Importance of Renal Sympathetic Tone in the Development of DOCA-Salt Hypertension in the Rat

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SUMMARY In many experimental models, acute increases in sympathetic nervous system activity produce disproportionately greater vasoconstriction in the renal vascular bed than occurs in most other vascular beds. Since increased sympathetic nervous system activity has been implicated in the pathogenesis of DOCA-salt hypertension in the rat, we hypothesized that an attenuation of renal sympathetic tone would delay the development of this form of hypertension. Renal denervation was carried out in 5-week-old uninephrectomized male Sprague-Dawley rats 1 week before beginning DOCA-salt treatment. Systolic blood pressures using the tail-cuff method in 32 sham-operated rats were significantly (p < 0.05) elevated above control by Day five (115 ± 3 vs 128 ± 3 mm Hg) of DOCA-salt administration and continued to rise, reaching a plateau by Day 21 (192 ± 5 mm Hg). In contrast, DOCA-salt administration in 32 renal denervated rats did not result in a significant elevation of blood pressure above control until Day 17 (121 ± 3 vs 135 ± 3 mm Hg, p < 0.05). During the first 2 weeks of DOCA-salt treatment, fractional urinary sodium excretion was significantly greater (p < 0.05) in renal denervated rats than in sham animals. During the third week of DOCA-salt administration, renal denervated rats had a rapid rise in blood pressure and a fall in fractional urinary sodium excretion to the level of the sham-operated animals. Coincident with the development of hypertension was a threefold increase in renal norepinephrine content (5.3 ± 0.4 ng/g on Day 14 vs 17.7 ± 3.0 ng/g on Day 24, p < 0.01), suggesting reinnervation. These data suggest that increased renal sympathetic tone in the DOCA-salt rats facilitates sodium retention and is necessary for the development of the hypertension. (Hypertension 2: 266-273, 1980)

KEY WORDS • DOCA-salt • sodium • renal denervation • sympathetic nervous system • Sprague-Dawley rat • hypertension

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sympathetic nervous system could initiate hypertension or allow hypertension to be sustained by preventing the occurrence of a pressure natriuresis.

When sympathetic nervous system activity is increased acutely, some vascular beds receive greater stimulation than others. This has been observed in a variety of experimental situations in which sudden increases in sympathetic activity produce a disproportionately greater vasoconstriction in the renal vascular bed than occurs in most other vascular beds. These data raise the possibility that in states of chronically increased sympathetic nervous system activity there may be disproportionately more sympathetic tone in the kidney than in other vascular beds. As was shown in the chronic intrarenal norepinephrine infusion studies, a disproportionate increase in renal sympathetic tone would facilitate retention of sodium and the development of hypertension.

Increased sympathetic nervous system activity has been implicated in the pathogenesis of desoxycorticosterone acetate (DOCA)-salt hypertension in the rat. If this increase is critical to the development of the hypertension, it is likely that there is a disproportionate increase of renal sympathetic tone. Accordingly, an attenuation of renal sympathetic activity should delay the development of the hypertension in these animals. The objective of this study was to examine the effect of renal denervation on the development of DOCA-salt hypertension in the rat.
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Methods

Animal Preparation

Male Sprague-Dawley rats (Southern Animal Farms, Prattville, AL; n = 85) were subjected to unilateral nephrectomy at 3 to 4 weeks of age. Following nephrectomy 14 days were allowed for compensatory renal hypertrophy to occur before a second operation (renal denervation or a sham operation) was performed. Renal denervation was accomplished in 40 rats through a flank incision by stripping the renal artery adventitia and painting the renal artery with 20% phenol (wt/vol) in ethanol. The sham operation carried out in 45 rats consisted of opening and closing the flank on the side of the remaining kidney. The kidney of sham rats was not handled in order to avoid traumatizing the renal artery and thus producing partial denervation.

One week after the second operation, DOCA-salt treatment was initiated in 32 denervated and 32 sham-operated rats. DOCA (Percorten Pivaltate, Ciba-Geigy Corporation, Summit, NJ) was administered weekly (by subcutaneous injection of 0.4 ml of a suspension containing, per ml of water: 25 mg DOCA, 10.5 mg methyl cellulose, 3 mg carboxymethylcellulose, 1 mg polysorbate 80, and 8 mg NaCl). Salt was given by substitution of 1% NaCl solution for drinking water. Animals were allowed to drink ad libitum. Control groups included eight uninephrectomized sham-operated rats that received weekly injections of the vehicle suspension without DOCA in order to follow the change in blood pressure due to growth, and eight uninephrectomized renal-denervated rats that received weekly injections of the vehicle suspension without DOCA in order to assess the effect of renal denervation per se on blood pressure.

Blood Pressure and Sodium Measurements

Throughout the study, animals were housed in a room with constant temperature (24° ± 1°C) and humidity (60 ± 5%) and light from 6 a.m. to 6 p.m. Systolic blood pressures of all animals were measured twice weekly using the tail-cuff method without anesthesia. Animals were weighed weekly, and body weight was used for normalization of kidney weight.

To examine the effects of renal denervation on the development of DOCA-salt hypertension, 32 sham-operated DOCA-salt-treated, 32 renal-denervated DOCA-salt-treated, eight sham-operated vehicle-treated, and eight renal-denervated vehicle-treated rats had blood pressures measured over a period of 34 days. The effects of renal denervation on sodium handling during the initial 24 days of DOCA-salt treatment were determined by measuring urinary sodium excretion in eight sham-operated and nine renal-denervated rats. These animals were housed individually in metabolic cages (Hoeltge-Acme, Cincinnati, OH) that had drinking bottles and food cups on the outside of the cage to avoid contamination of urine collections. The rats remained in the cages continuously throughout the 24-day period except for short periods on 2 days out of 7 during which they were removed for blood pressure measurements, DOCA injections, and weighings. Accordingly, 24-hour urinary sodium excretions were measured on those 5 out of 7 days in which the animals were undisturbed.

Rats in the metabolic cages were given 1% saline in drinking water ad libitum. The food was a purified basal diet (Ralston Purina Company, Richmond, IN) containing 0.29% sodium, ground, and mixed with 2 ml of distilled water to form a paste. This disturbed the rat from bringing food from the cup into the cage, thus contaminating the urine collection. Urine was collected under mineral oil to avoid evaporation. Total 24-hour sodium intake was calculated from the volume of 1% saline drunk and the amount of food consumed. Daily urinary sodium excretion was calculated from the volume and the urinary sodium concentration (mEq/liter) measured by flame photometry. Fecal sodium excretion was not measured because it normally represents only 1–2% of total sodium excretion and would be expected to be the same in both denervated and sham-operated groups. Urinary sodium excretion was expressed by the two methods recommended for growing rats. 1) percent of intake (24-hour urinary sodium excretion divided by 24-hour total sodium intake × 100); and 2) weekly sodium retention per gram of body weight gained (mean daily sodium intake for the week × 7 days minus mean daily urinary sodium excretion for the week × 7 days divided by body weight gain per week).

Biochemical Studies

After 14, 24, or 35 days of DOCA-salt administration, 21 of the sham-operated and 27 of the renal-denervated rats were systematically sacrificed, to measure the renal norepinephrine content for confirmation of the completeness of renal denervation, and to correlate changes in blood pressure and urinary sodium excretion with renal norepinephrine content as reinervation occurred. The animals were sacrificed by decapitation without anesthesia. Blood was collected in plain tubes for measurement of serum creatinine (n = 23) or in iced tubes containing 0.2 ml EDTA (4% wt/vol) for determination of plasma renin activity (n = 15). The kidneys were removed and rapidly frozen in liquid nitrogen for subsequent determination of renal norepinephrine content and wet weight. Renal norepinephrine content was measured (Cat-A-Kit, Upjohn) in our laboratory using a modification of the radio-enzymatic method of Peuler and Johnson. Plasma renin activity was measured by radioimmunoassay of generated angiotensin I according to the method of Haber et al.

Numerical results are expressed as means ± 1 se. Statistical analysis of the data was performed using Student's paired and unpaired t tests and regression analysis according to standard procedures. Changes are reported as significant if the p value was less than 0.05.
Results

Development of Hypertension

During 24 days of DOCA-salt administration, 32 sham-operated and 32 renal-denervated rats were observed for changes in blood pressure; in 24 and 18 of these animals respectively, observations were continued for a total of 34 days. As shown in figure 1, DOCA-salt administration in the sham group produced a rise in systolic blood pressure from 115 ± 3 to 128 ± 3 mm Hg (p < 0.05) within 5 days. Systolic blood pressure continued to rise, reaching 192 ± 5 mm Hg (p < 0.001) after 21 days of DOCA-salt administration and remaining elevated for the duration of the experiment (p < 0.001). In contrast, DOCA-salt administration in the renal-denervated rats did not result in a significant elevation of blood pressure above control until Day 17 (p < 0.05). Comparison of the two groups (fig. 1) showed that blood pressures of sham animals were significantly higher (145 ± 3 vs 126 ± 5 mm Hg, p < 0.01) by Day 9 and remained so through Day 30. By Day 34 of DOCA-salt administration, blood pressures of the renal-denervated animals had increased so that there was no significant difference between the groups (sham = 189 ± 6 mm Hg; denervated = 178 ± 6 mm Hg).

Eight control sham-operated and eight renal-denervated rats given vehicle injections and tap water were observed for 34 days. Control systolic blood pressures of the sham-operated (118 ± 4 mm Hg) and renal-denervated rats (122 ± 6 mm Hg) were not significantly different from the control blood pressures of the groups that received DOCA-salt treatment. Over the subsequent 34 days of observation, blood pressure ranged between 116 ± 4 and 127 ± 5 mm Hg in the sham-operated rats and between 121 ± 4 and 129 ± 5 mm Hg in the renal-denervated group, representing no significant change from control.

Urinary Sodium Excretion Studies During DOCA-Salt Administration

As shown in table 1, there was no significant difference in daily fluid intake or daily total sodium intake between the sham-operated (n = 8) and renal-denervated (n = 9) rats studied in metabolic cages during the first 24 days of DOCA-salt administration. In addition, there was no significant difference in weekly weight gain between the groups (table 2).

The time course of development and degree of hypertension in these animals (fig. 2) was similar to that observed in the larger groups not housed in metabolic cages (fig. 1). The sham-operated group developed an increase in pressure earlier (by Day 7) and maintained a higher pressure than the renal-denervated group through Day 24 (fig. 2). Sham-operated and renal-denervated rats excreted 30 ± 4% of the ingested sodium during the first 2 days of DOCA-salt treatment; from Days 3 through 7, fractional urinary sodium excretion in sham animals remained unchanged from control, while blood pressure increased significantly (p < 0.05). In con-
Male Sprague-Dawley Rats During Days 1–SS of DOCA-Salt Administration

contrasting, fractional urinary sodium excretion increased significantly in renal-denervated rats when compared to control (p < 0.01), while blood pressure remained at control levels; this excretion remained significantly greater than that of sham-operated rats between Days 7 and 14, a time when blood pressure was not increased in renal-denervated rats but was significantly so in sham-operated animals. In contrast, between Days 15 and 24, fractional urinary sodium excretions in the two groups were not significantly different. During this period, blood pressures in the renal-denervated group were significantly (p < 0.01) greater than that of sham-operated rats between Days 15 and 24, fractional urinary sodium excretion increased significantly (p < 0.01) while blood pressure remained significantly in renal-denervated rats when compared to control during the first and second weeks of DOCA-salt administration (p < 0.05). This difference disappeared during the third week.

Further examination of the weekly sodium retention per gram of body weight gained within groups (fig. 3) reveals evidence of a natriuresis during the second week of DOCA administration in both groups: the parameter decreased by 44% in the sham group (p < 0.01) and by 41% in the denervated group (p < 0.01). During the third week of DOCA-salt administration, weekly sodium retention per gram of body weight gained for sham animals was not significantly different than that observed during the first week, but was significantly greater (15%; p < 0.05) for the renal-denervated animals.

Multiple linear regression models relating sodium intake to sodium excretion were fit to the data for each week. In all cases, F tests for overall regression were found to be significant (p < 0.0001) and R² (multiple correlation coefficient) values were all greater than 0.90.

Table 2. Weekly Body Weights of Eight Sham and Nine Renal-Denervated (Denerv) Male Sprague-Dawley Rats During DOCA-Salt Administration

<table>
<thead>
<tr>
<th>Week</th>
<th>Sham (g)</th>
<th>Denerv (g)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184.0 ± 4.1</td>
<td>181.2 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>1</td>
<td>215.5 ± 4.4</td>
<td>217.0 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>248.0 ± 4.2</td>
<td>255.0 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>279.8 ± 5.6</td>
<td>285.2 ± 5.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are means ± SE.
A significant difference in slopes between sham-operated and denervated animals was found during the first week (0.27 vs -0.72, p < 0.003) and second week (0.49 vs 0.62, p < 0.04), with no difference found during the third week (0.49 vs 0.54, NS).

Renal Norepinephrine Content

There was no significant difference in kidney weight/body weight ratios between sham-operated and renal-denervated rats sacrificed at 14, 24, and 35 days of DOCA-salt administration (table 3). Renal norepinephrine contents at these time periods are shown in figure 4. At 21 days after denervation (Day 14 of DOCA-salt treatment), renal norepinephrine content was 14% that of sham-operated controls. It increased progressively and by 42 days after denervation (Day 35 of DOCA-salt treatment) there was no difference in renal norepinephrine content between the groups. There was a highly significant (p < 0.01) positive correlation between the change in blood pressure during DOCA-salt treatment and renal norepinephrine content in renal-denervated rats (fig. 5).

The sham-operated animals showed a progressive decline in renal norepinephrine content during DOCA-salt administration: renal norepinephrine content was significantly lower at 35 days of DOCA-salt administration than at 14 days (30.3 ± 3.3 vs 37.7 ± 2.4 ng/g, p < 0.05). In contrast to the renal-denervated rats, there was a highly significant (p < 0.01) inverse correlation between the change in blood pressure (blood pressure at sacrifice minus control blood pressure) during DOCA-salt treatment and renal norepinephrine content in sham-operated animals (fig. 6).

Serum Creatinine and Plasma Renin Activity

There was no significant difference in the serum creatinine of nine sham-operated rats after 24 days of DOCA-salt administration (0.61 ± 0.03 mg/100 ml) compared to the serum creatinine of 14 renal-denervated rats after 24 days of DOCA-salt (0.56 ± 0.03 mg/100 ml). There was no significant difference in plasma renin activity in nine sham-operated rats (0.17 ± 0.08 ng/ml/hr) compared to six renal-denervated rats (0.23 ± 0.14 ng/ml/hr) after 35 days of DOCA-salt administration.

Table 3. Kidney Weight/Body Weight Ratio in Sham and Renal-Denervated (Denerv) Rats Receiving DOCA-Salt

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Sham-operated rat.</th>
<th>Renal-denerv rat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.99 ± 0.05</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>24</td>
<td>0.87 ± 0.05</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>35</td>
<td>0.76 ± 0.04</td>
<td>0.83 ± 0.04</td>
</tr>
</tbody>
</table>

*Kidney weight/body weight × 100, in means ± se.
Discussion

Previous investigators have implicated the sympathetic nervous system in the pathogenesis of DOCA-salt hypertension in the rat.7-22 Our study supports these findings and emphasizes the importance of intact renal sympathetic nerves in the development of hypertension in this model. We found that renal denervation delayed the onset and attenuated the severity of the hypertension. This delay was related to a greater fractional excretion of sodium than in the sham-operated group. The subsequent development of hypertension in denervated rats was correlated with decreased fractional excretion of sodium and with increasing renal norepinephrine stores, suggesting reinnervation. These data suggest that increased renal sympathetic tone in the DOCA-salt rat facilitates sodium retention and is necessary for the development of the hypertension.

During the first week of DOCA-salt administration, both sham-operated and renal-denervated rats were in positive sodium balance. The renal-denervated group, however, had a significantly greater urinary sodium excretion than the sham rats. In conscious normotensive animals under basal conditions, the urinary excretion of sodium in response to salt-loading and the amount of sodium retained in response to mineralocorticoid administration are the same in a denervated kidney as in one with intact nerves.20,22 Since it has been previously demonstrated8 that activation of the sympathetic nervous system precedes the rise in blood pressure in the DOCA-salt model, the greater sodium retention seen in the sham rats was most likely due to the presence of increased renal sympathetic tone.

During the second week of DOCA-salt administration, both groups remained in positive sodium balance. Despite a significant rise in arterial blood pressure, the sham rats retained more sodium than the denervated animals whose pressures remained unchanged from control levels. Studies in the isolated perfused rat kidney have demonstrated that an increase in perfusion pressure of 30 mm Hg or greater results in a natriuresis.10 In spite of increments in systolic blood pressure of 40 mm Hg, the sham rats retained more sodium than the denervated animals in our experiments. This suggests that the blood pressure-sodium excretion curve for the kidney of sham rats had shifted to the right, resulting in a blunted pressure natriuresis.14 From these data we conclude that increased renal sympathetic tone was responsible for this change in the blood pressure-urinary sodium excretion relationship in the sham rats.

There was, in addition, an increase in urinary sodium excretion in both the sham-operated and renal-denervated animals during the second week of DOCA-salt treatment compared to the first week. Since this increase in sodium excretion was of similar magnitude in both groups and thus was apparently independent of blood pressure or renal sympathetic tone, it most likely represents mineralocorticoid escape.81

During the third week of DOCA-salt administration, renal-denervated rats had a rapid rise in systolic blood pressure and a fall in fractional urinary sodium excretion to the level of the sham-operated animals. Coincident with the development of hypertension in renal-denervated rats was a threefold increase in renal norepinephrine content, suggesting reinnervation. Regrowth of adrenergic nerve fibers begins in the rat heart as early as 1 week following 6-hydroxydopamine treatment and is complete by 2 months.13,14 In this preparation, regrowth of nerve fibers parallels the increase in endogenous norepinephrine levels.22 However, recovery of functional activity (as judged by
the response to tyramine) is almost completely restored 2 weeks after 6-hydroxydopamine treatment, at which time endogenous norepinephrine levels have not returned to control.\(^7\) These data suggest that the threefold increase in renal norepinephrine content seen in the fourth week following renal denervation (third week of DOCA-salt) in our experiments is compatible with the time course of reinnervation demonstrated in the rat heart. The highly significant positive correlation between renal norepinephrine content and the change in systolic blood pressure in the renal denervated rats is consistent with the notion that the increase in blood pressure seen in this group during the first 17 days of DOCA-salt treatment is due to reinnervation of the kidney. Reinnervation also can explain the similarity of fractional urinary sodium excretion between sham and denervated rats during the third week of DOCA-salt treatment. Extrapolation of the line in figure 3 to the y axis yields a value of 14 mm Hg. This is identical to the increase in systolic blood pressure seen in rats during the first 17 days of DOCA-salt treatment, suggesting that, if it were possible to produce permanent selective renal denervation, a more severe hypertension would not occur over a long period of DOCA-salt treatment.

Our observation that renal norepinephrine content in sham-operated rats decreased significantly as DOCA-salt administration continued confirms previous reports.\(^7\) In addition, we found that renal norepinephrine content in this group correlated inversely with the development of hypertension. Similar changes in tissue norepinephrine content in relation to blood pressure have been described in the hearts of DOCA-salt treated rats and are thought to be the result of increased turnover of norepinephrine secondary to chronic increased sympathetic activity.\(^5\) These data provide additional evidence for the importance of sympathetic tone in the development of DOCA-salt hypertension.

It has previously been shown that the administration of 6-hydroxydopamine, antisera to nerve growth factor, chlorisondamine, or clonidine prior to the initiation of DOCA-salt treatment prevents the development of hypertension in the rat.\(^5\) We delayed the onset and markedly attenuated the severity of the hypertension by selectively eliminating peripheral sympathetic tone from the renal vascular bed. Our study thus supports the work of others implicating the sympathetic nervous system in the pathogenesis of DOCA-salt hypertension and emphasizes the importance of increased renal sympathetic tone in the development of hypertension in this model.

Increased renal sympathetic nervous activity facilitates the retention of sodium by two mechanisms: a direct tubular effect, and renal vasoconstriction.\(^5\) From our study we are unable to determine the relative contribution of either mechanism. Nevertheless, sodium retention mediated by increased renal sympathetic tone appears important in facilitating the development of DOCA-salt hyperten-

The importance is further emphasized when one considers that there were no differences in fluid and total sodium intake, dose of mineralocorticoid, kidney size, serum creatinine, or activity of the renin-angiotensin system (other factors that have the potential of facilitating a hypertensive process) between the sham-operated and renal-denervated rats.

The influence of the renal nerves in the long-term regulation of salt and water balance is negligible in the conscious animal under basal conditions.\(^7\) However, our experiments suggest that the renal nerves influence salt and water homeostasis when there is chronically increased sympathetic nervous system activity. It has been shown that for a positive sodium balance to occur and sustained hypertension to develop, sympathetic activity must be disproportionately greater in the renal vascular bed in order to blunt a pressure natriuresis.\(^7\) It is well known that there is rich sympathetic innervation in the rat kidney. The effect of selective renal denervation on DOCA-salt-treated rats observed in our experiments suggests that a chronic disproportionate increase in renal sympathetic tone compared to other vascular beds is necessary for the development of hypertension in this model. Although it is likely that multiple etiologic factors contribute to the pathogenesis of essential hypertension, there is much evidence implicating increased renal sympathetic tone early in the course of the hypertensive process.\(^4\) This suggests that disproportionately greater stimulation to the kidney by the sympathetic nervous system could be an important mechanism for developing elevated blood pressure in essential hypertension.

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