Resistance to Mineralocorticoid-Induced Hypertensive Vascular Disease

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SUMMARY To support our contention that the Wistar-Furth rat is resistant to mineralocorticoid hypertension, we assessed the effects of deoxycorticosterone (DOC) administration or renal artery stenosis on the development of hypertension in the Sprague-Dawley and Wistar-Furth rat strains. Weekly administration of mineralocorticoid in the form of DOC pivalate resulted in rapid, severe hypertensive cardiovascular disease in Sprague-Dawley rats. Within 5 weeks the mean conscious systolic blood pressures in steroid-treated and control rats were 186 ± 4 and 118 ± 5 mm Hg, respectively. In contrast, blood pressures of Wistar-Furth rats were only moderately elevated, even after 10 weeks of DOC pivalate administration (136 ± 2 vs 116 ± 2 mm Hg for controls). Furthermore, none of the steroid-treated Wistar-Furth animals exhibited cardiovascular lesions. In parallel studies, littermates of these rat strains were subjected to renal artery stenosis and blood pressures were determined weekly in conscious rats. Silver clip constriction of the left renal artery, in the presence of the contralateral kidney, resulted in a rapid, sustained elevation of blood pressure in both Sprague-Dawley and Wistar-Furth rat strains (177 ± 4 and 176 ± 5 mm Hg, respectively). Corticosteroid levels were also determined in DOC-treated Sprague-Dawley and Wistar-Furth rats. The regimen employed resulted in a 10-fold increase in DOC levels as compared with controls, and the levels achieved were comparable in both strains. Thus, the Wistar-Furth rat appears to be selectively resistant to mineralocorticoid hypertensive vascular disease and thus affords a model for studying mechanisms of steroid hypertension. (Hypertension 10: 176-180, 1987)

KEY WORDS • hypertension • deoxycorticosterone • resistance • Wistar-Furth rats

WE have previously reported that adrenal regeneration hypertension fails to develop in the Wistar-Furth rat strain.1 Wistar-Furth rats (W/Fu) do exhibit cardiac and renal hypertrophy, but systolic blood pressure (BP) is minimally elevated and there is a virtual absence of cardiovascular lesions. This model of experimental hypertension was described by Skelton2 in 1955 and is generally thought to be mediated by mineralocorticoid excess.3 The identity of the hormone (or hormones) involved has not been clearly defined, although several steroids, including 11-deoxycorticosterone (DOC) and 19-nordeoxycorticosterone have been implicated.4-7 Bergon and Brownie8 found that serum DOC levels were elevated in adrenal-enucleated W/Fu 5 weeks after operation as compared with those of controls. Again, BP was mildly elevated but cardiovascular lesions were absent. This study and our previous one3 suggested the possibility that the W/Fu are resistant to mineralocorticoid hypertension.

In contrast to our observations, Hall et al.9 reported that W/Fu exhibit adrenal regeneration hypertension that is indistinguishable from that seen in Sprague-Dawley rats (SD). However, in a follow-up study, the same authors found that adrenal regeneration hypertension developed in SD at a much faster rate and with greater intensity than in the W/Fu.10 In fact, BPs of the W/Fu were not into the hypertensive range until the fifth week following adrenal enucleation, as compared with 2 weeks for the SD. Cardiovascular lesions were not evaluated. Hall et al.9,10 have attributed the discrepancy between our findings and theirs to our use of anesthetized rats for BP measurements; they used conscious animals. The different mode of response of the W/Fu in the two studies by Hall et al.9,10 was attributed to variability in the response of animals to adrenal enucleation from experiment to experiment.
It was clear to us that further studies on the W/Fu were warranted to resolve these discrepancies. To avoid any possible variability in the response of rats to adrenal enucleation, in the present study we chose to investigate the susceptibility of the W/Fu to two other well-known models of hypertension; DOC-salt and the two-kidney, one clip model of renal artery stenosis. The present article reports the results of these experiments and discusses the suitability of the W/Fu for studying mechanisms of mineralocorticoid hypertensive vascular disease.

Materials and Methods

Animal Procedures

The study used 35-day-old female SD weighing 120 to 140 g (Holzman, Madison, WI, USA), and age-matched female W/Fu weighing 80 to 100 g (Harlan Sprague-Dawley, Indianapolis, IN, USA). All animals were individually caged in three animal holding rooms at 22 ± 1°C with 12-hour light and dark cycles. Rats from each experimental group were randomly assigned to one of the three rooms. Animals were handled daily throughout the treatment period to acclimate them to removal from cages and thereby minimize stress at the time of BP measurement and at the termination of the experiment. Both W/Fu and SD were subjected to the following regimens.

Mineralocorticoid Treatment

DOC-treated rats and their controls were subjected to left unilateral nephrectomy under ether anesthesia 48 hours before the start of the treatment. They were then given 1% sodium chloride as drinking fluid and commercial rat chow ad libitum. DOC (Percorten pivalate; CIBA-Geigy, Summit, NJ, USA) was administered weekly by subcutaneous injection at a dose of 25 mg/kg body weight. Control rats received vehicle only. In studies in which steroid levels were determined, a third group of W/Fu receiving DOC, 50 mg/kg, was added to the regimen described.

Renal Artery Stenosis

W/Fu and SD were subjected to left renal artery clipping to produce stenosis. A laparotomy was performed on all animals under ether anesthesia, and the left renal artery was exposed. In the experimental group, a silver clip was gently placed across the artery just below the junction with the aorta. The right kidney was untouched. Because the SD are larger than the age-matched W/Fu, a clip with a 0.22 mm inside diameter was used in the former and one with a 0.20 mm inside diameter was used in the latter to achieve successful renal artery stenosis. Rats were maintained on tap water and commercial rat chow ad libitum.

BP Measurement

Systolic BPs were measured 2 days before operation and weekly throughout the course of the experiment in conscious, restrained rats that had been warmed to 37°C. Measurements were made by the tail-cuff method using an electrosphygmomanometer and physiograph recorder (Narco Biosystems, Houston, TX, USA). To compensate for difference in size between the two strains, a tail-cuff size of ⅛ in. inside diameter was used for the W/Fu and a ⅜ in. inside diameter was used for the SD. The average of three BP measurements was taken as the BP value for that day. BP measurements were performed over a 3-day period between 0900 and 1200 to minimize any possible influence of a diurnal variation in BP. All rats were subjected to weekly BP determination.

Collection of Serum and Steroid Analysis

After 5, 8, or 10 weeks of treatment, rats were killed by decapitation at 0800, which is the low point of the circadian rhythm. Exposure to stress was minimized by handling the animals daily throughout the treatment period. A quiescent kill was achieved by using several people, who entered the holding room at the same time. Rats were removed from their cages and killed in the immediately adjacent hall within 6 seconds of entering the room and removing the rat from its cage. Trunk blood was collected from individual rats and allowed to clot on ice. The serum was separated by centrifugation and stored at -20°C until assayed.

For the determination of DOC, a 2.0-ml aliquot of serum containing 4,000 dpm [3H]DOC as internal standard was extracted with 10 ml of methylene chloride. The extract was washed with 0.1 N sodium hydroxide and water, evaporated to dryness under nitrogen, and dissolved in acetonitrile and water (1:3, vol/vol). DOC was separated by high performance liquid chromatography (HPLC) (Model 5000; Varian, Palo Alto, CA, USA). DOC in the fractions collected was measured by radioimmunassay.

Levels of corticosterone were determined by direct radioimmunoassay. Corticosterone antibody and [3H]corticosterone were obtained from Radioassay Systems Laboratories (Carson, CA, USA) and New England Nuclear Research Products (Boston, MA, USA), respectively.

Histology

At the time of death, the hearts and kidneys of all animals were removed and fixed in 10% formalin. Following fixation, organs were trimmed, blotted, and weighed on an analytical balance. The organs were sectioned and stained with periodic acid–Schiff or hematoxylin and eosin for histological examination. After examination, the percentage of incidence and severity of microscopic lesions were calculated. Lesions were graded without knowledge of the experimental group being assessed. The severity of lesions was graded by a semiquantitative method based on a scale of 0 to 4 , taking into account the frequency and extent of lesions. The severity index was obtained by dividing the total score for each group by the theoretical maximum score for each group.

Statistics

All data are recorded as means ± SEM. For statistical comparisons in all studies, a one-way analysis of
variance was used to compare values in experimental and control rats. A p value below 0.05 was regarded as significant.

**Results**

**BP Response to Treatment**

BPs were comparable in all groups before treatment. The BP response to DOC pivalate administration and renal artery stenosis in the W/Fu is summarized in Figure 1. After 10 weeks of DOC administration, the W/Fu showed only a modest elevation in BP, even when treatment was extended for 10 weeks. At 10 weeks, mean systolic BP of DOC-treated W/Fu was 136 ± 2.1 mm Hg, as compared with 116 ± 1.9 mm Hg for control rats. Even in W/Fu treated with a larger dose of DOC pivalate (50 mg/kg), there was only a moderate increase in BP. Mean systolic BP of these DOC-treated rats after 5 weeks was 123 ± 5 mm Hg, as compared with 108 ± 2 mm Hg for controls. However, stenosis of the left renal artery in this strain resulted in a rapid rise in BP in the first week. This elevation was sustained throughout the course of treatment. At the end of 10 weeks mean systolic BP of W/Fu with renal artery stenosis was 176 ± 5 mm Hg, as compared with 110 ± 3 mm Hg for control rats.

Weekly administration of DOC pivalate (25 mg/kg) to SD resulted in a rapid and progressive increase in BP, which was significantly higher than that of control rats after 1 week of treatment (Figure 2). The mean systolic BP of DOC-treated SD after 5 weeks was 186 ± 4 mm Hg, as compared with 118 ± 1.7 mm Hg for control rats. Similarly, BP of SD undergoing renal artery stenosis rose sharply after the first week, and at 8 weeks the mean systolic BP of this group was 177 ± 3.6 mm Hg, as compared with 106 ± 3 mm Hg for control rats.

**Organ Weights**

Relative heart and kidney weights for SD and W/Fu are given in Table 1. Kidney weights more than doubled in DOC-treated SD as compared with control rats. Mild but significant renal and cardiac hypertrophy was present in DOC-treated W/Fu and in both SD and W/Fu that had undergone renal artery stenosis.

**Incidence and Severity of Microscopic Lesions**

Cardiovascular lesions were more prevalent and severe in DOC-treated SD than in any other group of rats (see Table 1). There was an absence of hypertensive cardiovascular disease in DOC-treated W/Fu. Cardiovascular lesions were present in both SD and W/Fu with renal artery stenosis, although these animals demonstrated a decrease in incidence and a lower severity index as compared with those in DOC-treated SD.

In both models of hypertension, renal damage was most severe in the juxtamedullary zone and consisted of fibrinoid necrosis, sclerosis, and pericapsular fibrosis of glomeruli. Some glomeruli, most notably those of DOC-treated SD, were completely obliterated by fibrosis. Tubular hyaline casts and medial hypertrophy of arterioles were also prominent in affected kidneys.

The coronary arteries of DOC-treated SD showed hyalinization and sclerosis of the media. Focal areas of fibrous connective tissue were present in the myocardium of DOC-treated SD and in both rat strains with renal artery constriction.
Table 1. Effect of DOC Treatment and Renal Artery Stenosis on Various Parameters in Sprague-Dawley and Wistar-Furth Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Wistar-Furth</th>
<th>Sprague-Dawley</th>
<th>Wistar-Furth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>DOC (n = 16)</td>
<td>Control (n = 10)</td>
<td>DOC (n = 18)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>118 ± 2</td>
<td>180 ± 15*</td>
<td>116 ± 6</td>
<td>136 ± 9*</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>246 ± 11</td>
<td>228 ± 7</td>
<td>189 ± 3</td>
<td>187 ± 11</td>
</tr>
<tr>
<td>Organ weight (mg/100 g body wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>416 ± 16</td>
<td>533 ± 15*</td>
<td>372 ± 11</td>
<td>421 ± 8*</td>
</tr>
<tr>
<td>Kidney</td>
<td>477 ± 28</td>
<td>1046 ± 64*</td>
<td>593 ± 25</td>
<td>717 ± 15*</td>
</tr>
<tr>
<td>Renovascular lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence (%)</td>
<td>0</td>
<td>68.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Severity index</td>
<td>0</td>
<td>0.31</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>Cardiac lesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence (%)</td>
<td>0</td>
<td>50.0</td>
<td>0</td>
<td>25.0</td>
</tr>
<tr>
<td>Severity index</td>
<td>0</td>
<td>0.22</td>
<td>0</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are means ± SEM. 2KIC = two-kidney, one clip. For explanation of severity index, see Materials and Methods (Histology).

Serum Steroid Levels

In SD and W/Fu, DOC pivalate administration (25 mg/kg) resulted in at least a 10-fold increase in blood DOC levels relative to control rats (Table 2). DOC levels achieved in the two strains were not significantly different (11.6 ± 2 vs 13.4 ± 4 ng/ml in SD and W/Fu, respectively). Blood levels of DOC achieved in W/Fu treated with larger doses of DOC pivalate (50 mg/kg) were nearly twice the levels seen in W/Fu treated with the smaller dose (22.3 ± 4 vs 13.4 ± 4 ng/ml). Serum corticosterone values were within the normal range in all groups of rats.

Discussion

Our previous studies on the development of adrenal regeneration hypertension in the W/Fu suggested that this strain may be resistant to mineralocorticoid hypertension. The present study lends support to this hypothesis. The administration of DOC pivalate and saline to SD evoked a rapid and progressive increase in systolic BP (see Figure 2) that was accompanied by severe cardiac and renal hypertrophy and extensive cardiovascular lesions typical of malignant hypertension. In sharp contrast, W/Fu given the same DOC-salt regimen showed only a modest elevation in BP (see Figure 1), even though the duration of treatment was twice as long. Marked renal and cardiac hypertrophy did occur in the W/Fu, but cardiovascular lesions were absent. The observed cardiac and renal enlargement in DOC pivalate-treated W/Fu cannot be readily understood. It may be reflective of the small but significant increase in BP that occurred in these animals. Alternatively, DOC pivalate may independently stimulate cardiac and renal hypertrophy. A dissociation of the effects of aldosterone and DOC on heart weight from their effects on BP has previously been reported by Komanicky and Melby.

These results cannot be attributed to intrinsic differences in absorption of the DOC ester by SD and W/Fu. The regimen employed resulted in a 10-fold increase over controls in serum DOC, and the levels achieved were comparable in both strains (see Table 2). Further-

Table 2. Systolic BP, Serum DOC, and Corticosterone Levels in DOC-Treated Sprague-Dawley and Wistar-Furth Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sprague-Dawley</th>
<th>Wistar-Furth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 7)</td>
<td>DOC-treated (n = 8)</td>
</tr>
<tr>
<td>Steroid dose (mg/kg body wt)</td>
<td>—</td>
<td>25</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>109 ± 6</td>
<td>172 ± 5*</td>
</tr>
<tr>
<td>Serum DOC (ng/ml)</td>
<td>0.781 ± 0.3</td>
<td>11.6 ± 2*</td>
</tr>
<tr>
<td>Serum corticosterone (μg/dl)</td>
<td>1.9 ± 0.9</td>
<td>4.8 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.001, †p < 0.05, ‡p < 0.10, compared with values in control rats.
more, the administration of twice the concentration of DOC pivalate to the W/Fu resulted in a doubling of serum DOC levels, which was again accompanied by only a mild elevation in BP. The levels of serum DOC reported here are comparable or even exceed values reported in adrenal regeneration hypertension \(^6\) or in DOC acetate hypertension. \(^11\) It is evident from the corticosterone levels (see Table 2) that rats of all groups were quiescent; thus, a stress-induced increment in serum DOC was unlikely.

These studies show that the W/Fu are selectively resistant to mineralocorticoid hypertension. In parallel studies both the W/Fu and SD exhibited hypertension and organomegaly in response to renal artery stenosis, a model of hypertension thought to be mediated by angiotensin II. \(^{21-22}\) Cardiovascular lesions were also present in both strains, although the incidence was lower and the severity milder in the W/Fu than in the DOC-salt treated SD. This finding confirms the reports by others \(^{21, 24}\) that renal artery stenosis produces a less severe form of hypertension than does the DOC-salt model.

Resistance to hypertension does not imply immunity to its development. The Dahl salt-resistant rat strain, for example, does manifest hypertension, albeit in a milder form than the salt-sensitive strain, when given DOC acetate–salt for long periods. \(^8\) In the Long-Evans strain, which Hall et al. \(^9\) reported to be resistant to DOC acetate–induced hypertension, we found that hypertension developed in response to free DOC administration, which presumably results in higher blood DOC levels. \(^25\) Therefore, it is conceivable that, with larger doses and prolonged treatment, free DOC-salt hypertension also could develop in the W/Fu.

In summary, the W/Fu are sensitive to renal hypertension but resistant to DOC-salt treatment, whereas the SD are sensitive to both. The mechanism by which the W/Fu withstand the effects of mineralocorticoid excess is still not known.

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