Renal denervation chronically lowers arterial pressure independent of dietary sodium intake in normal rats

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Renal denervation chronically lowers arterial pressure independent of dietary sodium intake in normal rats. Am J Physiol Heart Circ Physiol 284: H2302–H2310, 2003. First published February 27, 2003; 10.1152/ajpheart.01029.2002.—The present study was designed to test the hypothesis that renal nerves chronically modulate arterial pressure (AP) under basal conditions and during changes in dietary salt intake. To test this hypothesis, continuous telemetric recording of AP in intact (sham) and renal denervated (RDNX) Sprague-Dawley rats was performed and the effect of increasing and decreasing dietary salt intake on AP was determined. In protocol 1, 24-h AP, sodium, and water balances were measured in RDNX (n = 11) and sham (n = 9) rats during 5 days of normal (0.4% NaCl) and 10 days of high (4.0% NaCl) salt intake, followed by a 3-day recovery period (0.4% NaCl). Protocol 2 was similar with the exception that salt intake was decreased to 0.04% NaCl for 10 days after the 5-day period of normal salt (0.4% NaCl) intake (RDNX; n = 6, sham; n = 5). In protocol 1, AP was lower in RDNX (91 ± 1 mmHg) compared with sham (101 ± 2 mmHg) rats during the 5-day 0.4% NaCl control period. During the 10 days of high salt intake, AP increased <5 mmHg in both groups so that the difference between sham and RDNX rats remained constant. In protocol 2, AP was also lower in RDNX (93 ± 2 mmHg) compared with sham (105 ± 4 mmHg) rats during the 5-day 0.4% NaCl control period, and AP did not change in response to 10 days of a low-salt diet in either group. Overall, there were no between-group differences in sodium or water balance in either protocol. We conclude that renal nerves support basal levels of AP, irrespective of dietary sodium intake in normal rats.

sympathetic nervous system; angiotensin II; renal vascular resistance

THE KIDNEYS PLAY A PROMINENT role in fluid and electrolyte regulation and therefore arterial pressure homeostasis. One mechanism by which the kidneys are thought to maintain fluid homeostasis is by the renal sympathetic nerves. Fluctuations in the degree of renal sympathetic nerve activity (RSNA) modulate renin secretion from juxtaglomerular cells, sodium reabsorption from renal tubular cells, and renal hemodynamics (13).

Many lines of evidence have implicated sympathetically mediated mechanisms in the development of hypertension in several rat models (23, 26, 27, 46). Accordingly, in some models of hypertension, complete renal denervation delayed the development of hypertension (23, 27–30). These results indirectly support a role of afferent and efferent renal nerves in the pathogenesis of hypertension. However, evidence suggesting that renal nerves are important in the long-term control of blood pressure in normotensive rats is not well established. For example, under conditions of increases in dietary sodium intake, is the reduction of RSNA important in the maintenance of a normotensive state? Although this is a crucial question, it has not been answered due to technical limitations for long-term recording of sympathetic nerve activity in conscious rats.

The results of several studies (6, 33) tend to suggest that RSNA is modulated by changes in baroreceptor afferent nerve activity. The role of the baroreflex in regulating RSNA is in part supported by the observation that sinoaortic denervation (SAD) rats exhibit salt-sensitive hypertension (22, 37, 38). Furthermore, recent studies in dogs suggest that baroreceptor reflex chronically suppresses renal nerve activity under conditions of increased dietary salt (32) and plasma angiotensin II (33). These findings are consistent with a recent report (15) that baroreceptor denervation impairs the regulation of renal salt excretion. Taken together, these results suggest that disruption of the negative feedback loop between baroreceptor afferents and RSNA could lead to impaired regulation of fluid balances, resulting in an elevation in arterial pressure. If this is correct, then disruption of autonomic activity to the kidneys should also increase the salt sensitivity of arterial pressure.

In the present study, we tested this hypothesis by comparing the cardiovascular and fluid balance responses to chronic increases and decreases in salt intake of rats with intact renal nerves with rats subjected to bilateral renal denervation. Arterial pressure was monitored 24 h/day throughout the protocol in conscious nontethered rats with the use of radiotelemetry. We hypothesized that if changes in renal nerve activity in response to changes in dietary salt were critical to the regulation of arterial pressure, then renal denervated rats would exhibit larger salt-induced changes in arterial pressure than rats with intact renal nerves.
MATERIAL AND METHODS

General Procedures

Male Sprague-Dawley rats (250–275 g) were purchased from Harlan Sprague Dawley (Charles River Laboratory; Wilmington, MA) and housed until the time of study in our animal housing facility with controlled temperature and lighting. Standard rat chow and distilled water were provided ad libitum. After sham or renal denervation and transmitter implantation (described below), rats were individually housed in our laboratory in metabolic cages (Nalgene, Nalge Nunc International; Rochester, NY) and were under a 12:12-h light-dark cycle for the duration of the experiment (25 days). Rats were allowed ad libitum access to distilled water and food and were anesthetized with pentobarbital sodium (50 mg/kg) and given atropine sulfate (0.4 mg/kg) with a single intraperitoneal injection. Gentamicin sulfate (10 mg/kg) was administered intramuscularly before the surgery. The surgical procedure for renal denervation has been described by Trostel and Osborn (44). Briefly, a ventral midline abdominal incision was performed to expose the renal arteries and veins of each kidney. Under a dissecting microscope, visible renal nerves, that varied in sodium content according to the experimental protocol. 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.

Protocol 1. Cardiovascular and Fluid Balance Responses to Increased Dietary Salt in Sham and Renal Denervated Rats

Surgical procedures. RENAL OR SHAM DENERVATION. Rats were fasted overnight before the surgery. Before surgical procedures, rats were randomly assigned to the renal denervated (RDNX, n = 11) or sham (n = 10) groups. Rats were anesthetized with pentobarbital sodium (50 mg/kg) and given atropine sulfate (0.4 mg/kg) with a single intraperitoneal injection. Gentamicin sulfate (10 mg/kg) was administered intramuscularly before the surgery. The surgical procedure for renal denervation has been described by Trostel and Osborn (44). Briefly, a ventral midline abdominal incision was performed to expose the renal arteries and veins of each kidney. Under a dissecting microscope, visible renal nerves, that varied in sodium content according to the experimental protocol. 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.

Protocol 2. Cardiovascular and Fluid Balance Responses to Decreased Dietary Salt in Sham and RDNX Rats

The effect of renal denervation on cardiovascular and fluid balance responses to sodium restriction was studied in 12 rats (RDNX, n = 6; sham, n = 6). Surgical procedures and the experimental protocol were similar as described in protocol 1, with the exception that sodium intake was decreased for 10 days after the 5-day control period by switching the rats from the 0.4% NaCl diet to 0.04% NaCl. This was followed by a 3-day recovery period (0.4% NaCl) as in protocol 1.

Verification of Renal Denervation

Completeness of renal denervation was quantified by assay for renal norepinephrine content. At the end of each protocol, rats were euthanized with an overdose of pentobarbital given intraperitoneally and both kidneys were removed and inspected for signs of hydronephrosis or infection. Hydronephrosis was observed in the right kidney of one rat in the sham group in protocol 2 and all data collected from this rat were disregarded. Kidneys were weighed, wrapped in aluminum foil, immediately frozen in liquid nitrogen, and then stored at −70°C. Norepinephrine was extracted from...
the tissue and assayed for norepinephrine content by HPLC with electrochemical detection, performed as previously described (44).

Data Analysis and Statistics

All values are presented as means ± SE. Statistical analysis was performed with the use of analysis of variance for repeated measures. When treatment/time interaction was significant, Scheffé’s post hoc test was used to determine the time points where a significant difference between groups occurred. To establish whether significant changes in arterial pressure occurred within each group during high salt intake, we compared the 24-h average MAP for each time point to the average MAP during control period by using a post hoc test (Bonferroni’s). Comparison between groups for renal norepinephrine content was performed with the use of an unpaired t-test. Significance for all tests was defined as P < 0.05.

RESULTS

Protocol 1. Cardiovascular and Fluid Balance Responses to Increased Dietary Salt in Sham and Renal Denervated Rats

Effect of renal denervation on arterial pressure and heart rate. We initially analyzed the 12:12-h day-night periods of arterial pressure and heart rate to determine whether significant differences existed between groups during the day/night cycle. In the sham group, daytime 12-h mean MAP was 99 ± 2 mmHg during the control period and 103 ± 2 mmHg at night (difference of ~4 mmHg). When the rats were fed the 4.0% salt diet, daytime MAP was 102 ± 2 mmHg and 108 ± 2 mmHg at night (difference of ~6 mmHg). In RDNX rats, 12-h daytime average for MAP was 89 ± 1 mmHg during the control period and 92 ± 1 mmHg at night (difference of ~3 mmHg). When RDNX rats were fed a high-salt diet (4.0%), 12-h daytime average for MAP was 92 ± 1 mmHg compared with 96 ± 1 mmHg at night (difference of ~4 mmHg). Because the circadian rhythms of MAP were not significantly different between groups on a normal or high-salt diet, we chose to simplify the statistical analysis and data presentation by presenting 24-h averages for MAP and HR.

A statistically significant reduction in basal arterial blood pressure was observed in the RDNX compared with sham rats (Fig. 1A). During the 5-day 0.4% NaCl control period, MAP was ~10 mmHg lower in RDNX (91 ± 1 mmHg) compared with sham (101 ± 2 mmHg) rats. During the 10 days of 4.0% NaCl diet, MAP increased <5 mmHg in both groups so that the difference between sham and RDNX rats remained constant. Within each group, this salt-induced increase in arterial pressure was not significantly different from the control period. MAP returned to control values during the recovery period in both groups. Twenty-four-hour average heart rates were not significantly different between RDNX and sham rats at any time throughout the protocol (Fig. 1B).

Effect of renal denervation on sodium and water balance. No significant differences were observed between RDNX and sham rats for sodium intake over the study period (Fig. 2A). In addition, renal denervation did not significantly alter urinary sodium excretion compared with sham rats at any time during the protocol (Fig. 2B). On the first day of 4.0% NaCl diet (day 6), sodium balance for sham rats (3.98 ± 0.68 mmol/day) was significantly higher compared with RDNX rats (1.57 ± 0.27 mmol/day). However, there were no significant differences on sodium balance between the groups at any other time during the protocol (Fig. 2C). With regard to water balance, there were no differences between sham and RDNX rats for water intake (Fig. 3A), urine output (Fig. 3B), or water balance (Fig. 3C) at any time during the protocol.

Protocol 2. Cardiovascular and Fluid Balance Responses to Decreased Dietary Salt in Sham and RDNX Rats

Effect of renal denervation on arterial pressure and heart rate. Similar to that observed in protocol 1, MAP was significantly lower in RDNX (93 ± 2 mmHg) compared with sham (105 ± 4 mmHg) rats during the 5-day 0.4% NaCl control period (Fig. 4A). In addition, MAP did not change in response to 10 days of 0.04% NaCl in either group. No difference in 24-h average
heart rates was observed between RDNX and sham rats (Fig. 4B).

Effect of renal denervation on sodium and water balance. No significant differences in sodium intake (Fig. 5A), sodium urinary excretion (Fig. 5B), or sodium balance (Fig. 5C) were observed between RDNX and sham rats throughout the study. Throughout most of the protocol, water consumption was similar between both groups, although on days 12, 14, and 16, water intake was significantly higher in RDNX compared with sham rats (Fig. 6A). Urine output (Fig. 6B) and water balances (Fig. 6C) were similar in both groups.

Verification of renal denervation. Table 1 summarizes the results of the assay for renal norepinephrine content to verify completeness of renal denervation. Renal norepinephrine was markedly reduced in RDNX rats in both protocols. Interestingly, renal norepinephrine content was higher in sham rats in protocol 1 who received a high-salt diet for 10 days, compared with sham rats in protocol 2 who received a low-salt diet.

DISCUSSION

Effect of Bilateral Renal Denervation on Long-Term Basal Levels of Arterial Pressure

The results of our study did not support the hypothesis that impaired neural control of renal function...
results in an inability to regulate arterial pressure under conditions of changes in dietary salt intake. However, the most surprising and potentially most significant finding was that RDNX rats were chronically hypotensive. Although the difference in arterial pressure between RDNX and sham rats was only on the order of 10 mmHg, it was stable over time and was maintained whether rats consumed a 0.04%, 0.4%, or 4.0% NaCl diet. The fact that this 10-mmHg difference was stable over a 100-fold increase in salt intake argues against the idea that the hypotension was the result of denervation natriuresis and diuresis. If that were true, the difference should have been magnified when rats consumed a low-salt diet and diminished when they consumed a high-salt diet.

The concept that renal nerves are important in setting the basal level of arterial pressure in normal animals has not been supported by the literature. In fact, the results of previous studies, in which basal levels of arterial pressure were measured in intact and RDNX animals are conflicting. This may be due, in part, to the methods used to measure arterial pressure and how these measurements influence basal sympathetic activity. Such studies have included anesthetized surgically stressed animals (3), tail cuff measurements in conscious restrained rats (17), and direct recordings of arterial pressure in conscious tethered rats (21) or dogs (35). The strongest demonstration of how measurement conditions alter the influence of renal nerves on renal function comes from studies in conscious rabbits, in which renal nerve activity and renal blood flow were measured simultaneously in one kidney and blood flow was measured in the contralateral denervated kidney. Measurements made during

A

B

C

Fig. 4. Comparison of 24-h average mean arterial pressure (A) and heart rate (B) between RDNX and sham rats on normal (0.4%) and low (0.04%) NaCl diets.

Fig. 5. Comparison of 24-h sodium intake (A), sodium urinary excretion (B), and sodium balances (C) between RDNX and sham rats on normal (0.4%) and low (0.04%) NaCl diets.
brief periods when the rabbits were held in a restraining box showed that renal vascular resistance was greater in the innervated kidney compared with the denervated kidney (34). However, in a subsequent study (1) from the same laboratory, measurements made continuously in rabbits in their home cages showed no differences in basal renal blood flow or renal vascular resistance between the innervated and denervated kidneys.

With regard to how stress can determine the magnitude of the difference in arterial pressure between intact and RDNX rats, we analyzed data from a subset of rats in which arterial pressure was monitored immediately after implantation of the radiotelemetry transmitter (RDNX, n = 15; sham, n = 12) (see Fig. 7 for further details). These findings suggest that acute postsurgical stress alone, independent of restraint or tethering, may magnify differences in arterial pressure between intact and RDNX rats.

In our study, we minimized stress artifacts by continuously measuring arterial pressure in conscious unrestrained and nontethered sham and RDNX rats in their home cages. As such, our results provide strong evidence that renal nerves contribute to basal levels of arterial pressure under normal physiological conditions.

Potential Mechanisms of Hypotension in RDNX Rats

Although the mechanisms of the hypotensive response in RDNX rats are unknown, several possibilities exist. First, efferent renal nerves can directly stimulate tubular sodium reabsorption and it is known that renal denervation results in an acute natriuresis and diuresis (2, 4, 5, 10, 25, 39). However, we have shown (Fig. 7) that the difference in arterial pressure between groups is immediate and large in magnitude (−20 mmHg). Although, salt and water balance measurements were not performed during the 7 days preceding the control period, it is unlikely that this rapid decrease in arterial pressure observed in RDNX rats was solely the result of volume depletion due to renal denervation natriuresis-diuresis. This is further supported by the fact that the magnitude of the difference in arterial pressure between sham and RDNX rats remained constant over a 100-fold change in dietary salt intake. A second possibility is that the renin-angiotensin system is suppressed in RDNX rats, lowering arterial pressure. Several (7, 17, 20, 35), but not all (19, 21), investigators have reported that RDNX animals have low plasma renin activity compared with intact animals. In a recent study (11), we reported that

Table 1. Renal norepinephrine content between RDNX and sham rats

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Right Kidneys</th>
<th>Left Kidneys</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>n</td>
</tr>
<tr>
<td>Protocol 1 (high NaCl, %)</td>
<td>342 ± 61</td>
<td>9</td>
</tr>
<tr>
<td>Protocol 2 (low NaCl, %)</td>
<td>107 ± 14</td>
<td>4</td>
</tr>
</tbody>
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Values are means ± SE; n, number of kidneys. RDNX, renal denervated. Renal norepinephrine content was measured in ng/mg. *P < 0.05 compared with sham kidneys.
chronic blockade of angiotensin AT1 receptors with losartan decreased arterial pressure slowly, over a period of days, in conscious normotensive rats. However, in the present study, hypotension observed in RDNX rats occurred immediately after surgery (Fig. 7). These results tend to indicate that the sudden decrease in arterial pressure is not likely due to decreased plasma angiotensin II. However, one cannot exclude the possibility that a decrease in renin release may have contributed, in part, to the acute hypotensive effect of RDNX rats as well as the long-term effects of renal denervation on arterial pressure. A third possibility is that renal vasomotor tone is reduced in RDNX rats, resulting in an overall decrease in total peripheral resistance. Although studies in the rabbit suggest that the renal nerves do not influence basal renal vascular resistance (1), similar studies have not been conducted in the rat. Finally, renal afferent nerves may modulate vasopressin release (12, 41) and systemic sympathetic activity (8, 9, 36, 40, 42, 45). Thus renal denervation may chronically alter sympathetic outflow and therefore arterial pressure by interruption of afferent renal nerves. However, selective deafferentation has been performed in several experiments and, to our knowledge, did not cause a notable decrease in arterial pressure in normal rats. Interestingly, in a recent study (24) where rats were subjected to either complete renal denervation, selective deafferentation, or sham surgery, MAP was significantly lower in complete renal denervation compared with selective renal deafferentation or sham-operated rats. Furthermore, in the spontaneously hypertensive rat model, selective deafferentation by dorsal rhizotomy did not attenuate the subsequent increase in arterial pressure. However, total renal denervation (both afferent and efferent nerve component of the renal sympathetic nerves) did (43).

**Effect of Bilateral Renal Denervation on Long-Term Salt Sensitivity of Arterial Pressure**

The results of this study clearly demonstrate that renal denervation does not alter the salt sensitivity of arterial pressure. There are two possible explanations as to why our data do not support this hypothesis. First, renal denervation does not impair the ability to regulate sodium and water balance, and therefore arterial pressure, during periods of salt excess or deprivation. Second, long-term changes in sodium balance do not necessarily affect arterial pressure.

We were unable to demonstrate sodium retention during periods of high salt intake or sodium loss during periods of sodium restriction in renal denervation compared with intact rats. Surprisingly few studies have been conducted on the effect of renal denervation on regulation of sodium and water balance during periods of chronic salt loading. One study, in which urine was continuously collected in conscious rats, showed that the rate of achieving sodium balance when rats were switched to a high-salt diet was attenuated in RDNX rats (21). More importantly, although RDNX rats retained more sodium during the first 3 days of high salt intake, there were no differences in arterial pressure between intact and RDNX rats. However, measurements were carried out for only 3 days, so it is not known whether differences in arterial pressure would have been observed over a longer time period. In a recent study in dogs with unilateral renal denervation using the split-bladder technique, intact renal nerves promoted sodium excretion compared with the contralateral denervated kidneys (32).

The effect of salt restriction on sodium balance after renal denervation has been studied in various species. Unfortunately, the results of these studies have also led to contradictory findings. Indeed, several investigators have observed that in dogs, rabbits, and rats, renal denervation impaired the ability of the kidneys to conserve sodium when animals were placed on a low-sodium diet (16, 18) but others did not (4, 17, 35). Such a discrepancy in renal sodium handling between studies is most likely due to variable degrees of sodium restriction with renal nerves becoming more important with more severe salt depletion (14). In one study, bilateral renal denervation impaired the ability to maintain sodium balance (16), but because arterial pressure was not measured, the link between sodium balance and arterial pressure could not be established. Although our results suggest that renal nerves are crucial in the long-term control in arterial pressure, the chronic hypotensive effect of RDNX in rats appeared to be independent of renal sodium or water balances. Furthermore, even if our ability to measure sodium and water balance accurately is not certain, it is clear that it did not have an effect on arterial pressure regulation.
Our laboratory and others (22, 37, 38) have demonstrated that SAD results in salt-dependent hypertension in the rat. However, the mechanisms underlying chronic salt-induced increases in arterial pressure in the SAD rat have not been investigated. One hypothesis is that SAD impairs reflex control of renal nerve activity and sodium excretion when salt intake is increased. However, we were unable to detect differences in sodium balance between SAD and sham-operated rats fed an 8.0% NaCl salt diet (38). In contrast, a recent study reported that SAD rats had an impaired ability to excrete a chronic salt load compared with intact rats (15). In that study, rats consumed salt in their drinking water, without access to free water, to increase the accuracy of the balance measurements. It is unclear whether the dichotomy between results is due to the magnitude of the salt load, the precision of the balance measurements or the manner in which the oral salt load was given (food vs. water). Nonetheless, this recent report (15) supports the concept that impaired neural control of renal excretory function may be correlated with salt-dependent hypertension.

On the basis of this rationale, we predicted that renal denervated rats would exhibit salt-induced changes in arterial pressure, but the results of the present study do not support this hypothesis. Such a discrepancy between salt sensitivity of RDNX and SAD rats could be explained, in part, by the difference in basal levels of RSNA as well as reflex responses to increased dietary salt (6). For example, SAD rats may have a higher “fixed” level of RSNA due to the loss of negative feedback from arterial baroreceptors, resulting in an inappropriately high level of RSNA during periods of high salt intake. On the other hand, RSNA in RDNX rats is virtually absent. Consequently, it is true that both SAD and RDNX rats have impaired baroreflex control of renal function; differences in basal renal sympathetic tone may explain differences in the salt-sensitivity of arterial pressure.

Finally, one alternate explanation must also be considered. It is possible that impaired neural control of sodium balance is present in SAD rats but is not the cause of salt-dependent hypertension in this model. An equally logical hypothesis is that SAD impairs arterial and venous dilation during periods of high-salt intake and the salt-sensitive hypertension is not linked to impaired neural control of the kidney. This could be consistent with the findings of the present study, in which RDNX rats did not show signs of altered salt sensitivity of arterial pressure.

**Perspectives**

The importance of renal nerves in the control of basal arterial pressure in normotensive animals remains unresolved. A major, although unexpected, finding of our study is that a difference in arterial pressure was observed immediately after surgery and was sustained throughout the protocol. This observation strongly suggests that renal nerves play a crucial role in setting the long-term basal level of arterial pressure in unstressed normal animals consuming a normal salt diet. However, renal denervation did not impair the ability to chronically regulate body fluid balance or arterial pressure in response to a 100-fold change in dietary sodium intake. Taken together, these findings support the hypothesis that renal nerves influence resting levels of arterial pressure, but they do not determine the long-term salt sensitivity of arterial pressure in normal rats. The mechanisms underlying the influence of renal nerves on basal levels of arterial pressure remain to be investigated.

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**REFERENCES**