Role of renal nerves in development of hypertension in DOCA-salt model in rats: a telemetric approach

Frédéric Jacob, Leah A. Clark, Pilar Ariza Guzman, and John W. Osborn

Department of Physiology, Lillehei Heart Institute, University of Minnesota, Minneapolis, Minnesota

Submitted 2 March 2005; accepted in final form 25 May 2005

Jacob, Frédéric, Leah A. Clark, Pilar Ariza Guzman, and John W. Osborn. Role of renal nerves in development of hypertension in DOCA-salt model in rats: a telemetric approach. Am J Physiol Heart Circ Physiol 289: H1519–H1529, 2005. First published June 3, 2005; doi:10.1152/ajpheart.00206.2005.—Centrally mediated hyperactivity of the autonomic nervous system contributes to DOCA hypertension; however, the targeted peripheral vascular bed(s) remain unclear. We propose that if renal sympathetic activity is a factor in the development of DOCA-salt hypertension, then renal denervation (RDNX) should attenuate the hypertensive response. In protocol 1, uninephrectomized RDNX (n = 9) and sham-denervated (n = 6) Sprague-Dawley rats were allowed free access to 0.9% NaCl solution and 0.1% NaCl diet. Mean arterial pressure (MAP) and heart rate were telemetrically recorded for 4 days before and 36 days after DOCA (100 mg/rat) implantation; sodium and water balances were recorded daily. Protocol 2 was similar except that saline intake in sham rats (n = 7) was matched to that observed in RDNX rats of protocol 1 for 30 days; for the last 10 days, the rats were allowed free access to saline. Before DOCA in protocol 1, MAP was lower (P < 0.05) in RDNX rats (99 ± 1 mmHg) compared with sham rats (111 ± 3 mmHg); however, heart rate and sodium and water balances were similar between groups. RDNX attenuated the MAP response to DOCA by ~50% (ΔMAP = 22 ± 3 mmHg, where Δ is change in MAP) when compared with sham rats (ΔMAP = 38 ± 6). RDNX rats consumed significantly less saline than sham rats, and cumulative sodium and water balances were reduced by 33% and 23%, respectively. In protocol 2, a similar pattern in MAP elevation was observed in RDNX and saline-restricted, sham-denervated rats even when saline restriction was removed. These results indicate that the renal sympathetic nerves are important in hypertension development but that other factors are also involved.

Deoxycorticosterone-salt hypertension; renal denervation; sympathetic renal nerves

Primary hypertension is an important risk factor for the development of cardiovascular diseases; however, the mechanisms underlying the initiation and maintenance of the hypertensive process remain unclear (4). Emerging evidence suggests that sympathetic nervous system overactivity plays a crucial role in the development of some forms of primary hypertension (6, 8, 18). One long-standing hypothesis proposes that neurogenic hypertension is the result of alterations in renal function exclusively via the actions of renal nerves on renal vascular resistance, tubular sodium reabsorption, and renin release (12, 22). Alternatively, other reports suggest that increased sympathetic activity to non-renal vascular beds could lead to a primary increase in peripheral vascular resistance and hypertension (13, 38).

In the DOCA-salt model, substantial evidence exists that centrally mediated hyperactivity of the sympathetic system is essential in the pathogenesis of this model of hypertension (5, 19). However, the peripheral vascular beds targeted by increased sympathetic output remain to be clearly established. It has been suggested that increased renal sympathetic nerve activity is responsible for the developmental phase of this model because renal denervation (RDNX) delayed, but did not abolish, the elevation in arterial pressure (27, 28, 38). However, this has not been a consistent finding because it has also been reported that RDNX had no effect on the development of hypertension in the DOCA-salt model (13).

There are three plausible explanations for the inconsistencies observed in these previous studies (13, 27, 28, 38) regarding the role of renal nerves in DOCA-salt hypertension. First, although this model is neurogenic, it may be driven by increased sympathetic activity to vascular beds other than the kidney. Second, it is possible that the model is not neurogenically driven but rather is due to the direct actions of DOCA on renal function. Finally, part of the disagreement on the role of renal nerves in DOCA-salt hypertension may be due to methodological differences in the measurement of arterial pressure. In many of the previous studies, arterial pressure was recorded indirectly with the tail-cuff method in restrained animals (13, 27, 28, 38) and, in some of these reports, in conjunction with direct arterial pressure measurements in anesthetized rats (13, 38). Not only do these techniques acutely alter sympathetic nerve activity and mean arterial pressure (MAP), they also only capture a “snapshot” of arterial pressure. As a result, these methods may not accurately reflect true differences in basal levels of arterial pressure over time.

Recently, methods for telemetric recording of arterial pressure in conscious unrestrained rats have become available. This technique provides a more reliable and accurate method to investigate subtle, physiologically relevant changes in arterial pressure over long periods of time. For example, using this method, we have recently reported that bilateral RDNX results in a modest but sustained decrease in arterial pressure in normal rats (24, 25), an observation that had not been previously reported in studies that used other arterial pressure recording techniques.

In the present study, we used telemetric recording of arterial pressure to test the hypothesis that renal nerves contribute to the development of DOCA-salt hypertension. To test this hypothesis, we compared the arterial pressure and fluid balance responses to DOCA-salt in RDNX and sham-denervated (sham) rats.
MATERIALS AND METHODS

General Procedures and Timeline

Male Sprague-Dawley rats (275–300 g) were purchased from Harlan Sprague Dawley (Charles River Laboratory, Wilmington, MA) and housed in our animal room with controlled temperature and 12:12-h light-dark cycle.

Right unilateral nephrectomy was performed (described in Surgical Procedures), and a 14-day period was allowed for compensatory renal hypertrophy to occur in the left kidney (Fig. 1). Standard rat chow and distilled water were provided ad libitum during this period. RDNX or sham denervation was then performed, and radio telemetry transmitters were placed during the same surgical procedure (described in Surgical Procedures). During the 7-day recovery period after RDNX, the rats were individually housed in our laboratory in metabolic cages (Nalge Nunc International, Rochester, NY) and allowed ad libitum access to distilled water and 0.4% NaCl powdered food (Research Diets, New Brunswick, NJ). At the start of the protocol, the rats were provided ad libitum access to a 0.9% NaCl drinking solution and 0.1% NaCl powdered food (Research Diets, New Brunswick, NJ). On day 5 of the protocol, silicone implants impregnated with DOCA were placed subcutaneously on the dorsum of each rat (described in Surgical Procedures).

All procedures were approved by the institutional Animal Care and Use Committee and were conducted in accordance with the institutional and National Institutes of Health guidelines.

Surgical Procedures

Unilateral nephrectomy. Rats were anesthetized with a single injection of pentobarbital sodium (50 mg/kg ip) and atropine sulfate (0.2 mg/kg). Right unilateral nephrectomy was performed with the use of a retroperitoneal approach. After a right flank incision, the right kidney was visualized. The right ureter and renal vessels were isolated and then sectioned between two ligatures. The fat and connective tissue surrounding the right kidney were removed while care was taken to avoid damaging the adjacent adrenal gland. The flank incision was closed with 3-0 silk suture materials. Postoperatively, a single injection of ampicillin sodium (22 mg/kg im) was given, and pain management was provided with a single intramuscular injection of buprenorphine hydrochloride (0.05 mg/kg).

RDNX or sham denervation. Before surgery, the rats were randomly assigned to either the RDNX (n = 9) or the sham-operated (n = 6) group. Rats were anesthetized with a single injection of pentobarbital sodium (50 mg/kg ip) and atropine sulfate (0.2 mg/kg). The surgical procedure for RDNX has been previously described (40). Briefly, a ventral midline abdominal incision was performed to expose renal arteries and veins of the left kidney. Under a dissecting microscope, visible renal nerves, fat, and connective tissue were stripped from the renal vessels. Renal vessels were then painted with a 10% phenol in alcohol solution to ensure the destruction of any remaining nerves. In sham rats, a ventral midline abdominal incision was made and the left kidney was exposed briefly.

Implantation of telemetry transmitters. Continuous cardiovascular data acquisition was performed with the use of a commercially available telemetry system (Data Sciences International, St. Paul, MN). To determine whether an offset in the telemetry transmitter signal was present before implantation, the transmitter was placed onto its selected receiver, values (preimplantation) for each transmitter were recorded for 2 h, and a mean value was calculated. During the same surgical procedure, as described in RDNX or sham denervation, the abdominal aorta was exposed for implantation of the telemetry transmitter unit (model TA11PA-C40). The unit consisted of a fluid-filled catheter attached to the transmitter. The catheter was then inserted directly into the terminal aorta with the use of a 21-gauge needle as a catheter introducer and advanced cranially so that the tip was caudal to the renal arteries. The catheter was then glued into place with cyanoacrylate adhesive. The transmitter was sutured to the abdominal wall during closure of the wound. The skin was then closed with 9-mm surgical wound clips.

DOCA silicone implantation. DOCA silicone implants were made at least 72 h before surgical implantation. DOCA (100 mg) was added to 2 ml of silicone (Sylgard 184 silicone elastomer base; Dow Corning, Midland, MI) and mixed for 10 min until homogeneous. Silcone elastomercurig agent (0.2 ml) was then added to the concoction. The DOCA implants were left to cure at room temperature for 24 h and were then refrigerated at 4°C until the day of the surgery. On day 5 of the protocol, the rats were anesthetized with isoflurane, and each 100-mg DOCA silicone implant was cut into 2- to 3-mm cubes that were then placed subcutaneously between the scapular blades in each rat (100 mg DOCA/rat). The surgical procedure was performed in 15 min and was executed between 10:00 AM and 12:00 PM.

Experimental Protocols

Protocol 1. Effect of RDNX on cardiovascular and body fluid balance responses to unlimited access to salt in DOCA-salt hypertension. MAP and heart rate (HR) were recorded continuously in the sham and RDNX rats 4 days before DOCA implantation, on day 5 of DOCA implantation, and 35 days after DOCA implantation. Rats consumed a 0.1% NaCl diet and 0.9% NaCl drinking solution ad libitum throughout the 40-day protocol.

Sodium and water balances were measured throughout the protocol. Twenty-four-hour sodium intake was obtained by adding the sodium intake from the saline solution to the sodium from the food intake. The sodium intake from the saline solution was calculated by multiplying the saline intake (in ml) by the sodium concentration (0.9% = 0.154 mmol/ml). Dietary sodium intake was calculated by multiplying the food intake (in g) by the sodium content of the food (0.1% = 0.0175 mmol/g). Urine was collected over 24 h, and urine...
output was determined gravimetrically. Urinary sodium concentration was measured using a NOVA-5 + sodium-potassium analyzer (Biomedical, Waltham, MA). Twenty-four-hour urinary sodium excretion was calculated as the product of urine flow and urine sodium concentration. Sodium and water balances were calculated as the difference between intake and urinary excretion of sodium and water, respectively. Cumulative sodium and water balances were obtained by successively adding daily sodium balance or water balance recorded for each day of the protocol.

The transmitter signal was monitored by a receiver (model RLA 1010) located directly behind the metabolic cage. The receiver was connected to a BCM 100 consolidation matrix, which was connected to an IBM-compatible computer (Presario 850; Compaq). Data acquisition and analysis were performed with the use of Dataquest IV software (Data Sciences International). MAP and HR were sampled for 10 s (sampling rate of 100 samples/s) every 4 min throughout the protocol. Subsequently, the 24-h average of MAP and HR was determined. MAP and HR recordings were not stopped while daily food and water intake measurements were performed. These measurements were obtained between 10:00 and 11:00 AM and usually took 30 min to complete.

At the end of the study, the rats were euthanized and transmitters were removed from the caudal aorta. To determine whether there was an offset in the telemetry transmitter signal after removal of the device from the abdominal cavity, the transmitter was placed onto its selected receiver, values (postimplantation) for each transmitter were recorded for 2 h, and a mean value was calculated. Pre- and postimplantation values were then averaged, and the obtained value was added (when negative) or subtracted (when positive) from all recorded pressures. This offset was no more than 2 ± 1 mmHg during the experimental protocol.

Protocol 1. Effect of RDNX on Cardiovascular and Body Fluid Balance Responses to Unlimited Access to Salt in DOCA-Salt Hypertension

Cardiovascular responses in DOCA-salt hypertension. With regard to the impact of RDNX on arterial pressure, three main findings were observed (Fig. 2, top). First, basal MAP was significantly lower in RDNX rats (99 ± 1 mmHg) compared with sham rats (111 ± 3 mmHg) during the 4-day control period. Second, the MAP response to DOCA-salt was biphasic. There was an immediate response characterized by a rapid elevation in MAP, which was followed by a slower increase in arterial pressure. Finally, after DOCA silicone implantation, MAP significantly increased from control values in both groups. However, RDNX significantly attenuated the rise in MAP by ~50% in RDNX rats (ΔMAP from control = 22 ± 3 mmHg, where Δ is change in MAP) compared with sham rats (ΔMAP from control = 38 ± 6 mmHg).

When compared with their respective 4-day averaged control MAP, a significant rise in MAP was observed in both groups within 48 h after DOCA implantation. The magnitude of this pressor response was similar in both groups (~12

![Fig. 2. Comparison of 24-h mean arterial pressure (MAP, top) and heart rate (HR, bottom) between RDNX and sham rats. DOCA was implanted on the morning of day 5 in all rats (solid arrow). * Significant difference between groups at predetermined point intervals; † and ¥, significant within-group difference in MAP and HR from 4-day averaged control values in RDNX and sham rats, respectively.](http://ajpheart.physiology.org/)

AJP-Heart Circ Physiol • VOL 289 • OCTOBER 2005 • www.ajpheart.org
mmHg) so that by day 8, MAP peaked at 112 ± 2 mmHg in RDNX rats and 123 ± 4 mmHg in sham rats.

Chronically, RDNX significantly attenuated the development of hypertension. By the midpoint of the study (approximately day 20), the DOCA-induced δ was significantly less in RDNX rats (ΔMAP from control = 13 ± 2 mmHg) compared with sham rats (ΔMAP from control = 23 ± 5 mmHg). Interestingly, from this point in the protocol (day 20) onward, the differences in MAP between RDNX and sham rats became more pronounced and a notable dichotomy in HR responses and sodium and fluid balances was also observed between groups (discussed in more detail in Body fluid balance responses in DOCA-salt hypertension). By day 40, the absolute levels of MAP remained significantly lower in RDNX rats (121 ± 1 mmHg) compared with sham rats (149 ± 8 mmHg).

During the control period, 24-h average basal HR was not different between groups. HR responses after DOCA implantation were initially similar between RDNX and sham rats (Fig. 2, bottom). By the study midpoint (approximately day 20), a dichotomy in HR responses, relative to increases in MAP, began to appear. More specifically, in sham rats, HR remained stable despite a continuous rise in MAP. Conversely, HR in RDNX rats steadily declined despite a slower rise in MAP and was significantly lower when compared with sham rats on days 28-30, 32, 39, and 40. On day 40, HR in sham rats was 348 ± 11 beats/min (ΔHR from control = −57 ± 7 beats/min) compared with 319 ± 8 beats/min in RDNX rats (ΔHR from control = −88 ± 6 beats/min).

Body fluid balance responses in DOCA-salt hypertension. During the 4-day control period, averaged basal sodium intake (Fig. 3, top) and urinary sodium excretion were similar between groups (Fig. 3, middle). After implantation of DOCA, a threefold increase in sodium intake was observed in both groups. However, after this initial phase, RDNX rats tended to consume significantly less sodium compared with sham rats. This difference was notable as early as day 11 and remained significantly lower when compared with sham rats from day 26 to the end of the study. The decline in sodium intake over time in RDNX rats was of such magnitude that for the last 10 days of the protocol, salt intake was similar to their 4-day averaged control values.

Similar to what was observed for sodium intake, urinary sodium excretion rose sharply in both groups after DOCA implantation. When compared with sham rats where urinary sodium excretion remained elevated throughout the protocol, a time-dependent decrease in urinary sodium excretion was observed in RDNX rats. Consequently, significant differences in urinary sodium excretion between groups were consistently observed from day 26 to the end of the protocol. The decline in urinary sodium excretion in RDNX rats was of such magnitude that for the last 10 days of the protocol, salt intake was similar to their 4-day averaged control values.

Sodium balance was similar between RDNX and sham rats during the control period, and no significant difference in sodium balance was observed between groups after DOCA implantation (Fig. 3, bottom). Although positive sodium balance was noted in both groups after DOCA implantation, it was more apparent in sham rats because most data points were positive compared with control but not in RDNX rats. In addition, periodic positive peaks (notably days 8, 15, 22, and 36) of sodium balance were observed in sham rats but with lesser frequency and magnitude in RDNX rats (day 8).

Because the pattern of fluid intake, urine output, and water balance was very similar to the one described for sodium balances, they will only be succinctly described here. During the control period, no significant difference in saline intake (Fig. 4, top) or urine output (Fig. 4, middle) was observed between groups. The implantation of DOCA caused saline intake and urine output to significantly increase in both groups. However, as noted for sodium intake and urinary sodium excretion, saline intake and urine output tended to decrease over time in RDNX rats but not in sham rats. No significant difference in water balance was observed during the control period or after DOCA implantation (Fig. 4, bottom). However, sham rats remained in positive water balance when compared with their own control for most days but not RDNX rats. In addition, periodic positive peaks (notably for days 8, 15, 22, and 36) of water balance were observed in sham rats but with lesser frequency and magnitude in RDNX rats (day 8).
Figure 5 shows cumulative sodium (top) and water balances (bottom) in both groups. A time-dependent gradual divergence in cumulative sodium balance was observed between groups (approximately day 20) so that RDNX significantly attenuated cumulative sodium balance from days 32 to 40 when compared with sham rats. By day 40, cumulative sodium balance was reduced by 32% in RDNX rats compared with sham rats (58/1006 6 mmol in RDNX rats and 58/1006 10 mmol in sham rats). Similarly, RDNX significantly reduced cumulative water balance over time (i.e., from days 25 to 40) compared with rats with intact renal nerves. As observed in protocol 1, MAP in RDNX rats was significantly lower (~8 mmHg) than in rats with intact renal nerves. However, HR and all variables for sodium and water balance were similar in both groups. Because we were interested in comparing the response of both groups to DOCA, data in Figs. 6, 7, and 8 are plotted as changes from these control values.

Verification of RDNX. Renal norepinephrine content was reduced by 95% in RDNX rats (2.7 ± 0.6 ng/g) compared with sham rats (51.8 ± 7.0 ng/g; P < 0.05).

Protocol 2. Effect of RDNX on Cardiovascular Responses and Body Fluid Balances to Limited Access to Salt in DOCA-Salt Hypertension

In protocol 1, RDNX rats consumed less saline than sham rats. This raised the possibility that the attenuation of hypertension in RDNX rats was secondary to reduced saline intake. Protocol 2 was conducted to investigate this possibility. The protocol was repeated in sham rats in which saline intake was restricted to that observed in RDNX rats in protocol 1 until the last 10 days of the protocol, when they were allowed free access to saline. The results are presented with data from RDNX rats in protocol 1. Shown in Table 1 are control data for MAP and HR as well as variables for sodium and water balances. As observed in protocol 1, MAP in RDNX rats was significantly lower (~8 mmHg) than in rats with intact renal nerves. However, HR and all variables for sodium and water balance were similar in both groups. Because we were interested in comparing the response of both groups to DOCA, data in Figs. 6, 7, and 8 are plotted as changes from these control values.

Figure 5 shows cumulative sodium (top) and water balances (bottom) in both groups. A time-dependent gradual divergence in cumulative sodium balance was observed between groups (approximately day 20) so that RDNX significantly attenuated cumulative sodium balance from days 32 to 40 when compared with sham rats. By day 40, cumulative sodium balance was reduced by 32% in RDNX rats compared with sham rats so that absolute values of cumulative sodium balance were 40 ± 6 mmol in RDNX rats and 58 ± 10 mmol in sham rats. Similarly, RDNX significantly reduced cumulative water balance over time (i.e., from days 25 to 40) compared with rats with intact renal nerves. By day 40, a 23% reduction in cumulative water balance in RDNX rats (580 ± 31 ml) was observed when compared with sham rats (747 ± 66 ml).

Body weight responses in DOCA-salt hypertension. No significant differences in body weight were observed between RDNX (385 ± 6 g) and sham (373 ± 7 g) rats at the beginning of the protocol. Although RDNX rats tended to gain more weight over the 40-day protocol (RDNX = +191 ± 8; sham = +177 ± 16 g), no significant differences in body weights were observed at the end of the study between RDNX (576 ± 12 g) and sham (550 ± 10 g) rats.
Cardiovascular responses in DOCA-salt hypertension. After DOCA implantation, the pattern of ΔMAP was similar in both groups (Fig. 6, top). When saline restriction was removed in saline-restricted sham rats (SHAM-R; day 30), a small but insignificant rise in MAP was observed (Table 2). On day 40, despite the fact that SHAM-R rats were allowed unlimited access to saline, the magnitude of rise in MAP was similar between groups.

The pattern of decline in HR was comparable in RDNX and SHAM-R rats such that on day 29, the ΔHR was similar in both groups (Fig. 6, bottom). Allowing unlimited access to salt in SHAM-R rats had no significant impact on HR, and by day 40, the degree of decline in HR was not significantly different between SHAM-R and RDNX rats (Table 2).

Body fluid balance responses in DOCA-salt hypertension. Because saline intake in SHAM-R was matched to saline intake in RDNX rats during the 4-day control period and the 26-day period after DOCA implantation, no differences in changes in sodium intake were observed between groups (Fig. 7, top). When the SHAM-R rats were given free access to the saline solution, a threefold increase in sodium intake was observed within the first 24 h after the restriction was removed. This acute response was followed by a slower decline in the changes in sodium intake, which appear to reach a plateau from

Table 1. Comparison of cardiovascular and fluid balance parameters during control period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RDNX</th>
<th>Sham-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>99±1</td>
<td>107±3*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>408±5</td>
<td>408±5</td>
</tr>
<tr>
<td>Sodium intake, mmol/24 h</td>
<td>5.9±0.5</td>
<td>5.1±0.1</td>
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<td>Urinary sodium excretion, mmol/24 h</td>
<td>5.2±0.4</td>
<td>4.7±0.1</td>
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<tr>
<td>Sodium balance, mmol/24 h</td>
<td>0.7±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Saline intake, ml/24 h</td>
<td>35.3±3.0</td>
<td>30.7±0.5</td>
</tr>
<tr>
<td>Urine output, ml/24 h</td>
<td>19.0±2.5</td>
<td>18.1±0.5</td>
</tr>
<tr>
<td>Water balance, ml/24 h</td>
<td>16.3±0.6</td>
<td>12.6±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE of the 4-day control period before DOCA implantation. RDNX, renal denervation; Sham-R, sham rats restricted in saline intake. MAP, mean arterial pressure; HR, heart rate. *P < 0.05, significant differences between groups.

Fig. 6. Comparison of 24-h change in (Δ) MAP (top) and HR (bottom) between RDNX and saline-restricted sham (SHAM-R) rats. DOCA was implanted on the morning of day 5. On day 30, the rats were allowed unlimited access to saline (open arrow).

Fig. 7. Comparison of 24-h change in total sodium intake (top), urinary sodium excretion (middle), and sodium balance (bottom) between RDNX and SHAM-R rats. See Fig. 6 for open arrow significance; χ², significant difference between groups at predetermined point intervals.

The pattern of decline in HR was comparable in RDNX and SHAM-R rats such that on day 29, the ΔHR was similar in both groups (Fig. 6, bottom). Allowing unlimited access to salt in SHAM-R rats had no significant impact on HR, and by day 40, the degree of decline in HR was not significantly different between SHAM-R and RDNX rats (Table 2).
day 37 until the end of the protocol. By the end of the study (day 40), changes from control in sodium intake were significantly different between groups (Table 2).

During the saline restriction period, changes in urinary sodium excretion were not different between RDNX and SHAM-R rats (Fig. 7, middle). When the saline restriction was removed, the pattern of changes in sodium excretion closely followed the changes in sodium intake. By day 40, the changes in urinary sodium excretion were significantly higher in SHAM-R rats compared with RDNX rats (Table 2). With the exception of day 30, no significant differences in changes in sodium balance were observed between groups (Table 2 and Fig. 7, bottom).

Because the pattern of saline intake, urine output, and water balance was similar to the pattern for sodium balance, they will only be briefly described here. When the sodium intake in SHAM-R rats was matched to the sodium intake in RDNX rats, no significant differences were observed during the 29-day period after DOCA implantation for changes in saline intake (Fig. 8, top), urine output (Fig. 8, middle), and water balance (Fig. 8, bottom). When SHAM-R rats were allowed free access to saline, changes in saline intake and urine output significantly increased compared with RDNX rats. The peak of saline intake and urine output observed within the first 24 h was followed by a slower decline (Table 2). With the exception of one point (day 30), no significant differences in water balances were observed between SHAM-R and RDNX rats (Table 2 and Fig. 8, bottom).

Figure 9 compares the 24-h changes in cumulative sodium (top) and water (bottom) balances among the RDNX, sham, and SHAM-R rats until day 29 of the protocol (i.e., before removal of saline restriction in SHAM-R rats). Over time, SHAM-R rats tended to accumulate less sodium compared with RDNX and sham rats, and this response was significantly different from days 20 to 29 of the protocol. By day 29, cumulative sodium balance was 16.8 ± 2.3 mmol in SHAM-R rats compared with 31.5 ± 4.2 mmol in RDNX rats and 34.5 ± 6 mmol in sham rats. Similarly, saline restriction significantly reduced cumulative water balance in SHAM-R rats compared with RDNX rats (i.e., from days 16 to 29) and sham rats (i.e., from days 12 to 29). By day 29, the cumulative water balance in SHAM-R rats was 284 ± 86 ml compared with 411 ± 23 ml in RDNX rats and 505 ± 43 ml in sham rats.

Table 2. Comparison of changes from control period in cardiovascular and fluid balance parameters at specific time intervals

<table>
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<tr>
<th>Parameters</th>
<th>Day 30</th>
<th>Day 40</th>
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<tr>
<td></td>
<td>RDNX</td>
<td>SHAM-R</td>
</tr>
<tr>
<td>Δ MAP, mmHg</td>
<td>16 ± 3</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Δ HR, beats/min</td>
<td>−84 ± 6</td>
<td>−72 ± 3</td>
</tr>
<tr>
<td>Δ Sodium intake, mmol/24 h</td>
<td>6.7 ± 1.6</td>
<td>28.7 ± 2.5*</td>
</tr>
<tr>
<td>Δ Urinary sodium excretion, mmol/24 h</td>
<td>6.7 ± 1.6</td>
<td>25.3 ± 2.5*</td>
</tr>
<tr>
<td>Δ Sodium balance, mmol/24 h</td>
<td>0.0 ± 0.2</td>
<td>3.4 ± 1.1*</td>
</tr>
<tr>
<td>Δ Saline intake, ml/24 h</td>
<td>43.9 ± 10.3</td>
<td>185.7 ± 16.5*</td>
</tr>
<tr>
<td>Δ Urine output, ml/24 h</td>
<td>47.8 ± 9.6</td>
<td>163.7 ± 15.8*</td>
</tr>
<tr>
<td>Δ Water balance, ml/24 h</td>
<td>−3.9 ± 1.5</td>
<td>22.0 ± 6.8*</td>
</tr>
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</table>

Values are mean changes ± SE from control period value at specific times during the study. Δ, change from control period. *P < 0.05, significant differences between groups.
The DOCA-salt model of hypertension has been studied for decades in various species; however, the underlying mechanisms leading to the development and maintenance of the hypertensive process remain controversial. Although there is some indication to the contrary (11, 14), several lines of evidence support the hypothesis that the rise in arterial pressure in this model involves a centrally mediated hyperactivity of the sympathetic nervous system (5, 16, 19, 20).

The specific peripheral vascular beds targeted by this sympathetic hyperactivity remain to be identified. In the early 1980s, the contribution of renal sympathetic nerves in mineralocorticoid hypertension was suggested since two laboratories reported that complete RDNX attenuated DOCA-salt hypertension (27, 28, 38). However, subsequent studies by other investigators were unable to reproduce these results (13), and so the importance of renal nerves in DOCA-salt hypertension remains a matter of debate. The present study was conducted, using a different method of arterial pressure measurement, to address this controversy.

There were several significant findings in the present study. First, RDNX rats had a significantly lower arterial pressure during the control period compared with sham rats. Second, RDNX attenuated the severity of DOCA-salt hypertension by ~50% compared with rats with intact renal nerves. Third, control of HR was significantly impaired in RDNX rats compared with sham rats, particularly in relation to DOCA-associated changes in arterial pressure. Fourth, RDNX attenuated DOCA-induced changes in saline intake compared with sham rats. Finally, RDNX reduced cumulative sodium and water balances compared with sham rats after DOCA treatment. These results are consistent with an important role of renal nerves in mediating DOCA-salt hypertension. Moreover, as discussed in Role of Efferent Sympathetic Renal Nerves in DOCA-Salt Hypertension and in Role of Afferent Sympathetic Renal Nerves in DOCA-Salt Hypertension, these results suggest that not only are renal efferent nerves involved in this model of hypertension but that renal afferent nerves may play a role as well.

Role of Renal Nerves in Normotensive Rats

Centrally mediated autonomic function plays a prominent role in fluid and electrolyte regulation and therefore arterial pressure homeostasis. One mechanism by which the sympathetic activity maintains fluid homeostasis is efferent renal nerves. Fluctuations in renal sympathetic nerve activity modulate renin secretion from juxtaglomerular cells, sodium reabsorption from renal tubular cells, and renal vascular resistance (12). Afferent renal nerves modulate central autonomic activity (10, 32, 45) and appear to be also involved in long-term regulation of body fluid regulation because bilateral afferent RDNX was associated with sodium-dependent hypertension in rats (30).

In the present study, control levels of arterial pressure were significantly lower in RDNX rats (~10 mmHg) compared with sham rats (Fig. 2, top, and Table 1). This finding is consistent with our previous report in which the 10-mmHg difference in MAP between RDNX and sham rats was constant over various ranges of salt intake (24). Furthermore, we have also reported that a unilateral RDNX results in a 5-mmHg reduction in arterial pressure compared with sham rats (25). The finding of the present study was surprising because the rats were uninephrectomized and consuming 0.9% saline. Nonetheless, the findings in this study combined with the previous observations solidify the concept that renal nerves support basal levels of arterial pressure irrespective of dietary sodium intake in normal rats (24, 25). The mechanisms whereby RDNX causes a decrease in arterial pressure are not completely understood. The results of our previous reports suggest that the decrease in arterial pressure can neither be explained by natriuresis/diuresis after RDNX nor solely by a decrease in renin secretion (24, 25). Other mechanisms that may mediate a decrease in arterial pressure after RDNX include decreased renal vascular resistance and/or interruption of renal afferent input to the central nervous system. These possibilities remain to be investigated.

Role of Efferent Sympathetic Renal Nerves in DOCA-Salt Hypertension

In the present study, RDNX delayed the development of DOCA-salt hypertension by ~50% compared with sham rats. This observation suggests that renal nerves contribute to the developmental phase of this model of hypertension. This con-
clusion is consistent with some (27, 28, 38) but not all (13) studies.

Some of our findings are consistent with the idea that efferent control of sodium and water balances contributes to DOCA-salt hypertension (27, 28, 38). After DOCA implantation, it was observed that for most days, positive sodium and water balances tended to be higher in sham rats compared with RDNX rats. Periodic positive peaks of sodium and water balances were observed in sham rats throughout the study but with lesser frequency and magnitude in RDNX rats. This resulted in a gradual divergence in cumulative sodium and water balances between groups (approximately day 20), which is consistent with the role for efferent renal nerves in DOCA-salt hypertension. Interestingly, this divergence coincided with an accelerated increase in arterial pressure in sham rats compared with RDNX rats. The observation that the reduction in cumulative sodium and water balances in RDNX and SHAM-R rats compared with sham rats was associated with a lesser degree of elevation in arterial pressure (Fig. 9) further supports the concept that the magnitude of hypertension is related to the control of sodium and water balances. Although blood volume was not measured in our study, it is conceivable that the difference in sodium excretion between groups resulted in a divergence in blood volume and, ultimately, arterial pressure.

However, the evidence that volume overload plays a significant role in the development of mineralocorticoid hypertension is meager at best. An elegant study by Fink et al. (15) recently suggested that in the DOCA-salt hypertensive rat, mean circulatory filling pressure was increased, which was due in part to modest increases in blood volume. However, the increase in mean circulating filling pressure was also mediated by an increase in sympathetic venomotor tone (15).

Efferent renal sympathetic nerves also regulate renal vascular resistance and renin release (12). Therefore, a reduction in renal vasomotor tone after RDNX could have resulted in an overall decrease in total peripheral resistance and arterial pressure. To our knowledge, no long-term studies on the impact of RDNX on renal vascular resistance have been performed in the DOCA-salt hypertensive model. Several lines of evidence tend to suggest that renin does not play a role in the development or maintenance of mineralocorticoid-induced hypertension (23, 28, 42).

Role of Afferent Sympathetic Renal Nerves in DOCA-Salt Hypertension

The kidney is not simply the target of renal efferent sympathetic activity, but, because it contains mechano- and chemoreceptors, is also a sensory organ capable of sending information via afferent renal nerves to the central nervous system. Most brain stem regions involved in cardiovascular control and several regions of the anterior hypothalamus implicated in regulation of body fluid balances receive input from renal afferent nerves (36). This suggests that centrally mediated regulation of arterial pressure can be modulated by information relayed through renal afferent sympathetic nerve activity. In fact, electric stimulation or chemical stimulation of renal afferent fibers has been shown to excite and, less frequently, to inhibit hypothalamic neurons involved in control of sympathetic outflow and vasopressin secretion (9, 29, 37, 41). The role of afferent renal nerves in arterial pressure control is further supported by the findings of a recent study (30) in which bilateral dorsal rhizotomy in male Sprague-Dawley rats was associated with sodium-dependent hypertension. Selective deafferentation also effectively attenuated the development of hypertension in one-kidney, one-clip Goldblatt hypertensive rats (44). However, the role of renal afferent nerves in the DOCA-salt hypertensive model is unclear.

In the present study, two findings suggest a role of renal afferents in mineralocorticoid hypertension. First, we observed that RDNX caused a significant reduction in ad libitum saline intake compared with sham rats. This finding was somewhat surprising, and the mechanisms underlying this difference in salt intake are unclear. Although the role of afferent renal nerves was not directly investigated in the present study, it is conceivable that disruption of renal afferent fibers alters salt appetite (18, 34) or alters central vasopressinergic mechanisms by decreasing antidiuretic hormone secretion (7), which could result in a modification in “water-seeking” behavior. This hypothesis is supported by the observation that when the saline restriction was removed in sham rats, saline intake increased dramatically and remained significantly higher compared with RDNX rats. Our findings are supported by other reports in which renal nerves were found to be critical for normal salt appetite response to a hypovolemic/hypotensive challenge (39). On the other hand, selective bilateral renal deafferentation did not alter sodium appetite when compared with rats with intact afferent renal nerves (30), indicating that afferent renal nerves may not play an important role in modulation of salt appetite. However, by design, the study from Kopp et al. (30) and ours were notably different because the salt-retaining hormone DOCA was given to rats in the present protocol. Therefore, it is possible that mineralocorticoid and afferent renal nerves interact synergistically on central structures to modulate salt appetite in DOCA-salt hypertension.

We also observed that control of HR was dysfunctional because the bradycardic response that followed DOCA-induced increases in arterial pressure was enhanced in RDNX rats. In other words, the rats with intact afferent renal nerves had an attenuated bradycardic response to DOCA-salt hypertension because it occurred in the face of a greater increase in arterial pressure. Although arterial baroreflex was not specifically evaluated in the present study, our findings tend to indicate that RDNX alters the baroreflex response to DOCA-salt hypertension, further supporting a role of afferent nerves in the modulation of central nuclei involved in the autonomic control of HR in the DOCA-salt model (26). Such a finding also indirectly supports the concept that sympathetic activity is elevated in mineralocorticoid hypertension because HR in sham rats was inappropriately elevated for the magnitude of hypertension.

Attenuation But Not Abolishment of Mineralocorticoid Hypertension: Beyond the Role of Renal Nerves

Because hypertension was only attenuated by 50% after RDNX, what other mechanisms are involved in the developmental phase of mineralocorticoid hypertension? There are three possibilities.

First, increased sympathetic activity in DOCA-salt hypertension may be a more generalized phenomenon, and nonrenal vascular beds (e.g., splanchnic, musculocutaneous, or cardiac)
may also contribute to the hypertensive process (5, 33, 35, 42, 46, 47). Second, whole body vascular reactivity to various pressor substances is increased in the DOCA-salt model and may play a synergistic role, in conjunction with the autonomic nervous system, to increase total peripheral resistance (1, 2, 43). Although the relative contribution of different vascular beds in the hypertensive process was not investigated here, our results tend to support the concept of a generalized increase in autonomic activity rather than being limited to a single vascular bed (1, 5, 35). A third mechanism involves the direct antinatriuretic effect of mineralocorticoids on renal tubular cells (17). By promoting sodium (and water) reabsorption, DOCA causes an increase in blood volume and hypertension (1, 3, 15, 21, 31).

**Perspectives**

It is now well established that centrally mediated sympathetic activity plays an important role in the pathogenesis of DOCA-salt hypertension. However, the vascular beds targeted by an increase in sympathetic output remain controversial. In the present study, the observation that RDNX attenuated the elevation in arterial pressure by ~50% compared with sham rats indicates that renal nerves are significantly involved in the hypertensive process. This attenuation in hypertension was due to the loss of renal sympathetic efferent control of sodium and water balances after RDNX. However, the present study strongly suggests a role for afferent renal nerves in DOCA-salt hypertension because salt intake was reduced and the brady-cardiac response to elevation in arterial pressure was altered in RNDX rats compared with sham rats.

The observation that RDNX attenuated but did not abolish the development of mineralocorticoid hypertension implies that other mechanisms are involved in mineralocorticoid hypertension. These could include, but are not limited to, increased sympathetic activity to nonrenal vascular beds, increased vascular reactivity to vasoactive hormones, and direct mineralocorticoid effect on renal tubular cells. The relative contribution of these mechanisms in DOCA-salt hypertension needs to be further investigated.

**ACKNOWLEDGMENTS**

We thank Vivian Mylander for technical assistance with this project. Present address of F. Jacob: Georgetown University School of Medicine, 4000 Reservoir Rd., Bldg. D, Rm. 333, Washington, DC 20057 (e-mail: fj24@georgetown.edu).

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant HL-64178.

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