Afferent renal denervation impairs baroreflex control of efferent renal sympathetic nerve activity

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Kopp UC, Jones SY, DiBona GF. Afferent renal denervation impairs baroreflex control of efferent renal sympathetic nerve activity. Am J Physiol Regul Integr Comp Physiol 295: R1882–R1890, 2008. First published October 22, 2008; doi:10.1152/ajpregu.90529.2008.—Increasing efferent renal sympathetic nerve activity (ERSNA) increases afferent renal nerve activity (ARNA), which decreases ERSNA to prevent sodium retention. High-sodium diet enhances ARNA, suggesting an important role for ARNA in suppressing ERSNA during excess sodium intake. Mean arterial pressure (MAP) is elevated in afferent denervation by dorsal rhizotomy (DRX) rats fed high-sodium diet. We examined whether the increased MAP in DRX is due to impaired arterial baroreflex function. In DRX and sham DRX rats fed high-sodium diet, arterial baroreflex function was determined in conscious rats by intravenous nitroprusside and phenylephrine or calculation of transfer function gain from arterial pressure to ERSNA (spontaneous baroreflex sensitivity). Increasing MAP did not suppress ERSNA to the same extent in DRX as in sham DRX, −60 ± 4 vs. −77 ± 6% Maximum gain, −4.22 ± 0.45 vs. −6.04 ± 0.90% ΔERSNA/mmHg, and the maximum value of instantaneous gain, −4.19 ± 0.45 vs. −6.04 ± 0.81% ΔERSNA/mmHg, were less in DRX than in sham DRX. Likewise, transfer function gain was lower in DRX than in sham DRX 3.9 ± 0.2 vs. 6.1 ± 0.5 NU/mmHg. Air jet stress produced greater increases in ERSNA in DRX than in sham DRX, 35,000 ± 4,900 vs. 20,900 ± 3,410% of area under the curve. Likewise, the ERSNA responses to thermal cutaneous stimulation were greater in DRX than in sham DRX. These studies suggest impaired arterial baroreflex suppression of ERSNA in DRX fed high-sodium diet. There were no differences in arterial baroreflex function in DRX and sham DRX fed normal-sodium diet. Impaired arterial baroreflex function contributes to increased ERSNA, which would eventually lead to sodium retention and increased MAP in DRX rats fed high-sodium diet.

dorsal rhizotomy; high-sodium diet; renorenal reflexes; spontaneous baroreflex sensitivity; reflex renal nerve stimulation

IN THE KIDNEY, THE MAJORITY of the renal sensory nerves are located in the renal pelvic wall (27, 28, 32). The afferent renal nerves project to the ipsilateral dorsal root ganglia at the T6–L2 level with the majority of the cell bodies of the afferent renal nerves being at the T6–L1 level (3, 6, 14). These nerves are activated by increases in renal pelvic pressure within the physiological range (26). The increase in afferent renal nerve activity (ARNA) produced by increased renal pelvic pressure leads to a reflex decrease in efferent renal sympathetic nerve activity (ERSNA) and a natriuresis, i.e., a renorenal reflex response (29).

The responsiveness of the afferent renal nerves is enhanced by high-sodium and suppressed by low-sodium diet by a mechanism at the peripheral nerve endings (26). In conditions of increased sodium intake, the threshold for activation of the renal mechanosensory nerves is < 3 mmHg (26), suggesting that the afferent renal nerves are tonically active during these conditions (19, 34, 39). The low-activation threshold of the renal mechanosensory nerves together with the natriuretic nature of the renorenal reflexes would suggest that activation of these reflexes is an important component of the spectrum of renal mechanisms involved in the renal control of water and sodium homeostasis.

Not only do increases in ARNA decrease ERSNA but increases in ERSNA increase ARNA (28, 30). The increase in ARNA exerts a powerful negative feedback control of ERSNA via activation of the renorenal reflexes (29) in the overall goal of maintaining low ERSNA to prevent sodium retention. These findings suggest that ERSNA would be inappropriately elevated in rats lacking the inhibitory renorenal reflex control of ERSNA, which, during high dietary sodium intake, would result in sodium retention and increased mean arterial pressure (MAP). This hypothesis was tested in our previous studies in afferent renal denervated rats [dorsal rhizotomy (DRX)], which showed elevated MAP and impaired pressure natriuresis when fed high-sodium diet (24). The question arising from those studies is why the increased MAP and/or increased ERSNA were not buffered by activation of the arterial baroreflex, whose function has been shown to be enhanced by high-sodium diet (10).

We hypothesized that the increased MAP in DRX rats was related to an impairment of the arterial baroreflex control of ERSNA. It has long been argued that the baroreflexes do not play a role in the long-term control of MAP and sympathetic nerve activity due to resetting (7). However, recent studies employing long-term activation of the arterial baroreceptors have challenged this concept (33, 42). Long-term recordings of ERSNA showed no evidence for impaired baroreflex control of ERSNA during experimentally induced hypertension (2). In view of these studies, we reasoned that the mechanisms involved in an impairment of the arterial baroreflex control of ERSNA in DRX rats fed high-sodium diet would be related to lack of afferent renal nerve signals to the supraspinal centers involved in cardiovascular control. There is considerable evidence for the control of ERSNA being influenced by a central interaction between the neural input from the afferent renal nerves and neural input from the arterial baroreceptors (4, 16, 40, 43, 44).

We examined arterial baroreflex function in conscious rats by two different methods: 1) the pharmacological method...
involved the dorsal roots were not sectioned.

normal Na^+ /H11003 cut for recording of ERSNA. The ERSNA signal was amplified

catheter placed in the femoral vein for drug infusion, and a bipolar

involving analysis of the baroreceptor curve, air jet stress, and thermal

cutaneous stimulation (somatic).

METHODS

The experimental protocols were approved by the Institutional

Animal Care and Use Committee and performed according to the

“Guide for the Care and Use of Laboratory Animals” from the

National Institutes of Health.

Male Sprague-Dawley rats, 51 ± 1 days old (38–69 days) weighing

211 ± 3 g (152–285 g), were anesthetized with pentobarbital

sodium (0.2 mmol/kg ip; Abbott Laboratories). Bilateral DRX (24, 31) (n = 68) or sham DRX (n = 71) was performed in litter mates at the T3–L1 level. The sham DRX procedure was identical to DRX except the dorsal roots were not sectioned.

Following surgery, DRX and sham DRX rats were placed on normal Na^+ pellets (Na^+ = 163 meq/kg; Teklad) with 0.9% NaCl drinking fluid (high-sodium diet, n = 87) or tap water drinking fluid (normal-sodium diet, n = 52) (26). Age distribution was similar among the four groups of rats.

Recording of Pulsatile Arterial Pressure in Conscious Rats

Three weeks following DRX and sham DRX surgery, rats were

anesthetized with methohexital sodium (0.14 mmol/kg ip; Jones Pharma). A PE-10 polyurethane catheter was placed in the femoral artery and tunneled subcutaneously to the back of the neck and exteriorized. The catheter was filled with an isotonic saline solution containing 5% glucose and 200 U heparin/ml. The catheter was placed as close as possible to the aorta and glued in place, allowing blood to

perfuse the leg distally to the catheter. Following recovery from anesthesia, the rats were placed in individual cages and the arterial catheters were connected to pressure transducers via swivels allowing free roaming. Arterial blood pressure was recorded 24 h/day for 3 days. Although the catheters were flushed twice daily with iso-

tonic saline containing 50 U heparin/ml, there was frequent cloting of the arterial catheters after midnight (24:00). Because this result in inconsistent quality of the recordings, the data recorded between midnight and 8:00 were omitted in the calculations of average daily MAP.

Recording of ERSNA in Conscious Rats

Following arterial pressure recordings, the rats were anesthetized (pentobarbital sodium, 0.2 mmol/kg ip + 0.04 mmol·kg^-1·h^-1 iv), a catheter placed in the femoral vein for drug infusion, and a bipolar silver electrode placed on the central portion of one renal nerve branch cut for recording of ERSNA. The ERSNA signal was amplified 20,000 × and filtered between 30 and 3,000 Hz. It was then full-wave rectified and integrated with a 20 ms time constant, and the background noise (postmortem death) was subtracted. All data were collected at 500 Hz and averaged over 2 s in the experiments involving analysis of the baroreceptor curve, air jet stress, and thermal cutaneous stimulation (see below). In the transfer function analysis studies, integrated ERSNA and arterial pressure were also sampled at 500 Hz but then reduced to 50 Hz by calculation of average values over 10 consecutive points (9–11, 21). Because our previous studies have shown that cutting one renal nerve branch for recording of ARNA does not modify the ERSNA and ARNA responses to reflex renal nerve stimulation (28) or the renorenal reflex responses to activation of renal mechanosensory nerves (29), it is unlikely that cutting one renal nerve branch for recording of ERSNA would modify ARNA responses to any significant extent in the sham DRX rats in the present studies. Heart rate (HR) was recorded with a linear cardiotachometer triggered by the arterial pressure wave form.

Experimental Protocol

Five to six hours following recovery of anesthesia, the rats were

placed in a device that allowed forward and backward movements. A steady-state period of at least 30 min was allowed to elapse before the experimental protocols (described below) were started. The studies were divided into two main groups. In the first group, we compared the arterial baroreflex control of ERSNA and HR in DRX and sham DRX rats fed high-sodium diet by measuring changes in ERSNA and HR in response to changes in MAP produced by intravenous injection of nitroprusside and phenylephrine. In the second group, we compared spontaneous baroreflex sensitivity in DRX and sham DRX rats using either high- or normal-sodium diet by calculation of the gain of the transfer function between arterial pressure and ERSNA during baseline steady-state conditions (21). In this group of rats we also compared the increases in ERSNA produced by somatic and environmental stressor reflex inputs in DRX and sham DRX rats fed either high- or normal-sodium diet. The quality of the ERSNA recording was assessed by its pulse synchronicity and the magnitude of the decrease in the ERSNA signal in response to norepinephrine, as tested at the end of each experiment. All data were recorded on tape for off-line analysis.

Group I: arterial baroreflex function in DRX and sham DRX rats, pharmacological method. The experiments were performed in 14 DRX and 12 sham DRX rats fed high-sodium diet. Following a 10-min control period, MAP was lowered from the control level to ~50 mmHg with an intravenous infusion of nitroprusside, 0.4 μg/min for 45–60 s, and increased from that level to ~180 mmHg with an intravenous infusion of phenylephrine, 2–5 μg/min for 45–60 s (10). The time between termination of the nitroprusside infusion and start of the phenylephrine infusion was 10–20 s.

Analysis of arterial baroreflex curve. MAP was plotted against integrated ERSNA or HR, both expressed in percentage of its baseline value during the control period. The data were collected over the MAP range from the maximum induced fall in MAP produced by nitroprusside up to the maximum increase in MAP produced by phenylephrine. The resultant sigmoidal relationships, representing the overall arterial baroreflex, were analyzed with a four-parameter logistic regression equation (10, 22),

\[
y = y_{0} + \frac{y_{m} - y_{0}}{1 + \frac{(x - x_{0})}{p_{3}}} \\
\]

where \( y \) is % ERSNA or % ΔHR and \( x \) is MAP. The parameters represent \( p_{1} \), full range of change in ERSNA/HR; \( p_{2} \), the slope coefficient; \( p_{3} \), MAP at midrange of the curve, and \( p_{4} \), the lower plateau of the curve. From these data, maximal gain was calculated as \( -p_{1}p_{2}/p_{4} \). Values of \( p_{1}p_{2} \) were derived for each rat; group average was calculated and used to plot a mean curve for each group. The instantaneous gain over the full range of MAP was obtained from the first derivative of the four-parameter logistic regression equation.

Group IIA: spontaneous arterial baroreflex control of ERSNA in DRX and sham DRX rats. In 21 DRX and 24 sham DRX rats fed high-sodium diet and 17 DRX and 24 sham DRX rats fed normal-sodium diet, transfer function spectra were calculated from the input (arterial pressure) to output (ERSNA) signals during the 10-min control period preceding either air jet stress (group IIB) or thermal cutaneous stimulation (group IIC) using the method described by...
Kanbar et al. (21). The transfer function was taken as the quotient of the cross spectrum of input and output divided by the power spectrum of the input. Power spectra were calculated using the Welch algorithm on blocks of 512 data points, which were 50% overlapped and subjected to a Hamming window; this yielded a frequency discrimination of 0.098 Hz. Transfer function gain values were normalized by the mean ERSNA calculated over the same 10-min control period. Coherence is a frequency domain estimate of a linear correlation (i.e., squared coherence, akin to coefficient of determination) between two signals indicating the degree to which the variance in one signal can be explained by a linear operation on the other signal. The coherence spectra were calculated from input and output. The coherence function was taken as the quotient of the square of the cross spectrum of input and output divided by the product of the power spectrum of the input times the power spectrum of the output. The algorithm involved mean detrending and a Hamming window with 50% overlap of blocks of 512 data points yielding a frequency discrimination of 0.098 Hz. To determine the threshold for coherence above which it exceeds zero with a certain significance level, the method described by Koopmans (23), which depends on the total number of samples, the total number of blocks and the nature of the tapering window was used. In this study, coherence values > 0.2 are significantly different from zero at $P < 0.01$.

The arterial pressure power spectra were used to locate the frequency at which maximum spectral power density occurred in the frequency band encompassing HR (usually 5–8 Hz in rats). Coherence, gain and phase were then noted at this particular frequency. MAP, HR, and ERSNA were calculated for the same data set as used for the transfer function analysis.

**Group IIB: ERSNA responses to air jet stress in DRX and sham DRX rats.** Following a 10-min control period, a 15-min experimental period was begun during which acute environmental stress consisting of continuous air jet stream toward the dorsum of the rat’s head (9) was applied to 21 DRX and 21 sham DRX rats fed high-sodium diet and 16 DRX and 20 sham DRX rats fed normal-sodium diet. The experimental period was followed by a 30-min recovery period.

**Group IIC: ERSNA responses to thermal cutaneous stimulation in DRX and sham DRX rats.** In these experiments, performed in 9 DRX and 10 sham DRX rats fed high-sodium diet and 8 DRX and 8 sham DRX rats fed normal-sodium diet, the rat’s tail was placed in a beaker with water throughout the experiment. Following a 10-min control period, a 3-min experimental period was begun during which the rat’s tail was moved from a beaker containing room-temperature water to a beaker containing 42°C water. After the end of the experimental period, the tail was again placed in the beaker containing room-temperature water for a 10-min recovery period. Pilot experiments had shown that placing the rat’s tail in water at 42°C produced an increase in overall sympathetic activity, as judged by increases in MAP, HR, and ERSNA, without eliciting a pain response in the conscious rat. The absence of pain was judged by the lack of tail flick and/or movement of the rat.

**Statistical Analysis**

Because of differences in the time interval from the start of the stress period to the peak increase in ERSNA between rats, the increase in ERSNA, MAP, and HR produced by the two stimuli, air jet stress and placing the tail in 42°C water, were evaluated by calculating the area under the curve of each parameter vs. time, with baseline being the average value of the control period. This approach takes into account changes in both magnitude and duration of response. Because not all data sets were normally distributed, as determined by the D’Agostino and Pearson omnibus normality tests, nonparametric statistical analysis was used. Friedman two-way ANOVA followed by multiple comparisons were used to calculate differences between groups and the Mann-Whitney U-test was used to determine differences between DRX and sham DRX rats fed a high-sodium diet. A significance level of 5% was chosen. Data in text and figures are expressed as means ± SE (38).

**RESULTS**

Body weight was similar among all four groups of rats 3 wk following DRX and sham DRX surgery, 286 ± 3, 278 ± 3, 277 ± 5, and 275 ± 5 g in DRX and sham DRX fed high-sodium diet, DRX, and sham DRX fed normal-sodium diet, respectively.

**Group I: Arterial Baroreflex Function in DRX and Sham DRX Rats-Pharmacological Method**

In view of the enhanced sensitivity of the arterial baroreflex during high-sodium diet (10) one may have anticipated that DRX would not alter MAP in conditions of high-sodium dietary intake. However, our previous studies showing increased MAP in DRX rats fed high-sodium diet (24) do not support this assumption; rather, these data may suggest that there is an interaction between the renorenal reflex and arterial baroreflex control of ERSNA. We tested this hypothesis by comparing the arterial baroreflex curves in DRX and sham DRX rats fed high-sodium diet. MAP in unrestrained conscious rats, averaged over 3 days, was greater in DRX than sham DRX rats fed high-sodium diet (Fig. 1). Likewise, MAP was increased in both groups of rats before the start of the acute experiment, i.e., following recovery from surgery/anesthesia, MAP being greater in the DRX than in sham DRX rats, 135 ± 4 vs. 121 ± 3 mmHg ($P = 0.01$). As shown in Fig. 2A, increasing MAP did not suppress ERSNA to the same level in DRX rats as in sham DRX rats, the lower plateau ($p4$) being $-60 ± 4$% in DRX and $-77 ± 6$% in sham DRX rats ($P < 0.05$). The slope coefficients ($p2$) were similar, 0.09 ± 0.01 and 0.11 ± 0.01 mmHg⁻¹. Maximum gain, calculated as $-(p1/p2)/4$, tended to be lower in DRX, 4.22 ± 0.45% ΔERSNA/mmHg, than in DRX, 10.22 ± 0.33 mmHg⁻¹.

**Fig. 1.** Mean arterial pressure (MAP), averaged over 16–18 h/day for 3 days, in conscious renal denervated by dorsal rhizotomy (DRX) and sham DRX rats. Statistical analysis was performed in 9 DRX and 10 sham DRX rats fed high-sodium diet and 8 DRX and 8 sham DRX rats fed normal-sodium diet, the rat’s tail was placed in a beaker with water throughout the experiment. Following a 10-min control period, a 3-min experimental period was begun during which the rat’s tail was moved from a beaker containing room-temperature water to a beaker containing 42°C water. After the end of the experimental period, the tail was again placed in the beaker containing room-temperature water for a 10-min recovery period. Pilot experiments had shown that placing the rat’s tail in water at 42°C produced an increase in overall sympathetic activity, as judged by increases in MAP, HR, and ERSNA, without eliciting a pain response in the conscious rat. The absence of pain was judged by the lack of tail flick and/or movement of the rat.
sham DRX, 6.04 ± 0.90% ΔERSNA/mmHg, (P < 0.05, one tail). Figure 2B showing the instantaneous gain (i.e., the gain at each MAP interval) over the same range of MAP demonstrates that the maximum value of instantaneous gain was less in DRX rats compared with sham DRX rats (P < 0.05). Baseline ERSNA (i.e., % ΔERSNA = 0 in Fig. 2A) occurred at −22 ± 3 and −14 ± 5 mmHg (P < 0.01) in the DRX and sham DRX rats, respectively; the corresponding value for instantaneous gain was less in DRX rats, 3.4 ± 0.3% ΔERSNA/mmHg, than in sham DRX rats, −5.5 ± 0.9% ΔERSNA/mmHg (P < 0.02). Instantaneous gain at baseline MAP (i.e., ΔMAP = 0 in Fig. 2A) tended to be lower in DRX rats than in sham DRX rats, but this difference did not quite reach statistical significance (P = 0.057).

In contrast to the impaired arterial baroreflex control of ERSNA in DRX rats, the arterial baroreflex control of HR was similar in the two groups of rats (Fig. 3). Baseline HR was slightly higher in DRX than in sham DRX rats, 422 ± 11 vs. 371 ± 15 beats/min (P < 0.05).

**Group IIA: Spontaneous Arterial Baroreflex Control of ERSNA in DRX and Sham DRX Rats**

In agreement with the results in group I, MAP in conscious unrestrained rats in group II was greater in DRX rats fed high-sodium diet than in sham DRX rats fed high-sodium diet (Fig. 1). There was no difference in MAP among the sham DRX rats fed high-sodium diet and DRX and sham DRX rats fed normal-sodium diet.

Because the studies in group I suggested that the arterial baroreflex control of ERSNA is impaired in DRX rats fed high-sodium diet, we wanted to further explore these findings by examining the spontaneous arterial baroreflex control of ERSNA by calculating the gain of the transfer function between arterial pressure and ERSNA at HR frequency during steady-state conditions. One of the advantages with this technique is that it does not involve arterial pressure changes produced by acute administration of pharmacological, vasoactive agents. The frequency at which maximal spectral power density occurred was near HR frequency, which was similar in the four groups of rats (Table 1). The coherence was similar in all groups and statistically different from zero, supporting the assumption of a linear relationship between oscillations in arterial pressure and ERSNA. As shown in Fig. 4, transfer function gain of arterial pressure to ERSNA was lower in DRX rats fed high-sodium diet compared with all other groups. The coefficient of variation for MAP was similar in DRX and sham DRX during the control period (Table 1).

Although comparing baseline ERSNA among groups of rats is difficult due to the signal, being derived from multifiber recordings, the impaired arterial baroreflex control of ERSNA in DRX rats fed high-sodium diet (Figs. 2 and 4) may suggest that the increased value of ERSNA in DRX rats fed high-sodium diet (Table 1) represents a true increase in baseline ERSNA in these rats.

The impaired arterial baroreflex control of ERSNA in DRX rats fed high-sodium diet shown by two different techniques suggests the likelihood of an increased ERSNA response to reflex sympathetic nerve stimulation. We tested this hypothesis by exposing conscious rats to one of two different interventions: air jet stress, an environmental stimulus (9), and thermal...
cutaneous stimulation (placing the rat’s tail in 42°C water), a somatic afferent stimulus (28).

**Group IIB: ERSNA Responses to Air Jet Stress in DRX and Sham DRX Rats**

Air jet stress produced a greater increase in ERSNA in DRX rats than in sham DRX rats (both groups fed high-sodium diet) \( (P < 0.05) \) (Fig. 5). The enhanced ERSNA response was partly due to increased duration of the response, being 1,350 ± 110 s in DRX and 790 ± 90 s in sham DRX rats \( (P < 0.01) \). The increase in MAP produced by air jet stress was similar in the two groups. Likewise, the HR responses to air jet stress were similar in DRX and sham DRX rats, 53,180 ± 7,800 vs. 52,570 ± 6,570 beats·min\(^{-1}\)·s\(^{-1}\). In DRX and sham DRX rats fed normal-sodium diet, the increases in ERSNA and MAP produced by air jet stress were of similar magnitudes (Table 2).

**Group IIC: ERSNA Responses to Thermal Cutaneous Stimulation in DRX and Sham DRX Rats**

Placing the rat’s tail in 42°C water produced a greater increase in ERSNA in DRX rats than in sham DRX rats (both groups fed high-sodium diet) \( (P < 0.05) \) (Fig. 6). The enhanced ERSNA response in DRX rats was partly due to increased duration of the response being 370 ± 70 s in DRX and 170 ± 40 s in sham DRX rats \( (P < 0.02) \). The increases in MAP produced by placing the tail in 42°C water were of similar magnitudes in the two groups. Likewise, the HR responses to thermal cutaneous stimulation were similar in DRX and sham DRX rats, 12,810 ± 4,180 vs. 11,450 ± 4,860 beats·min\(^{-1}\)·s\(^{-1}\). In DRX and sham DRX rats fed normal-sodium diet, placing the tail in 42°C water resulted in increases in ERSNA and MAP of similar magnitudes (Table 2).

**DISCUSSION**

The present study shows that the arterial baroreflex control of ERSNA is impaired in DRX rats fed high-sodium diet. These rats also showed increased ERSNA responses to environmental and somatic stimulation. These data suggest that in conditions of high-sodium dietary intake, activation of the afferent renal nerves contributes to the arterial baroreceptor-mediated suppression of ERSNA in the overall goal of preventing sodium retention and maintaining water and sodium homeostasis.

**Renorenal and Arterial Baroreflexes**

Increases in ERSNA increase ARNA, which, in turn, decreases ERSNA via activation of the inhibitory renorenal reflexes, i.e., a negative feedback loop (28–30). In conditions of high-sodium diet, increases in renal pelvic pressure < 2.5 mmHg, i.e., within the range of basal renal pelvic pressure (19, 34, 39), activate renal sensory nerve fibers with an increase in ARNA (26), suggesting that the afferent renal nerves are tonically active. Support for this hypothesis is derived from early studies showing that total (i.e., efferent plus afferent)

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### Table 1. Baseline data during the 10-min control period preceding air jet stress or placement of the rat’s tail in 42°C water in conscious renal denervated by dorsal rhizotomy (DRX) and sham DRX rats fed either high- or normal-sodium diet, group IIA

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>HR, Hz</th>
<th>ERSNA, mV</th>
<th>Coherence</th>
<th>MAP, Coefficient of Variation, %</th>
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</thead>
<tbody>
<tr>
<td>DRX-high sodium</td>
<td>21</td>
<td>7.81±0.22</td>
<td>13.90±1.51*</td>
<td>0.51±0.04</td>
<td>10.3±0.3</td>
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<tr>
<td>Sham DRX-high sodium</td>
<td>24</td>
<td>7.17±0.24</td>
<td>8.98±0.63</td>
<td>0.57±0.05</td>
<td>11.0±0.5</td>
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<tr>
<td>DRX-normal sodium</td>
<td>17</td>
<td>7.83±0.33</td>
<td>7.88±0.98</td>
<td>0.53±0.06</td>
<td>11.2±0.7</td>
</tr>
<tr>
<td>Sham DRX-normal sodium</td>
<td>24</td>
<td>7.05±0.30</td>
<td>9.61±0.82</td>
<td>0.52±0.04</td>
<td>12.6±0.8</td>
</tr>
</tbody>
</table>

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Data are expressed as means ± SE. N, number of rats; HR, heart rate; ERSNA, efferent renal sympathetic nerve activity (×10,000); MAP, mean arterial pressure. *\( P < 0.02 \), DRX rats vs. sham DRX rats fed high-sodium diet.
unilateral renal denervation increases contralateral ERSNA and decreases contralateral urinary sodium excretion in saline volume expanded rats (12). The enhanced responsiveness of the afferent renal nerves in high-sodium dietary conditions (26) suggests that activation of the renorenal reflexes contributes to the suppression of ERSNA to facilitate sodium excretion in the overall goal of maintaining sodium and fluid balance during excess sodium intake. The importance of the inhibitory renorenal reflexes regulating water and sodium homeostasis was shown by our previous studies demonstrating that in the absence of intact afferent renal innervation, rats become hypertensive when exposed to a high-sodium diet (24). The enhanced responsiveness of the afferent renal nerves produced by high-sodium diet is not unique to the afferent renal nerves but has also been demonstrated for the afferent carotid sinus nerves (10). Thus, the question that arises from our previous studies in DRX rats fed high-sodium diet is why the increased MAP is not counteracted by an enhanced arterial baroreflex activation. We hypothesized that the answer to this question involves an interaction between the afferent renal and arterial baroreceptor neural inputs. In the rat, the majority of the cell bodies of the afferent renal nerves are located in ipsilateral DRG at the T5–L1 levels (3, 6, 14). Within the spinal cord, the afferent renal nerves project to the ipsilateral dorsal horn in laminae I, III-V (6). In these areas, the afferent renal nerve fibers synapse with interneurons projecting to sites within the central nervous system associated with cardiovascular regulation, including nucleus tractus solitarius (NTS), rostral ventrolateral medulla (RVLM), subfornical organ, and paraventricular nucleus of hypothalamus (40). However, there is also evidence for a monosynaptic projection of the afferent renal nerves to areas within the brain stem (45). Because the majority of the afferent carotid sinus and aortic nerves project to NTS (8), it seems likely that an interaction between the renorenal and arterial baroreflexes would occur at a supraspinal level, possibly within the medulla (8). Although, the present studies in conscious rats did not allow for recordings from neurons in the brain stem in response to activation of the afferent renal nerves, there is considerable anatomical and electrophysiological evidence for such an interaction to occur at a supraspinal level (8). Evidence for supraspinal integration of the renorenal reflexes involving neurons in the medulla is derived from studies in rabbits showing that decreases in ERSNA produced by electrical stimulation of the afferent renal nerves were blocked by renal denervation or spinal cord transection at C2 but not by transection of the brain stem at the pontine-medullary junction, suggesting an involvement of medullary structures in renorenal reflexes (37). Further studies showed that electrical afferent renal nerve stimulation produced a wide-spread decrease in efferent sympathetic nerve activity, including renal, cervical, and cardiac nerve activity in association with a decrease in MAP (36). A majority of the neurons in the ventral lateral medulla (VLM) that decrease their activity in response to afferent renal nerve stimulation also respond to stimulation of the central portion of the aortic nerves with a decrease in ERSNA (36). These neurons are also responsive to inputs from the carotid sinus nerves (41). Since the decreased activation of the neurons in VLM produced by stimulation of either the afferent renal or aortic nerves is mirrored by a fall in ERSNA, these data suggest an important role for these neurons in arterial baroreceptor and renorenal reflexes. Further support for a possible interaction between the arterial baroreceptor and renorenal reflexes at the medullary level is derived from studies in rats showing that electrical stimulation of the afferent renal nerves decreases MAP as well as the activity of neurons in the RVLM, which have pulse-synchronous activity and which decrease their activity in response to pressor doses of intravenous phenylephrine (15). Importantly, activation of the afferent renal nerves by physiological stimuli, including increases in renal pelvic pressure and ischemia, activates neurons in the RVLM (43).

### Arterial Baroreflex Function in DRX and Sham DRX Rats

Because of the convergence of afferent input from renal and carotid sinus nerves on neurons within the medulla, including NTS and RVLM (8), we hypothesized that the arterial baroreflex was altered in DRX rats. We speculated that the arterial baroreflex may be altered only in DRX rats fed high-sodium diet because the afferent renal nerves are tonically active in these rats (26). We used two different approaches, the pharmacological method that involves examining the acute alter-

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**Table 2. Effects of air jet stress for 15 min and placing the tail in 42°C water for 3 min in DRX and sham DRX rats fed normal sodium diet**

<table>
<thead>
<tr>
<th>Group</th>
<th>ERSNA, %/s (AUC)</th>
<th>MAP, mmHg/s (AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DRX</strong></td>
<td><strong>Sham DRX</strong></td>
<td><strong>DRX</strong></td>
</tr>
<tr>
<td>Group IIB</td>
<td>31,930±7,250</td>
<td>39,690±9,760</td>
</tr>
<tr>
<td>Group IIC</td>
<td>4,050±1,050</td>
<td>3,830±1,210</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. AUC, area under the curve. Group IIB: DRX, n = 16; sham DRX, n = 20; group IIC: DRX, n = 8; sham DRX, n = 8.
ations in MAP and ERSNA produced by intravenous administration of vasoactive agents (10) and determination of the spontaneous baroreflex sensitivity by calculation of the transfer gain from arterial pressure to ERSNA (21). The obvious advantage with the latter method is the absence of drug-induced circulatory changes. Studies by Kanbar et al. (21) in conscious, freely moving rats have validated the use of the transfer gain from arterial pressure to ERSNA to determine arterial baroreflex control of ERSNA. Their findings showed reduced transfer gain in rats with partial denervation of the sinoaortic nerves, the transfer gain being inversely related to arterial pressure variability and positively correlated with the gain calculated from the pharmacological determination of the arterial baroreflex sensitivity.

The results from the pharmacological method showed that increases in MAP failed to suppress ERSNA to the same level in DRX as in sham DRX (both groups fed high-sodium diet). Although maximum gain tended to be lower in DRX than in sham DRX rats, this difference did not quite reach statistical significance. However, calculations of instantaneous gain showed a more robust support for the maximum gain being less in DRX rats compared with sham DRX rats (both groups fed high-sodium diet). Values for instantaneous gain were less in DRX than sham DRX (both groups fed high-sodium diet) at baseline ERSNA and at baseline MAP, although this latter difference did not quite achieve statistical significance. The finding that baseline ERSNA was reached at MAP values, which were less than control MAP (MAP during the control period preceding injection of nitroprusside), is a reflection of the well-known hysteresis of the arterial baroreflex (5). Importantly, these data suggesting an impairment of the arterial baroreflex in DRX rats are supported by the studies using the transfer gain from arterial pressure to ERSNA to determine the spontaneous baroreflex sensitivity in different groups of rats. The transfer gain in the DRX rats fed high-sodium diet was less than that in sham DRX rats fed high-sodium diet. Taken together, these data suggest that increased sympathetic nerve activity to the renal and possibly other vascular beds contributes to the increased MAP in DRX rats fed high-sodium diet. Although data from multifiber recordings have to be interpreted with caution, it is noteworthy that the values of basal ERSNA were significantly increased in DRX rats fed high-sodium diet. The similar arterial baroreflex control of HR in DRX and sham DRX rats fed high-sodium diet may be related to HR being under the influence of both sympathetic and vagal nerve activity.

The lack of difference in the sensitivity of the arterial baroreflex control of ERSNA between DRX and sham DRX rats fed normal-sodium diet was not unexpected since the responsiveness of the afferent renal nerves is much less in conditions of normal than high-sodium dietary intake (26). These data suggest that there is very little interaction between the renorenal and arterial baroreflexes in normal-sodium dietary conditions. The similar coefficient of variation for MAP in DRX and sham DRX rats fed either high- or normal-sodium diet was also not unexpected because only one afferent neural input was removed. Previous studies showing increased MAP variability have been demonstrated in animals following bilateral or unilateral sinoaortic denervation or in conditions of marked hypertension (7, 21).

Responsiveness of ERSNA to External Stimuli in DRX and Sham DRX

Short-term acute stress stimuli, including air jet stress and placing the rat’s tail in warm water (thermal cutaneous stimulation), results in immediate increases in MAP and HR, the mechanisms involved being multifactorial, including activation of the central and peripheral nervous system and vasoactive hormone release (e.g., 9, 18, 20, 28). The concomitant acute increases in MAP, HR, and sympathetic nerve activity produced by acute stress suggest that the normal arterial baroreflex control of sympathetic nerve activity is overridden (17). However, it might be anticipated that the increased MAP response may, over time, eventually lead to arterial baroreflex-induced suppression of sympathetic nerve activity. In view of the impaired arterial baroreflex control of ERSNA in DRX rats fed high-sodium diet (24), we hypothesized that acute stress stimuli involving increases in MAP would result in less suppression of ERSNA in DRX rats than in sham DRX rats. We tested this hypothesis by exposing the conscious rat to one of two different stimuli, air jet stress and thermal cutaneous stimulation. Air jet stress or thermal cutaneous stimulation resulted in similar increases in MAP in DRX and sham DRX rats, allowing us to compare the ERSNA responses in the two groups of rats. The results show that air jet stress or thermal cutaneous stimulation resulted in greater ERSNA responses in DRX rats compared with sham DRX rats, despite similar increases in MAP. Interestingly, the greater ERSNA responses to both stimuli in DRX rats on high-sodium diet were to a large extent related to increased duration of the responses. These data suggest that the afferent renal nerves may play an important role in the attenuation or termination of the increases in sympathetic nerve activity to renal and possibly other vascular beds produced by external stimuli. We speculate that in conditions of high dietary sodium intake, the failure of stress-induced increases in MAP to attenuate or terminate the increases in ERSNA in DRX rats to a similar extent as in sham DRX rats eventually will lead to renal sodium retention and hypertension. Our previous studies in DRX rats, which involved measurements of daily urinary sodium excretion demonstrated similar sodium excretion during days 11–21 of high-sodium diet in DRX and sham DRX rats (24). Because the similar sodium excretion was achieved at different levels of MAP, these data suggested an impairment of the pressure-natriuresis mechanism. Because the surgery involved in DRX is rather extensive, urinary sodium excretion was not measured during the first 10 days of recovery. Therefore, we were not able to observe whether the increased MAP in the DRX rats fed high-sodium diet was preceded by positive sodium balance, as has been shown in sinoaortic denervated rats during the first five postoperative days on high-sodium diet (13). As expected from the results showing similar arterial baroreceptor function in DRX and sham DRX rats fed normal-sodium diet, there were no differences in the ERSNA responses to air jet stress or thermal cutaneous stimulation between the two groups of rats fed normal-sodium diet.

MAP in Conscious Unrestrained DRX and Sham DRX Rats

MAP values were significantly higher in DRX rats fed high-sodium diet than in sham DRX rats fed high-sodium diet. There was no difference in MAP between sham DRX and DRX rats fed normal-sodium diet. These data are in agreement with
our previous studies (24). However, the difference in MAP between DRX and sham DRX fed high-sodium diet was somewhat lower in the current than in our previous studies. In the previous studies, the MAP values represented 2-h averages over 4 days recorded while the rats were placed in devices allowing only forward and backward movement. A likely explanation for the lower MAP in the present studies is that the MAP values in the present studies represent 16–18 h/day averages over 3 days recorded, while the rats were in a cage that allowed free movement in a setting with controlled temperature and 12-h light and dark cycles. There was no difference between daytime and evening MAP values within each group. Importantly, MAP in sham DRX rats fed high-sodium diet and DRX and sham DRX rats fed normal-sodium diet were similar to those in our previous studies. Taken together, these data suggest that MAP in the DRX rats fed high-sodium diet is influenced by a combination of dietary sodium and environmental characteristics. This view is supported by the present studies showing enhanced ERSNA responses to air jet stress and thermal cutaneous stimulation in DRX rats fed high-sodium diet.

Possible Mechanisms Involved in the Increased Arterial Pressure in DRX Rats Fed High Sodium Diet

The results from the present studies do not allow a definitive conclusion about a possible supraspinal mechanism(s) contributing to the increased MAP in the DRX rats fed high-salt diet since the studies were performed in conscious rats precluding recording from brain stem cardiovascular areas of interest. However, there is considerable evidence for signals from the afferent renal nerves decreasing the activity of neurons in the VLM. Importantly, the activity of many of these neurons is also modulated by inputs from both the aortic and carotid sinus nerves (8). Considering the convergence of the afferent signals from the renal and carotid sinus nerves on neurons in several brain areas involved in cardiovascular control, including RVL (4, 16, 40, 42, 43), we speculate that the lack of afferent renal nerve input to cardiovascular sensitive neurons in these areas may contribute to an impairment of arterial baroreflex control of ERSNA, eventually leading to increased MAP in conditions of high-salt diet. In this context, it is of interest that sinoaortic denervated rats are also characterized by salt-sensitive hypertension (35). We speculate that the increased MAP in these rats may likewise involve impaired renorenal reflex control of sympathetic nerve activity.

There are likely several mechanisms involved in the high-sodium diet-induced impaired arterial baroreflex control of ERSNA in DRX rats. In intact rats, high-sodium diet enhances the activation of the afferent renal nerves by a mechanism involving decreased endogenous ANG II at the peripheral nerve terminals (26); ANG II inhibits the activation of afferent renal nerve fibers by a pertussis toxin sensitive mechanism at the peripheral afferent renal nerve terminals (25). In addition to peripheral afferent mechanisms being modulated by high-sodium diet, microinjection of excitatory (L-glutamate) and inhibitory (γ-aminobutyric acid) agents directly into the RVL showed that high-sodium diet enhanced both the excitatory (L-glutamate) and inhibitory (γ-aminobutyric acid) effects on ERSNA and MAP, presumably by local mechanisms within the RVL (1). We speculate that in intact rats fed high-sodium diet, increased ARNA would lead to increased inhibitory modulation of cardiovascular responsive neurons in medullary structures, including RVL, which would lead to enhanced arterial baroreflex control of sympathetic nerve activity, including ERSNA, in the overall goal of maintaining arterial pressure and preventing sodium retention. Thus, in conditions of high-dietary sodium intake, removing afferent renal innervation eliminates an important inhibitory influence on the responsiveness of excitatory RVL neurons eventually resulting in increased sympathetic nerve activity.

Perspectives and Significance

The renorenal reflex control of the circulation has received little attention. Increases in ERSNA increase ARNA, which in turn decrease ERSNA via activation of the renorenal reflex mechanisms (28). Our previous studies have suggested that the afferent renal nerves are tonically active in conditions of high-sodium diet (26). Thus, in these conditions the increased ARNA would provide an important contribution to the maintenance of low ERSNA, which is essential in preventing renal sodium retention. The results of the present studies provide support for this view in showing that MAP is increased in rats with disrupted afferent renal innervation, but only during conditions of increased dietary sodium intake. The current data further show an impairment of arterial baroreflex control of ERSNA in DRX rats fed high-sodium diet, which may suggest an altered regulation of sympathetic nerve activity to other vascular beds, in addition to the renal vascular bed. If so, these data would imply an important role for the afferent renal nerves in the long-term control of water and sodium homeostasis and the regulation of arterial pressure.

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