Dissociation of Renal Nerve and Excretory Responses to Volume Expansion in Prehypertensive Dahl Salt-Sensitive and Dahl Salt-Resistant Rats

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Prehypertensive Dahl salt-sensitive rats on low sodium diet have impaired baroreceptor and cardiopulmonary receptor function as compared with Dahl salt-resistant control rats. We tested whether these abnormalities influenced the ability of conscious prehypertensive Dahl salt-sensitive rats to excrete a moderate intravenous isotonic saline volume load (0.9% NaCl, 3% body weight i.v.). During control, 30-minute volume expansion, and 2-hour recovery periods, arterial blood pressure and heart rate did not change. Glomerular filtration rate and renal plasma flow remained stable throughout control and recovery. The amount of the volume load excreted as either water or sodium was the same for Dahl salt-sensitive and Dahl salt-resistant rats with either denervated or innervated kidneys. The time course of water and sodium excretion was similar in Dahl salt-sensitive and Dahl salt-resistant rats. Renal sympathetic nerve activity, however, showed different patterns. Although the initial decrease in renal sympathetic nerve activity during volume expansion was similar for both groups, Dahl salt-sensitive rats exhibited a prompt return of renal sympathetic nerve activity to control levels, whereas renal sympathetic nerve activity in Dahl salt-resistant rats remained decreased throughout the recovery period. The rapid recovery of renal sympathetic nerve activity to control levels after volume expansion in Dahl salt-sensitive rats supports the concept of impaired cardiopulmonary receptor function in these rats. The similar diuretic and natriuretic responses to moderate intravenous isotonic saline volume expansion in all four groups (Dahl salt-sensitive and Dahl salt-resistant, intact and denervated) despite markedly different renal sympathetic nerve activity responses suggest a decreased responsiveness of the kidney in both Dahl salt-sensitive and Dahl salt-resistant rats to altered neural input. (Hypertension 1989;13:822–827)
less sodium than all the other groups. These results emphasize the influence of the preceding dietary NaCl intake and the magnitude of the intravenous isotonic saline load on the natriuretic responses.

The major aims of our study were twofold, and the following questions were addressed: Do moderate intravenous isotonic saline loads (3% body weight over 30 minutes) elicit different renal sympathetic nerve activity responses in conscious DR and DS rats on a low NaCl diet? Are different renal sympathetic nerve activity responses associated with different diuretic and natriuretic responses to the intravenous isotonic saline load? Renal denervation was used to examine the relation between the changes in renal sympathetic nerve activity and the diuretic and natriuretic responses. In view of the possibility that the above-mentioned differences in results related to the magnitude of the intravenous isotonic saline load, we employed a more physiological 3% isotonic saline load.

Materials and Methods

Twenty-nine female DS and DR rats were obtained from Brookhaven National Laboratories (Upton, New York). At 4 weeks of age, rats were placed on a low NaCl diet (0.4% NaCl, Nutritional Biochemicals, Cleveland, Ohio) and allowed tap water for drinking ad libitum. The rats were divided into four groups by strain (DS and DR) and renal innervation (intact or denervated): DS intact, DS denervated, DR intact, and DR denervated.

Surgical Procedures

Bilateral renal denervation was performed 7 days before experimentation. Under methohexital anesthesia (Brevital, 30 mg/kg i.p., Eli Lily, Indianapolis, Indiana), bilateral flank incisions were made, and renal denervation was performed by surgically stripping the renal arteries and veins of adventitia, cutting all visible renal nerve bundles under a dissection microscope (×25), and coating the vessels with a solution of 10% phenol in 95% ethanol as previously described. This renal denervation procedure prevents the renal vasoconstrictor response to suprarenal lumbar sympathetic nerve stimulation, prevents the antinatriuretic response to environmental stress, reduces renal catecholamine histoautoradiography to nondetectable levels, and reduces renal norepinephrine concentration to less than 5% of control.

At 9–11 weeks of age, all rats (weight approximately 210 g) were anesthetized with methohexital and implanted with femoral arterial and venous catheters and a bladder cannula. All intact animals were also implanted with an electrode to record renal sympathetic nerve activity. The technical procedure of renal sympathetic nerve activity recording has been described previously. In short, the left kidney was exposed through a flank incision; a renal nerve bundle was dissected and placed on a bipolar stainless steel electrode (Cooner Wire Co., Chatsworth, California). Renal sympathetic nerve activity was amplified (×10,000–50,000) and filtered (low, 30 Hz; high, 1,000–3,000 Hz) with a Grass P511 bandpass amplifier and a Grass HIP511 high-impedance probe (Grass Instr. Co., Quincy, Massachusetts). The amplified and filtered signal was channeled to a Tektronix 5113 oscilloscope (Tektronix Inc., Beaverton, Oregon) and a Grass Model 7DA polygraph for visual evaluation, to an audio amplifier–loudspeaker (model AM8 Audio Monitor, Grass Instr. Co.) for auditory evaluation, and to a rectifying voltage integrator (model 7P10, Grass Instr. Co.).

The neurogram and integrated voltage signals were displayed on the polygraph. The quality of the renal sympathetic nerve activity signal was assessed by its pulse-synchronous rhythmicity and by the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with an intravenous injection of phenylephrine (1–3 μg). When an optimal renal sympathetic nerve activity signal was observed, the recording electrode was fixed to the nerve bundle with a silicone adhesive (Wacker Sil-Gel 604, Wacker-Chemie, Munich, West Germany). We then secured the electrode cable in position by suturing it to the abdominal trunk muscles. Finally, the electrode cable was tunneled to the back of the neck and exteriorized; the flank incision was closed in layers.

After surgical preparation, rats were placed in rat holders to permit steady-state urine collection. An intravenous infusion (20 μl/min) of isotonic saline containing sufficient quantities of inulin and p-aminohippurate (PAH) for determination of inulin and PAH clearances was then started. Four to 6 hours after surgery the arterial catheter was attached to a pressure transducer (model P23Db, Statham, Oxnard, California), the urinary bladder catheter was led to a collection beaker, and the experiment was begun.

Experimental Protocol

Throughout the experiment mean arterial blood pressure, heart rate, and renal sympathetic nerve activity were continuously recorded. During a 30-minute control period, two consecutive 15-minute urine collections and two venous blood samples (200 μl each) at the midpoint of each collection period were taken. Thereafter, all rats received intravenously over 30 minutes a volume of isotonic saline equal to 3% body weight. Thereafter, the 2-hour recovery period consisted of four consecutive 30-minute urine collection periods with midpoint venous blood samples. Finally, the rats were killed with an overdose of methohexital, and postmortem renal sympathetic nerve activity was recorded as a measure of background noise; these values were subtracted from all experimental values of renal sympathetic nerve activity. The kidneys were removed, decapsulated, drained, and weighed.
FIGURE 1. Left, responses of renal nerve activity (RNA, n=6), urinary flow rate (V, n=9), and urinary sodium excretion (UNaV, n=9) to 3% body weight isotonic saline volume expansion in conscious Dahl salt-sensitive (S) rats. Right, responses of renal nerve activity (n=6), urinary flow rate (V, n=8), and urinary sodium excretion (UNaV, n=8) to 3% body weight isotonic saline volume expansion in conscious Dahl salt-resistant (R) rats. C, control; VE, volume expansion. *p<0.05.

**Miscellaneous**

Data acquisition for mean arterial blood pressure, heart rate, and renal sympathetic nerve activity measurements was performed with a commercially available computer software package (Labtech Notebook, version 4.2, Lab. Technols. Corp., Wilmington, Massachusetts). Integrated renal sympathetic nerve activity was expressed as μV sec/1 sec interval. Because of the limitations of comparing values of multifiber renal sympathetic nerve activity between rats, the data are expressed as percentage change from control values.

Urine volume was determined gravimetrically. Urine and plasma sodium concentrations were measured by flame photometry (model 143, Instrumentation Laboratories, Lexington, Massachusetts). Urine and plasma inulin and PAH concentrations were determined by the anthrone and ethylenediamine methods, respectively.

Glomerular filtration rate (GFR) was measured as inulin clearance:

\[ C_{IN} = \frac{V \cdot U_{IN}}{P_{IN}} \]

where \( U_{IN} \) and \( P_{IN} \) are urine and plasma inulin concentrations, respectively, and \( V \) is urinary flow rate. Renal plasma flow (RPF) was determined by PAH clearance:

\[ C_{PAH} = \frac{V \cdot U_{PAH}}{P_{PAH}} \]

where \( U_{PAH} \) and \( P_{PAH} \) are urine and plasma PAH concentrations, respectively.

The data were statistically analyzed using one-way analysis of variance, repeated-measures analysis of variance, and Scheffe’s post hoc test. Significance was defined as \( p<0.05 \).

**Results**

The control values of mean arterial blood pressure were 109.9±3.6 mm Hg for intact DS rats (n=8) and 111.0±2.6 mm Hg for intact DR rats (n=8). The respective values for denervated DS rats (n=6) were 121.8±3.2 mm Hg and 115.7±3.7 mm Hg in denervated DR rats (n=6). Heart rate was 437±13 beats/min for intact DS rats (n=8) and 429±19 beats/min for intact DR rats (n=8); the respective values for denervated rats were 422±11 beats/min in DS (n=6) and 409±14 beats/min in DR rats (n=6). There were no significant differences in mean arterial blood pressure and heart rate among the groups throughout the experiment.

As displayed in Figures 1 and 2, urinary flow rate to the highest level during the first 30-minute period and were back to control values during the third 30-minute period of recovery. This pattern was similar for all four groups of rats with no significant differences among them. The peak excretion rates in DS rats were \( V, 33.1 \pm 4.9 \) μl/min/g kidney wt and \( U_{IN} V, 4.2 \pm 0.85 \) μeql/min/g kidney wt in intact rats (n=9) versus \( V, 38.3 \pm 6.2 \) μl/min/g kidney wt and \( U_{IN} V, 5.6 \pm 0.7 \) μeql/min/g kidney wt in denervated (n=6) rats. The peak excretion rates in DR rats...
Volume Loading in Prehypertensive Dahl Rats

V. R. M. et al.

Dahl S Rats

Dahl R Rats

\[ V (\mu l/min/g Kw) \]

\[ U_{\text{Na}} (\mu eq/min/g Kw) \]

\[ N = 6 \]

\[ N = 6 \]

Fig. 2. Responses of urinary flow rate (V) and urinary sodium excretion (U\(_{\text{Na}}\)V) to 3% body weight isotonic saline volume expansion in conscious Dahl salt-resistant (R) (n=6) and Dahl salt-sensitive (S) (n=6) rats. C, control; VE, volume expansion. *p<0.05.

were V, 35.8±3.6 \( \mu l/min/g \) kidney wt and \( U_{\text{Na}}V \), 4.0±0.86 \( \mu eq/min/g \) kidney wt in intact (n=8) versus V, 37.2±4.8 \( \mu l/min/g \) kidney wt and \( U_{\text{Na}}V \), 5.3±0.89 \( \mu eq/min/g \) kidney wt in denervated (n=6) animals.

As seen in Figure 3, the control values of GFR and RPF were not different among groups and remained stable throughout the experiment (intact animals n=7, denervated animals n=6).

Representative renal sympathetic nerve recordings are shown in Figure 4. Mean changes of renal sympathetic nerve activity in DS and DR rats during volume expansion and recovery are displayed in Figure 1. Basal renal nerve activity of DS rats (n=6) was 189±46 \( \mu V \cdot sec/sec \) and of DR rats (n=6) 134±36 \( \mu V \cdot sec/sec \); these values are not significantly different from each other. During the volume-expansion period, renal sympathetic nerve activity is shown at four sampling points at 7.5-minute intervals. In both DR and DS rats, the maximal decrease in renal sympathetic nerve activity occurred 15 minutes after onset of volume expansion; there was no significant difference between DR and DS rats throughout the 30-minute volume-expansion period. During the recovery period, renal sympathetic nerve activity is shown at 15-minute intervals. In the first 15 minutes after cessation of volume expansion, renal sympathetic nerve activity had returned to control values in DS rats and remained there for the duration of the recovery period. In DR rats, however, renal sympathetic nerve activity remained depressed and did not exhibit any recovery toward control values throughout the 2-hour recovery period. Renal sympathetic nerve activity was significantly different between DS and DR rats for the entire 2-hour recovery period.

Discussion

The major finding of this study is the dissociation of the renal sympathetic nerve activity and steady-state renal excretory responses to a moderate intravenous isotonic saline load in conscious prehypertensive DS and DR rats consuming a low NaCl diet. Despite similar degrees of inhibition of renal sympathetic nerve activity during the volume-expansion period, renal sympathetic nerve activity returned quickly to control values after volume expansion in DS rats, whereas it remained depressed for up to 2 hours after volume expansion in DR rats. However, the magnitudes of the diuretic and natriuretic responses to the volume expansion were not different between DS and DR rats. In both, V and \( U_{\text{Na}}V \) were back to control values at 1 hour after cessation of volume expansion. Renal denervation did not alter the diuretic or natriuretic responses to volume expansion in either DS or DR rats.
In prehypertensive DS rats, the prompt recovery of renal sympathetic nerve activity to control values after volume expansion suggests an impaired function of reflex neural mechanisms that participate in the control of volume homeostasis. Receptors that detect volume changes (transduced as pressure) and reflexively regulate sympathetic outflow are present in the left atrium and ventricle of rats\textsuperscript{11,12} and are part of the cardiopulmonary baroreceptor reflex. Ferrari et al\textsuperscript{1} demonstrated a blunted maximal splanchnic nerve response (approximately 35% decrease) during intravenous volume loading in anesthetized prehypertensive sinoaortic denervated DS rats compared with the nerve response of DR rats (approximately 49% decrease) that was explained in terms of an altered cardiopulmonary baroreceptor reflex. While a difference in peak decreases in renal sympathetic nerve activity was not detected in the present study, several differences in experimental design may explain this: anesthetized versus conscious state, presence or absence of sinoaortic denervation, and splanchnic (both preganglionic and postganglionic elements) versus renal (postganglionic) nerve. The nature of the fluid and its volume and rate of administration were different. Ferrari et al\textsuperscript{1} used intravenous bolus injections of dextran-75, 2.7 ml/100 g body wt and hemorrhage over short time intervals to rapidly change left ventricular end-diastolic pressure to assess cardiopulmonary baroreceptor control of splanchnic nerve activity. In contrast, we used the 3% body weight isotonic saline load given over 30 minutes to assess cardiopulmonary baroreceptor control of renal nerve activity and its contribution to the steady-state renal excretory responses. In addition, the colloid osmotic properties of dextran differ from isotonic saline, and the resultant fluid shifts into and out of the vascular space would be expected to differ. Thus, it is possible that the acute versus the prolonged nature of the volume-expansion stimulus may explain these various observations.

Although we observed no changes in mean arterial blood pressure or heart rate, an influence of the high pressure baroreceptors cannot be excluded. Since the high pressure baroreceptor reflex is also altered in prehypertensive DS rats,\textsuperscript{13,14} this may have contributed to the maximal response of renal sympathetic nerve activity in our prehypertensive DS rats. It was argued that the most likely explanation for the defective cardiopulmonary baroreceptor reflex in DS rats was an abnormality in the cardiac receptors themselves or their coupling to cardiac tissue.\textsuperscript{1}

During the 2-hour recovery period after the cessation of volume expansion, we could detect no differences in urinary sodium and water excretion between DS and DR rats, either denervated or intact. These results are in agreement with those of DiBona and Sawin,\textsuperscript{3} who demonstrated that conscious DS and DR rats on either a low or high NaCl diet had similar natriuretic responses to a 10% body weight intravenous isotonic saline load. It was postulated that the absence of an exaggerated natriuretic response to intravenous isotonic saline loading in hypertensive DS rats, regularly observed in spontaneously hypertensive rats, could be explained by abnormal cardiopulmonary baroreceptor reflex control of renal sympathetic nerve activity, in analogy with the findings of Ferrari et al\textsuperscript{1} in the splanchnic nerve.

If the prompt return of renal sympathetic nerve activity to control values observed in DS rats were of functional importance, one would expect the diuretic and natriuretic responses to be attenuated (certainly not exaggerated, see above) in intact DS rats and possibly augmented in denervated DS rats in comparison with intact DR rats; this attenuation of responses was not observed. In experiments with hypertensive DS rats, renal denervation failed to alter the further development of hypertension in contrast to other NaCl-dependent or NaCl-influenced rat models of hypertension\textsuperscript{15}; this occurrence suggests that DS rats do not require renal sympathetic nerves for the development of hypertension. It is known that the increased renal vascular resistance observed in hypertensive DS rats is not related to increased neurogenic tone.\textsuperscript{16}

The similar diuretic and natriuretic responses to moderate intravenous isotonic saline volume expansion in all four groups (DS and DR rats, intact and denervated) despite markedly different renal sympathetic nerve activity responses suggest a decreased responsiveness of the kidney in both DS and DR.
rds to altered neural input. In addition, since normal rats, dogs, and monkeys with renal denervation have attenuated diuretic and natriuretic responses to acute volume expansion, this would further support the view that the neural control of renal function is altered in DR and DS rats.

References

Key Words • volume loading • cardiac receptors • efferent renal nerve activity • kidney function
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