Role of the Renal Nerves in the Maintenance of DOCA-Salt Hypertension in the Rat
Influence on the Renal Vasculature and Sodium Excretion

RICHARD E. KATHOLI, M.D., ALLAN J. NAFTILAN, M.D., PH.D., SANFORD P. BISHOP, D.V.M., PH.D., AND SUZANNE OPARIL, M.D.

SUMMARY We previously observed that the renal nerves facilitate sodium retention and contribute to the development of DOCA-salt hypertension in the rat. To determine whether the renal nerves also participate in the maintenance of DOCA-salt hypertension, we studied the effects of renal denervation after 3 or 10 weeks of DOCA-salt treatment on systolic blood pressure, urinary sodium excretion, creatinine clearance, and precapillary arteriolar wall/lumen ratios of renal, hindlimb muscle, and cremaster muscle vascular beds. Systolic blood pressures of animals given DOCA-salt reached a plateau by 3 weeks of treatment at which time a sham operation or renal denervation was performed. Sham operation in hypertensive animals resulted in no change in systolic blood pressure and no change in percent sodium intake excreted. Wall/lumen ratio of the renal precapillary arteriole in sham-operated hypertensive animals was increased compared to similar sized vessels in hindlimb and cremaster muscle. In contrast, renal denervation resulted in a natriuresis and an attenuation of the hypertension (208 ± 7 to 187 ± 7 mm Hg; p < 0.01). Wall/lumen ratio of the renal capillary arterioles in renal denervated animals was no different than similar sized vessels in hindlimb and cremaster muscle and significantly less than that seen in sham-operated animals (0.85 ± 0.05 vs 1.03 ± 0.06; p < 0.05). In another group of animals, sham operation or renal denervation was performed after 10 weeks of DOCA-salt treatment. At this time neither operation altered systolic blood pressure or sodium balance. In contrast to 3-week DOCA-salt-treated hypertensive sham-operated animals, renal precapillary arteriolar wall/lumen ratio of 10-week animals was no different than similar sized vessels in hindlimb and cremaster muscle. In addition, renal precapillary arteriolar wall/lumen ratio of 10-week DOCA-salt-treated renal-denervated animals was no different than that seen in 10-week DOCA-salt-treated sham-operated hypertensive animals. Creatinine clearance of the 10-week DOCA-salt-treated sham-operated or renal-denervated animals was significantly (p < 0.01) lower than that of the 3-week DOCA-salt-treated groups (0.25 ± 0.14 vs 1.03 ± 0.10 ml/min). These data suggest that the renal nerves contribute to the early established phase of DOCA-salt hypertension by shifting the arterial pressure-renal sodium excretion curve to the right. With time, the renal nerves play a diminishing role in the maintenance of established DOCA-salt hypertension in the rat, while other renal factors, including decreased glomerular filtration rate and probable fixed renal vascular changes, play an increasingly important role. (Hypertension 5: 427-435, 1983)

KEY WORDS • sympathetic nervous system • wall/lumen ratio • pre-capillary arteriole • arteriole • creatinine clearance • plasma renin activity • renal norepinephrine content • splenic denervation • hindlimb and cremaster muscle vasculature

INCREASED sympathetic nervous system activity and, in particular, enhanced renal sympathetic activity have been implicated in the development of deoxycorticosterone acetate (DOCA)-salt hypertension in the rat.1-16 We previously have observed that renal denervation delayed the onset and attenuated the severity of hypertension in this model, indicating that intact renal sympathetic nerves are important in its developmental phase. The delay in the development of hypertension was related to a greater fractional excretion of sodium in renal denervated than in sham-operated animals. The subsequent development of hypertension in renal-denervated animals was correlated with a decreased fractional excretion of sodium and with increasing renal norepinephrine stores, suggesting renal reinnervation. These studies suggested that increased efferent renal sympathetic nerve activity in the DOCA-salt rat facilitates sodium retention and contributes to the development of hypertension by shifting the arterial pressure-renal sodium excretion curve to the right.
Recent studies from other laboratories have implicated additional factors in the pathogenesis of DOCA-salt hypertension, such as vasopressin, a circulating pressor substance that suppresses vascular sodium-pump activity, increased vascular reactivity, and structural changes in blood vessels.\textsuperscript{17-21} Whatever additional factors contribute to a hypertensive process, systems analysis of arterial pressure control emphasizes the important role of the kidney in both the development and the long-term maintenance of hypertension.\textsuperscript{22} The objective of the current study was to define the contribution of the renal nerves to the maintenance of DOCA-salt hypertension. To examine this question, kidneys were denervated 3 weeks after initiation of DOCA-salt administration, a time when the blood pressure had just reached a plateau, and 10 weeks after initiation of DOCA-salt treatment, a time when the hypertension was well established. Discontinuing DOCA-salt treatment after 10 weeks often fails to ameliorate the hypertension, suggesting that structural vascular and/or renal parenchymal changes have developed.\textsuperscript{23} Thus, we examined the effects of renal denervation not only on blood pressure and sodium excretion but also on creatinine clearance, plasma renin activity, and precapillary arteriolar and arteriolar wall/lumen ratios of the renal, cremaster muscle, and hindlimb muscle vascular beds in order to define the contribution of the renal nerves to the maintenance of fixed DOCA-salt hypertension in the rat.

\section*{Methods}

\subsection*{Animal Preparation}
Male Sprague-Dawley rats (Southern Animal Farms, Prattville, Alabama) were subjected to unilateral right nephrectomy at 3 weeks of age. Following nephrectomy, 14 days were allowed for compensatory renal hypertrophy to occur before DOCA-salt treatment began. DOCA-Percorten Pivalate (Ciba-Geigy Corporation, Summit, New Jersey) was administered weekly by subcutaneous injection of 0.4 ml of a suspension containing, per milliliter of water: 25 mg DOCA, 10.5 mg methyl cellulose, 3 mg carboxymethylcellulose, 1 mg polysorbate 80, and 8 mg NaCl. Salt was administered by substitution of 1\% of NaCl solution for drinking water. The normotensive control groups included age- and sex-matched uninephrectomized sham-operated or renal-denervated rats that received weekly injections of the vehicle suspension without DOCA and were allowed to drink tap water ad libitum in order to follow the change in blood pressure due to growth. Renal denervation or a sham operation was performed 3 or 10 weeks after initiation of DOCA-salt treatment. Renal denervation was accomplished 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 consisted of opening and closing the flank on the side of the remaining kidney. As an additional control, splenic denervation was performed 3 weeks after initiation of DOCA-salt treatment. This was done to rule out the possibility that the lowering of arterial pressure and transient increase in sodium excretion observed with renal denervation of 3-week DOCA-salt-treated animals was due to a nonspecific or nephrotoxic effect of phenol or to an effect of surgery or denervation of a visceral organ with an attendant natriuresis or decrease in peripheral resistance.

\subsection*{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 weekly using the tail-cuff method without anesthesia.\textsuperscript{24} Animals were weighed weekly.

To examine the effects of renal denervation on the maintenance of DOCA-salt hypertension, sham-operated DOCA-salt-treated rats, renal-denervated DOCA-salt-treated rats, splenic-denervated DOCA-salt-treated rats, sham-operated vehicle-treated rats, and renal-denervated vehicle-treated rats were compared. Renal denervation, splenic denervation, or sham operation was performed after 3 weeks of DOCA-salt treatment in some animals. Renal denervation or sham operation was performed after 10 weeks of DOCA-salt treatment in others. The effects of renal denervation on sodium handling were determined by measuring urinary sodium excretion in sham-operated, splenic-denervated, and renal-denervated rats. These animals were housed individually in metabolic cages (Hoeltge-Acme, Cincinnati, Ohio) that had drinking bottles and food cups on the outside of the cage to avoid contamination of urine collections. During sodium excretion studies, rats remained in the cages continuously except for short periods on 1 day 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 6 out of 7 days on 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, Indiana) containing 0.29\% sodium, ground, and mixed with distilled water to form a paste. This deterred 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. At the end of each 24-hour collection period, the collection funnel of each cage was rinsed with 50 ml of distilled water and the rinse saved for analysis of sodium content. 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 urine volume and urine sodium concentration (mEq/liter) plus the product of the wash volume and wash sodium concentration. Urine sodium concentration was measured by flame photometry. Fecal sodium excretion was not measured because it normally represents only 1\% to 2\% of total sodium excretion and would be expected to be the same in both denervated and sham-operated groups.\textsuperscript{25} Urinary sodium excretion was measured weekly using the tail-cuff method without anesthesia. Animals were weighed weekly.
tion was expressed as percent of intake (24-hour urinary sodium excretion divided by 24-hour total sodium intake × 100).

Vessel Measurements

After 5 and 12 weeks of DOCA-salt administration, four normotensive control, four sham-operated, and four renal-denervated animals were randomly selected for morphometric study of resistance vessels. The animals were anesthetized with ether and the thoracic aorta cannulated with an 18 gauge needle. The perfusion system was attached to a reservoir maintained at a height adjusted to provide 100 mm Hg hydrostatic pressure. Heparinized saline was infused into the aorta to wash blood from the systemic circulation, following which 2% phosphate buffered glutaraldehyde (330 mOsm) was infused for approximately 10 minutes for perfusion fixation of vessels. Fixed tissues from renal, cremaster muscle, and hindlimb muscle vascular beds were removed from the animal and fixed for an additional 2 hours in glutaraldehyde. These tissues were cut in a plane to obtain cross sections of as many parenchymal vessels as possible. The tissues were cut so that each vessel could only be present in one section. Tissues were stored in phosphate buffer wash, dehydrated through ascending grades of alcohol, and flat-embedded in Spur epoxy resin. Sections were cut with a Sorvall JB-4 microtome with glass knives at a thickness of 1 μm and were stained with toluidine blue for examination.

Morphometric studies were performed using a sonic digitizer (Science Accessories) interfaced to a programmable desktop calculator (Hewlett Packard 9825A, Andover, Massachusetts). The tissue sections were projected onto the digitizer work surface from a Zeiss mercury lamp microslide projector and measurements made with the digitizer directly from the projected image. The system was calibrated at each work session so that each vessel could only be present in one section. Sections were cut with a Sorvall JB-4 microtome with glass knives at a thickness of 1 μm and were stained with toluidine blue for examination.

We used the following designations in studying arterial vessels from the kidney, cremaster muscle, and hindlimb muscle. Vessels of 15 to 30 μm in outer diameter were designated as precapillary arterioles and those of 30 to 100 μm as arterioles. Outer diameter and wall thickness of a total of 3640 vessels from the three vascular beds were measured. Each vessel was measured four times at the shortest cross-sectional diameter, and mean values were calculated. Lumenal diameter was calculated by subtracting wall thickness × 2 from the outer diameter. Wall/lumen ratio was calculated as wall thickness divided by the lumenal radius of the vessel. Slides were coded so that the measurements were made "blind."

Biochemical Studies

After 5, 7, and 12 weeks of DOCA-salt administration, the animals not subjected to vessel studies were sacrificed to measure renal and splenic norepinephrine content for confirmation of the completeness of renal or splenic denervation and for measurement of plasma creatinine concentration and renin activity. Animals were sacrificed by decapitation without anesthesia. Blood was collected in iced tubes containing EDTA (1 mg/kg) for determination of creatinine and plasma renin activity. Kidneys and spleens were removed and rapidly frozen in liquid nitrogen for subsequent determination of renal and splenic norepinephrine content. Plasma renin activity was measured by radioimmunoadsay of generated angiotensin I according to the method of Haber et al. Renal and splenic norepinephrine content was measured (Cat-A-Kit, Upjohn, Kalamazoo, Michigan) using a modification of the radio-enzymatic method of Peuler and Johnson.

Numerical results are expressed as means ± 1 se. Statistical analysis of the blood pressure data was performed using analysis of variance based on a split-plot in time model. A one-way analysis of variance was performed on the means of the vessel measurements for each rat. Comparisons of group means from the morphometric studies were done by Duncan's multiple comparison procedures.

Results

Effect of Renal Denervation on the 3-Week DOCA-Salt Hypertensive Rat

Blood Pressure

DOCA-salt administration in 40 rats produced a rise in systolic blood pressure which reached a plateau by 3 weeks (fig. 1, upper panel) (p < 0.001). Twenty rats underwent a sham operation after 3 weeks of DOCA-salt treatment and were observed for changes in blood pressure for 2 weeks; eight of these were observed for a total of 4 weeks after operation. As shown in figure 1 (top panel) systolic blood pressure after sham operation remained unchanged throughout the period of observation.

Twenty rats underwent renal denervation after 3 weeks of DOCA-salt treatment and were observed for 2 weeks; eight of these were observed for a total of 4 weeks after operation. In contrast to the sham-operated animals, renal denervation resulted in a significant decrease in systolic blood pressure from 208 ± 7 to 187 ± 7 mm Hg (p < 0.01). By 4 weeks post renal denervation, however, blood pressure had increased again so that there was no difference between the renal-denervated and sham-operated groups (fig. 1, top panel). Eight additional rats underwent splenic denervation after 3 weeks of DOCA-salt treatment and were observed for 2 weeks. Similar to the sham-operated animals, systolic blood pressure after splenic denervation remained unchanged throughout the period of observation (209 ± 9 before and 207 ± 9 mm Hg after splenic denervation).

Twelve sham-operated and eight renal-denervated control rats given vehicle injections and tap water were observed for 5 weeks. Baseline systolic blood pressures of these animals (119 ± 4 mm Hg) were not significantly different from the baseline blood pressures of the rats that subsequently received DOCA-salt treatment. Over the 5 weeks of observation, systolic
blood pressure ranged between 115 and 130 mm Hg in the vehicle injection-tap water-treated control groups, representing no significant change from baseline.

Urinary Sodium Excretion

During the 3rd week of DOCA-salt administration, by which time the blood pressure had reached a plateau, the percentage of sodium intake excreted was 80% ± 4% (fig. 1 lower panel). During the 1st week after sham operation or renal denervation (four weeks of DOCA-salt), at a time when blood pressure was decreasing in renal denervated animals, the percentage of sodium intake excreted of the renal-denervated group was 13% (p < 0.01) greater than that of sham animals. Thereafter there was no significant difference between groups in percentage of sodium intake excreted. During the 4th week after renal denervation, at a time when blood pressure was increasing, percentage of sodium intake excreted in this group remained unchanged. Table 1 demonstrates that there was no significant difference in weekly fluid or sodium intake between sham-operated and renal-denervated rats during the 4 weeks after operation. There was no significant difference between the two groups in weekly weight gain.

Additional control experiments revealed that during the 2 weeks after operation in DOCA-salt-treated animals there was no significant difference in percentage of sodium intake excreted, weekly fluid or sodium intake or weekly weight gain between sham-operated and splenic-denervated rats. Further, renal denervation did not significantly alter weekly urinary sodium excretion or fluid or sodium intake in normotensive control rats.

Creatinine Clearance

There was no significant difference in creatinine clearance among renal-denervated (0.98 ± 0.26 ml/min; n = 8), splenic-denervated (0.91 ± 0.15 ml/min; n = 8) and sham-operated (1.03 ± 0.13 ml/min; n = 8) animals 2 weeks after operation (5 weeks of DOCA-salt administration). Creatinine clearances of renal-denervated, splenic-denervated, and sham-operated DOCA-salt-treated animals were significantly lower than those of one-kidney normotensive controls (1.71 ± 0.17 ml/min; n = 8) (p < 0.001) or those of one-kidney normotensive renal-denervated animals (1.59 ± 0.21 ml/min; n = 8) (p < 0.001).

Plasma Renin Activity

There was no significant difference in plasma renin activity in renal-denervated rats (0.19 ± 0.11 ng/ml/hr; n = 8) compared to splenic-denervated rats (0.15 ± 0.05 ng/ml/hr; n = 8) or sham-operated rats (0.21 ± 0.09 ng/ml/hr; n = 8) 2 weeks after operation (5 weeks of DOCA-salt administration). Plasma renin activity of renal-denervated, splenic-denervated, and sham-operated animals was significantly lower (p < 0.001) than that of one-kidney normotensive controls (1.3 ± 0.6 ng/ml/hr; n = 8) or of one-kidney normotensive renal-denervated animals (1.7 ± 0.8 ng/ml/hr; n = 8).

Renal Norepinephrine Content

Two weeks following operation (5 weeks of DOCA-salt treatment), renal norepinephrine content of renal-denervated animals (n = 8) was 91% lower than that of control rats (3.8 ± 1.4 vs 42.4 ± 3.5 ng/g; p < 0.001), while renal norepinephrine content of splenic-denervated animals (31.2 ± 2.2 ng/g; n = 8) and sham-operated animals (33.8 ± 3.2 ng/g; n = 8) was 20% lower (p < 0.01) than that of control rats (n = 8). Renal norepinephrine content of renal-denervated normotensive control animals was 4.1 ± 2.0 ng/g (n = 8).

Four weeks following operation (7 weeks of DOCA-salt treatment), renal norepinephrine content of renal-denervated animals (25.5 ± 3.1 ng/g; n = 7) was significantly (p < 0.01) greater than that seen in der-
Fluid and Total Sodium Intake and Urinary Sodium Excretion of Eight Sham and Eight Renal-Denervated Male Sprague-Dawley Rats During the 4th Through 7th Weeks of DOCA-Salt Administration (Following Renal Denervation Done After 3 Weeks of DOCA-Salt)

<table>
<thead>
<tr>
<th>Week</th>
<th>Fluid intake (ml)</th>
<th>Total Na intake (mEq)</th>
<th>Urinary Na excretion (mEq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Denerv</td>
<td>p</td>
</tr>
<tr>
<td>4</td>
<td>66±7</td>
<td>64±8</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>71±8</td>
<td>70±7</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>79±9</td>
<td>79±9</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>85±8</td>
<td>86±9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± se weekly values for daily intake or excretion.
p values represent comparisons between sham and denervated (Denerv) animals.

Table 2. Outer Diameter (O.D.) and Wall Thickness (W.T.) of Precapillary Arterioles (15–30 μ) and Arterioles (30–100 μ) of Kidney, Cremaster Muscle, and Hindlimb Muscle from Normotensive Control Animals and 5-Week Sham-Operated and Renal-Denervated (Denerv) DOCA-Salt-Treated Animals (2 Weeks After Operation)

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>Control</th>
<th>Sham</th>
<th>Denerv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precapillary arteriole*</td>
<td>23.44±0.73</td>
<td>23.36±1.42</td>
<td>23.43±0.73</td>
</tr>
<tr>
<td>O.D. μ</td>
<td>M. S.</td>
<td>5.32±0.23</td>
<td>5.37±0.26</td>
</tr>
<tr>
<td>W.T. μ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cremaster muscle</td>
<td>23.18±1.53</td>
<td>23.23±0.89</td>
<td>23.18±1.53</td>
</tr>
<tr>
<td>O.D. μ</td>
<td>24.29±0.60</td>
<td>24.04±0.43</td>
<td>24.25±0.62</td>
</tr>
<tr>
<td>W.T. μ</td>
<td>6.07±0.25</td>
<td>5.61±0.29</td>
<td>5.36±0.31</td>
</tr>
<tr>
<td>Hindlimb muscle</td>
<td>48.74±2.65</td>
<td>47.84±1.71</td>
<td>48.03±1.31</td>
</tr>
<tr>
<td>O.D. μ</td>
<td>47.76±2.72</td>
<td>47.65±1.82</td>
<td>47.51±2.11</td>
</tr>
<tr>
<td>W.T. μ</td>
<td>6.08±0.40</td>
<td>8.21±0.43</td>
<td>7.60±0.38</td>
</tr>
</tbody>
</table>

*Measurements were made in four animals in each group. Values represent means ± se.

Two weeks following operation (5 weeks of DOCA-salt treatment), renal norepinephrine content of sham-operated animals was 37.1 ± 3.8 ng/g (n = 8).

Spleen Norepinephrine Content

Two weeks following operation (5 weeks of DOCA-salt treatment), splenic norepinephrine content of splenic-denervated hypertensive animals (n = 8) was 94% lower than that of normotensive control rats (3.1 ± 0.5 vs 49.8 ± 4.5 ng/g; p < 0.01), while splenic norepinephrine content of sham-operated hypertensive animals (36.0 ± 6.0 ng/g; n = 8) was 28% lower (p < 0.01) than that of control rats (n = 8).

Vessel Diameter and Wall to Lumen Ratios

Precapillary Arterioles. Table 2 and figure 2 show that the wall/lumen ratios of precapillary arterioles in the cremaster muscle and hindlimb muscle were not different from control in either renal denervated or sham-operated animals. In contrast, wall/lumen ratios of renal precapillary arterioles of sham-operated hypertensive DOCA-salt-treated animals were significantly greater than those of control animals. The wall/lumen ratios of renal precapillary arterioles of the DOCA-salt-treated sham-operated animals were also significantly greater than those seen in similar sized vessels of cremaster muscle and hindlimb muscle.

Wall/lumen ratios of renal precapillary arterioles in renal-denervated animals were not significantly different than those in control animals or in cremaster muscle and hindlimb muscle of sham-operated hypertensive animals.

Figure 2. Wall/lumen ratio in precapillary arterioles of the kidney, cremaster muscle, and hindlimb muscle from normotensive control animals and 5-week sham-operated and renal-denervated DOCA-salt-treated animals (2 weeks after operation).

The asterisk represents p < 0.05 compared to control.
Arterioles. As shown in table 2 and figure 3, wall/lumen ratios of arterioles in the kidney, cremaster muscle, and hindlimb muscle were significantly greater in the sham-operated hypertensive DOCA-salt-treated rats compared to normotensive control animals. In contrast to the observation for precapillary arterioles, there was no difference in arteriolar wall/lumen ratios between the sham-operated and renal-denervated DOCA-salt-treated animals.

Effect of Renal Denervation on the 10-Week DOCA-Salt Hypertensive Rat

Blood Pressure

These animals (n = 24) became hypertensive by 3 weeks of DOCA-salt administration and remained hypertensive throughout the 10 weeks of observation prior to operation. As shown in figure 4 (upper panel), renal denervation resulted in no change in blood pressure over 2 weeks of observation. Systolic blood pressures of age- and sex-matched control rats (n = 12) given vehicle injections and tap water and observed for 12 weeks ranged between 115 and 135 mm Hg, representing no significant change from baseline.

Urinary Sodium Excretion

As shown in figure 4 (lower panel), renal denervation did not alter the percentage of sodium intake excreted. There was no significant difference in weekly fluid or sodium intake between sham-operated and renal-denervated rats during the 2 weeks after operation. In addition, there was no significant difference between the two groups in weekly weight gain.

Creatinine Clearance

There was no significant difference in creatinine clearance between renal-denervated (0.18 ± 0.05 ml/min; n = 8) and sham-operated hypertensive (0.25 ± 0.14 ml/min; n = 7) animals 2 weeks after operation (12 weeks of DOCA-salt). Creatinine clearances of renal-denervated and sham-operated hypertensive 12 week DOCA-salt-treated animals were significantly lower (p < 0.01) than those of one-kidney age- and sex-matched normotensive (1.60 ± 0.15 ml/min; n = 7) controls and of the 5-week DOCA-salt hypertensive groups (renal-denervated: 0.98 ± 0.26 ml/min; n = 8 and sham-operated: 1.03 ± 0.13 ml/min; n = 8) at 2 weeks after operation.

Plasma Renin Activity

There was no significant difference in plasma renin activity in renal-denervated rats (0.20 ± 0.09 ng/ml/hr; n = 8) compared to sham-operated hypertensive rats (0.18 ± 0.12 ng/ml/hr; n = 7) 2 weeks after operation (12 weeks of DOCA-salt administration). Plasma renin activity of renal-denervated and sham-operated animals was significantly lower than that of one-kidney normotensive controls (1.2 ± 0.6 ng/ml/hr; n = 6).

![Figure 3. Wall/lumen ratio in arterioles of the kidney, cremaster muscle, and hindlimb muscle from normotensive control animals and 5-week sham-operated and renal-denervated DOCA-salt treated animals (2 weeks after operation). The asterisks represent p < 0.05 compared to control.](image)

![Figure 4. Blood pressure and percentage of sodium intake excreted in DOCA-salt-treated rats. Arrows indicate the time of renal denervation or sham operation after 10 weeks of DOCA-salt treatment.](image)
TABLE 3. Outer Diameter (O.D.) and Wall Thickness (W.T.) of Precapillary Arterioles (15–30 μ) and Arterioles (30–100 μ) of Kidney, Cremaster Muscle, and Hindlimb Muscle from Normotensive Control Animals and 12-Week Sham-Operated and Renal-Denervated (Denerv) DOCA-Salt-Treated Animals (2 Weeks After Operation)

<table>
<thead>
<tr>
<th>Vascular Bed</th>
<th>Control</th>
<th>Sham</th>
<th>Denerv</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIDNEY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precapillary arteriole*</td>
<td>22.31 ± 0.85</td>
<td>23.89 ± 0.55</td>
<td>22.56 ± 1.24</td>
</tr>
<tr>
<td>Arteriole*</td>
<td>49.18 ± 1.78</td>
<td>50.87 ± 2.44</td>
<td>48.66 ± 1.92</td>
</tr>
<tr>
<td><strong>CREMASTER MUSCLE</strong></td>
<td>23.11 ± 0.99</td>
<td>23.94 ± 0.94</td>
<td>23.41 ± 0.87</td>
</tr>
<tr>
<td><strong>HINDLIMB MUSCLE</strong></td>
<td>5.14 ± 0.26</td>
<td>5.52 ± 0.3</td>
<td>5.22 ± 0.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular Bed</th>
<th>Control</th>
<th>Sham</th>
<th>Denerv</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIDNEY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precapillary arteriole*</td>
<td>23.40 ± 0.98</td>
<td>24.41 ± 0.70</td>
<td>23.10 ± 0.95</td>
</tr>
<tr>
<td>Arteriole*</td>
<td>47.36 ± 2.13</td>
<td>47.68 ± 1.97</td>
<td>46.43 ± 1.88</td>
</tr>
<tr>
<td><strong>CREMASTER MUSCLE</strong></td>
<td>5.10 ± 0.34</td>
<td>5.40 ± 0.3</td>
<td>5.27 ± 0.32</td>
</tr>
<tr>
<td><strong>HINDLIMB MUSCLE</strong></td>
<td>8.25 ± 0.36</td>
<td>8.25 ± 0.44</td>
<td>7.80 ± 0.38</td>
</tr>
</tbody>
</table>

Measurements were made in four animals in each group. Values represent means ± SE.

Renal Norepinephrine Content

Two weeks following operation (12 weeks of DOCA-salt), renal norepinephrine content of renaldenervated animals (n = 7) was 85% lower than that of normotensive control rats (6.2 ± 1.6 vs 41.1 ± 3.2 ng/g; p < 0.001). Renal norepinephrine content of sham-operated animals (40.0 ± 3.7 ng/g; n = 8) was not significantly different than that of control rats (n = 8).

Vessel Diameter and Wall-to-Lumen Ratios

Precapillary Arterioles. As shown in table 3 and figure 5, wall/lumen ratios of precapillary arterioles in the kidney, cremaster muscle, and hindlimb muscle of sham-operated and renal-denervated hypertensive animals were not different from control. Comparison of precapillary arteriolar wall/lumen ratios in the kidneys of 5- and 12-week sham-operated DOCA-salt-treated hypertensive animals revealed that the 5-week group (fig. 2) had significantly (p < 0.05) greater precapillary arteriolar wall/lumen ratios.

Arterioles. As shown in table 3 and figure 6, wall/lumen ratios of arterioles in the kidney, cremaster muscle, and hindlimb muscle were significantly greater in sham-operated hypertensive DOCA-salt-treated compared to normotensive control animals. There was no difference in arteriolar wall/lumen ratios between the sham-operated and renal-denervated groups. Comparison of both sham-operated and renal-denervated 5-week (fig. 3) and 12-week (fig. 6) DOCA-salt-treated animals revealed no difference in arteriolar wall/lumen ratios among the three vascular beds.

Discussion

Previous studies have implicated the sympathetic nervous system in the pathogenesis of DOCA-salt hypertension in the rat. We previously have emphasized the importance of intact renal nerves in the devel-
operand of hypertension in this model. In the current study, renal denervation in the DOCA-hypertensive rat after 3 weeks of treatment resulted in a decreased renal precapillary arteriolar wall/lumen ratio associated with a natriuresis and an attenuation of the hypertension. In contrast, renal denervation after 10 weeks of DOCA-salt treatment resulted in no change in blood pressure, sodium excretion, or in wall/lumen ratio of any of the vascular beds examined. These data provide evidence suggesting that the efferent renal sympathetic nerves play a diminishing role with time during the course of hypertension in the DOCA-salt rat.

During the early established phase of DOCA-salt hypertension (3 weeks of DOCA-salt treatment), the renal precapillary arteriole had a greater wall/lumen ratio than seen in comparable vessels in the cremaster or hindlimb muscles. An increased precapillary arteriole wall/lumen ratio could be due to medial smooth muscle hypertrophy and/or vasoconstriction. Regardless of the mechanism, an increased precapillary wall/lumen ratio would result in increased vascular resistance. It is probable that this increased renal precapillary arteriolar wall/lumen ratio was due to efferent renal sympathetic nerve influence since renal denervation decreased it to control levels. The reduction in precapillary arteriolar wall/lumen ratio seen in the kidney post-denervation was associated with a natriuresis and a decrease in blood pressure. These observations suggest that the decrease in systolic blood pressure with renal denervation in the 3-week DOCA-salt-treated rat is due to a leftward shift in the arterial pressure-sodium excretion curve for the kidney. The subsequent return of blood pressure to levels of sham-operated rats 4 weeks after renal denervation was associated with no change in the percentage of sodium intake excreted.

This suggests a relative retention of sodium in view of the increase in pressure. These changes in blood pressure and sodium excretion occurred in association with an increase in renal norepinephrine content, suggesting reinnervation and providing further evidence that the renal sympathetic nerves contribute to the maintenance of early established DOCA-salt hypertension by shifting the renal arterial pressure-sodium excretion curve rightward. Although these studies suggest that interruption of efferent renal sympathetic nerve pathways is an important factor in the attenuation of the hypertension, the possibility cannot be excluded that loss of afferent renal nerve pathways has occurred in the kidney due to the sustained hypertension. Furthermore, the decreased creatinine clearance of 12-week DOCA-salt-treated hypertensive animals compared to 5-week animals further suggests that renal parenchymal damage has occurred in the kidney due to the sustained hypertension. This loss of renal function would also facilitate the hypertensive process.

The precapillary arteriolar wall/lumen ratios found in 10-week DOCA-salt-treated animals were no different than control. Another factor contributing to the failure of renal denervation to decrease blood pressure in 10-week DOCA-salt animals could be the appearance of structural changes in the various vascular beds secondary to sustained hypertension. Consistent with this is the finding of increased arteriolar wall/lumen ratios in all three vascular beds. These changes could reflect increases in both renal vascular and total systemic vascular resistance that were not reversible by denervation. Furthermore, the decreased renal function would also facilitate the hypertensive process.

Renal denervation significantly decreased the renal precapillary arteriolar wall/lumen ratio, but did not alter the wall/lumen ratio of renal arterioles. These observations suggest that the efferent renal sympathetic nerves selectively influence the renal precapillary arteriole but not the renal arteriole at this stage in DOCA-salt hypertension. This selective effect of the renal nerves on the renal precapillary arteriole could be due to a greater sensitivity to locally (neurally) released vasoactive substances or to trophic influences. It has been previously observed in other vascular beds that precapillary arterioles have a higher sensitivity to vasoactive substances than do arterioles. Alternatively one might postulate that the efferent renal nerves selectively influence the renal precapillary arteriole but not the renal arteriole at this stage because of differences in innervation between these classes of renal vessels. The renal arteriole, which is thought to be the resistance vessel, had an increased wall/lumen ratio which was unaltered by renal denervation. This increase in renal vascular resistance could explain why blood pressure was attenuated but not normalized following renal denervation at this stage.

Although renal denervation significantly altered blood pressure, sodium excretion, and renal precapillary arteriolar wall/lumen ratios in 3-week DOCA-salt-treated rats, similar changes were not observed after renal denervation in 10-week animals, suggesting that the renal nerves are not important in maintaining late-established hypertension in this model. One possible explanation for the failure of renal denervation to decrease blood pressure in 10-week DOCA-salt animals is that efferent renal sympathetic nerve activity may not be increased at this time. Consistent with this interpretation is the observation that renal precapillary arteriolar wall/lumen ratios in 10-week DOCA-salt-treated animals were no different than control, suggesting that norepinephrine turnover was not altered secondary to chronically increased sympathetic activity. Another factor contributing to the failure of renal denervation to decrease blood pressure in 10-week DOCA-salt animals could be the appearance of structural changes in the various vascular beds secondary to sustained hypertension. Consistent with this is the finding of increased arteriolar wall/lumen ratios in all three vascular beds. These changes could reflect increases in both renal vascular and total systemic vascular resistance that were not reversible by denervation.
comparable vessels in both cremaster and hindlimb muscles and that this increase was reversed by renal denervation. These findings strongly suggest a disproportionate increase in efferent renal sympathetic nerve activity in the 3-week DOCA-salt rat which has disappeared by 10 weeks of DOCA-salt treatment.6, 21, 22, 37 These observations also support the work of others that the precapillary arterioles are important regulators of blood flow through vascular beds.33

Acknowledgments

The authors express their gratitude to Terry J. Koerner, Braxton C. Bowdoin, Janice D. Nauffian, and Kathleen Beul for their technical assistance; Dr. Charles R. Katholi for assistance in statistical analysis of the data; Drs. Salah El Dorccr and Robert Cosgrove of Southern Research Institute for the use of their metabolic cages; and Ciba-Geigy Corporation, Summit, New Jersey, for supplying Per-

References

Role of the renal nerves in the maintenance of DOCA-salt hypertension in the rat. Influence on the renal vasculature and sodium excretion.
R E Katholi, A J Naftilan, S P Bishop and S Oparil

Hypertension. 1983;5:427-435
doi: 10.1161/01.HYP.5.4.427

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/4/427

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/