the remainder of the study. At the age of 56 days, beginning at 8:00 AM and after 45–60 seconds exposure to ether, rats were bled by heart puncture for the determination of plasma renin activity, plasma aldosterone concentration, and hematocrit. Although ether anesthesia has been shown to stimulate plasma renin activity,8 the reproducibility of renin levels measured in the ether-anesthetized rat has been previously demonstrated.9 In addition, it has been shown that changes in renin activity can be detected despite the use of ether when exposure is limited to 45–60 seconds.9 After the initial bleeding, an additional 2-day balance study was again performed in all rats to permit recovery from the bleeding procedure. On completion of this study, the group of rats treated with DOC and the control group were fasted overnight in metabolism cages in preparation for an acute saline-loading study. The next morning, bladder emptying was induced in each rat by ether sniffing, and voided urine was discarded. The rats were then returned to their metabolism cages for a 4-hour control urine collection period. On completion of the control collection, bladder emptying was again induced and voided urine added to respective control collection samples. The rats were then weighed, and a volume of isotonic saline equal to 3% of body weight was administered to each rat by gavage. The rats were returned to metabolism cages for a 2-hour urine collection. Afterward, bladder emptying was induced and voided urine added to the postload sample. The volume of each sample and urinary concentrations of sodium and potassium were then measured. No further treatment was given to half the rats from each group, and these rats were allowed to recover for 1 month. The remaining rats were treated for an additional month. Once treatment was discontinued, these rats were also allowed to recover for 1 month. During the last week of the respective recovery periods (ages, 86 days or 115 days), all rats were again bled for the determination of plasma renin activity, plasma aldosterone concentration, and hematocrit. Another 2-day balance study was then performed in all rats. Additionally, the acute saline-loading study was repeated in rats previously treated with DOC and in the control group. Separate groups of six DOC-treated rats, six rats given a high sodium diet, and six control rats were also studied for measurement of extracellular fluid volume during respective treatment regimens. These rats were studied after 1 month of treatment.

Urinary concentrations of sodium and potassium were measured by flame photometry. Plasma renin activity was measured by the radioimmunoassay of angiotensin I generated from endogenous renin substrate per milliliter plasma per hour during sample incubation at 37°C in the presence of angiotensinase inhibitors. Radioimmunoassay materials were purchased from New England Nuclear, Boston, Massachusetts. Plasma aldosterone concentration was measured by direct radioimmunoassay using materials purchased from Abbott Laboratory, San Juan, Puerto Rico. Cross-reactivity of the antialdo-
TABLE 1. Systolic Blood Pressure During and After Treatment With Deoxycorticosterone

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (days)</th>
<th>42±3</th>
<th>56±3</th>
<th>86±3</th>
<th>145±3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>122±3</td>
<td>124±3</td>
<td>131±3</td>
<td>125±3</td>
</tr>
<tr>
<td>DOC (stopped at age 56 days)</td>
<td></td>
<td>153±8*†</td>
<td>170±8*†</td>
<td>128±5</td>
<td></td>
</tr>
<tr>
<td>DOC (stopped at age 86 days)</td>
<td></td>
<td>169±4*†</td>
<td>180±4*†</td>
<td>191±3*†</td>
<td>189±2*†</td>
</tr>
<tr>
<td>High Na+ diet (stopped at age 86 days)</td>
<td></td>
<td>132±3‡</td>
<td>142±3‡</td>
<td>151±9</td>
<td>131±9</td>
</tr>
</tbody>
</table>

*Values significantly different (p<0.001) from corresponding control value.
†Values significantly different (p<0.01) from corresponding high Na+ diet value.
‡Values significantly different (p<0.05) from corresponding control value.

sterone antibody has been reported at 100% with aldosterone and less than 0.01% with DOC. Extracellular fluid volume was measured with [3H]inulin dilution. Each rat was bilaterally nephrectomized precisely 30 minutes before administration of [3H]inulin, and this procedure resulted in only minimal loss of peritoneal fluid. Statistical analysis of the data was done with Student’s t test among groups and paired t analysis within groups. Differences between values were considered statistically significant when p values were less than or equal to 0.05. All values were expressed as the arithmetic mean±SEM. When no differences were observed between 5 and 8 weeks of DOC-treatment data from these two groups were pooled.

This protocol was reviewed and approved by the local institutional Animal Care and Use Committee. All procedures comply with the standards for the care and use of animal subjects as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Maryland, 1985, pp 3–55).

Results

SBP shown in Table 1 rose gradually in DOC-treated rats and was significantly increased at the age of 42 days (3 weeks of DOC treatment) as compared with values observed in the control group. When DOC treatment was discontinued in rats at the age of 56 days, blood pressure levels fell, and 1 month later, blood pressure in this group of rats (age, 86 days) was similar to that in control rats. In contrast, when DOC treatment was prolonged for an additional 1 month, SBP remained elevated after DOC was discontinued. Consequently, under these conditions, the DOC-induced rise in SBP was irreversible. In response to a high salt diet, blood pressure also increased. Under these conditions, however, blood pressure remained unchanged in seven of the rats studied, whereas increased blood pressure was observed in all rats during DOC treatment regardless of the duration of treatment. Additionally, the rise in blood pressure during high salt intake was not only labile from week to week in any given rat but also transient in all of the rats studied. In contrast, the rise in blood pressure in response to DOC was sustained in all rats during treatment. As shown in Figure 1, plasma renin activity was lower in rats given either DOC or high salt diet as compared with control rats during treatment. Regardless of the duration of treatment, when treatment was discontinued for 1 month, plasma renin activity rose in rats that had previously been given either DOC or a high salt diet such that renin activity was similar to values observed in control rats. Plasma aldosterone concentration (Figure 2) reflected lev-
els of renin activity under all conditions studied. Aldosterone concentration was lower in DOC and high salt groups during treatment as compared with values in the control group. When treatment was discontinued for 1 month, plasma aldosterone in all rats previously treated with either DOC (5 or 8 weeks' treatment) or a high salt diet rose to levels observed in the control group. The results of acute isotonic saline-loading studies in DOC-treated and control rats during the last week of respective treatment periods and 1 month after discontinuing DOC are summarized in Figures 3 and 4. An exaggerated natriuretic response to intragastric saline loading was observed in rats given DOC for 5 weeks or for 8 weeks as compared with respective control groups (Figure 3). Although the kaliuretic response to saline loading (not shown) tended to be blunted in DOC-treated rats, this difference was not statistically significant. Independent of the duration of DOC administration, when treatment was suspended and rats allowed to recover for 1 month, the natriuretic (Figure 4) and kaliuretic (not shown) responses to saline loading were similar in rats previously treated with DOC and the respective control groups. As shown in Table 2, extracellular fluid volume was similarly increased in DOC and high salt diet groups after 1 month of treatment as compared with values in control groups.

**Discussion**

These studies demonstrated the induction of DOC hypertension in the developing rat without reduction of renal mass or a high sodium diet. The hypertensinogenic effects of this steroid, however, were dependent on the length of the treatment period. Prolonged treatment for 2 months led to a sustained rise in blood pressure even after DOC administration was discontinued. Thus, in contrast to the mature rat in which chronic saline loading and uninephrectomy are necessary to induce DOC hypertension, in the weanling rat, DOC treatment alone led to the development of hypertension. The low renin–low aldosterone state observed during steroid treatment was associated with increased extracellular fluid volume, suggesting the possibility that such fluid volume expansion might participate in the initial pressor response to DOC. Additionally, the exaggerated natriuretic response to an acute intragastric saline load during DOC treatment is also consistent with expanded extracellular fluid volume. Thus, possible explanations for our findings are that the developing rat might be more sensitive to changes in extracellular volume, that the degree of steroid-induced volume expansion might be greater in the weanling than in the mature rat, or that both sensitivity to and degree of volume

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**Figure 3.** Scatterplot showing urinary sodium excretion before (Control) and after (Salt Load) intragastric saline loading during treatment with deoxycorticosterone (•) or vehicle (○). *Denote values significantly different (p<0.01) from corresponding values in vehicle-treated rats. †Denote values significantly different (p<0.001) from corresponding control value within same group.

**Figure 4.** Scatterplot showing urinary sodium excretion before (Control) and after (Salt Load) intragastric saline loading, 1 month after deoxycorticosterone (•) or vehicle (○) treatment was discontinued regardless of length of treatment period. †Denote values significantly different (p<0.01) from corresponding control value within same group.
change might be involved. Another possibility is supported by studies demonstrating that, in contrast to the mature rat, during the process of renal maturation in the developing rat, mineralocorticoids stimulate sodium-potassium-dependent adenosine triphosphatase (Na\textsuperscript{+},K\textsuperscript{+}-ATPase) activity in the proximal tubule.\textsuperscript{11} Such a steroid-induced increase in renal Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in a nephron segment receiving the entire filtered load of sodium might be expected to result in greater sodium and water reabsorption than in the adult rat where proximal tubular Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity is not influenced by mineralocorticoids.\textsuperscript{12} Thus, administration of DOC might lead to a more marked expansion of extracellular fluid volume in the weaning rat than in the mature rat.

We evaluated the possibility that increased susceptibility to steroid-induced hypertension during development might reflect a greater sensitivity to extracellular fluid volume expansion. Chronic saline loading through the administration of a high sodium diet to weaning rats led to a rise in SBP. These data suggest that the developing rat might be more sensitive to extracellular fluid volume expansion as compared with the mature rat in which a high sodium diet fails to influence blood pressure. Consequently, the rise in blood pressure during chronic saline loading in the present study is consistent with the concept of enhanced susceptibility to hypertensinogenic effects of excess dietary sodium during development.\textsuperscript{6} This rise in blood pressure during high sodium intake, however, was less in magnitude than that observed in response to DOCA hypertensive rats. Moreover, although volume expansion can contribute to the initial rise in blood pressure during DOCA treatment, it does not fully account for the increased sensitivity to DOCA in weaning rats.

Extrarenal effects of steroids might contribute to the increased hypertensinogenic effects of DOC in the weaning rat. Such extrarenal effects of adrenocortical steroids include enhanced vascular reactivity,\textsuperscript{13} increased vascular smooth muscle sodium permeability,\textsuperscript{14} as well as elevation of circulating vasopressin levels.\textsuperscript{15} The possibility of age-dependent changes in extrarenal sensitivity to DOC cannot be evaluated on the basis of the present study. Regardless of this, we have described a model of DOC hypertension in which neither saline loading nor uninephrectomy are required because of enhanced sensitivity of the developing rat to this adrenocortical steroid.

References


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