RENAL SYMPATHETIC NERVE ACTIVITY AND THE EXAGGERATED NATRIURETIC RESPONSE OF THE SPONTANEOUS HYPERTENSION RAT

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SUMMARY To evaluate the role of altered efferent renal sympathetic nerve activity in the exaggerated natriuresis of the spontaneous hypertensive rat (SHR), we measured efferent renal sympathetic nerve activity in anesthetized age- and sex-matched SHR and normotensive Wistar Kyoto (WKY) control rats during control and intravenous saline volume expansion. Mean arterial pressure was 40-60 mm Hg higher in SHR than in WKY. During volume expansion, the increment in urinary flow rate (17.5 ± 3.1 vs 42.8 ± 8.0 /L/100 g/min) and sodium excretion (8.1 ± 1.5 vs 15.2 ± 2.3 mEq/min) were greater in SHR than in WKY. During control, efferent renal sympathetic nerve activity was 12.2 ± 0.4 Hz in WKY and 12.0 ± 0.5 Hz in SHR and fell equally during volume expansion to 5.0 ± 0.6 Hz in WKY and to 4.0 ± 0.8 Hz in SHR. Thus, these studies demonstrate that the exaggerated natriuresis of SHR as compared to WKY cannot be attributed to differences in reflex withdrawal of renal sympathetic nerve activity. (Hypertension 3: 134-138, 1981)

KEY WORDS • sympathetic nervous system • natriuresis • renal nerve • volume expansion

WE HAVE recently demonstrated that the spontaneous hypertensive rat (SHR) displays an exaggerated diuretic and natriuretic response to intravenous saline volume expansion when compared to the response of age- and sex-matched normotensive Wistar Kyoto (WKY) control rats. The mechanism of this exaggerated diuretic and natriuretic response is unknown, but several lines of evidence suggest a possible role for changes in efferent renal sympathetic nerve activity to participate in the observed alteration in the renal tubular reabsorption of sodium and water.

Previous studies from our laboratory as well as others have demonstrated in normal rats and dogs that both direct and reflex-induced changes in efferent renal sympathetic nerve activity produce directionally similar changes in renal tubular sodium and water reabsorption in the absence of changes in renal hemodynamics. These findings concur with the electron microscopic and fluorescence histochemical demonstration that adrenergic nerve terminals are in direct contact with basement membranes of proximal and distal tubules in the monkey, rat, and dog kidney. Judy and colleagues and Coote and Sato have reported that sympathetic activity of multifiber preparations of renal nerves is increased in SHR and that the sensitivity to inhibitory high-pressure baroreceptor afferent input is decreased. In addition, Schad and Sellers have demonstrated that intravenous saline volume expansion decreases efferent renal sympathetic nerve activity in conscious cats.

Based on this information we considered the possibility that alterations in efferent renal sympathetic nerve activity occurring during intravenous saline volume expansion, a low pressure cardiopulmonary baroreceptor stimulus, might contribute to the exaggerated diuresis and natriuresis observed in SHR.

METHODS

SHR and Wistar-Kyoto rats (WKY) were obtained from Laboratory Supply Company, Inc., Indianapolis, Indiana. The WKY strain is descended directly from the colony from which the SHR were isolated and are currently believed to be the most appropriate genetic controls for defining distinguishing characteristics of the SHR strain.

Male SHR and WKY rats, age 14-16 weeks, were kept in metabolic cages for at least 1 week prior to use.
and provided with standard rat pellet diet (Purina) and tap water ad libitum. The animals were anesthetized with sodium pentobarbital (50 mg/ml), 50 ml/kg administered intraperitoneally and placed on a thermostatically regulated, heated table. Approximately the same level of anesthesia was attempted in all animals. In general, supplemental doses of anesthetic were not required; occasionally, a single additional dose of sodium pentobarbital, 12.5 mg/kg i.p., was required approximately 120 minutes after the initial anesthetizing injection. The left kidney was exposed through a left subcostal incision and after dissection from the surrounding tissue, was mounted in a lucite chamber. Care was taken to avoid compression of the renal neurovascular pedicle; visible assessment of the amplitude and frequency of renal pulsations and of capillary flow served as control criteria. The kidney was continuously bathed in 38° C mineral oil and illuminated with a fiberoptic light source. Catheters (P.E. 50 or greater) were placed in the left ureter, a jugular vein, and a carotid artery for collection of urine, infusion of solutions, and measurement of arterial blood sampling. Exposed tissues were covered with gauze pads moistened with 38° C 0.9% NaCl and an infusion of 0.9% NaCl was established at the time of jugular vein catheterization and maintained at 0.05 ml/min thereafter. Each animal received an intravenous priming dose of inulin to establish and maintain a plasma inulin concentration of 0.5 to 1.0 mg/ml.

Slips of renal nerve were isolated from the ventral surface of the renal neurovascular pedicle using a stereoscopic dissecting microscope. Recordings from multifiber preparations were made by placing the nerves on bipolar platinum electrodes; the nerves and electrodes were continuously bathed in a pool of 38° C mineral oil to prevent tissue drying. The efferent signals were recorded by means of a bandpass amplifier (Ortec Model 4660). The amplified signals were then displayed on an oscilloscope (Tektronix Type 564) and led sequentially to an instantaneous frequency/time meter (Ortec Model 4672) and a resetting voltage integrator (Beckman type 9873B) whose reset time was a function of discharge frequency. The resetting voltage integrator was used in the total mode to integrate groups of unipolar pulsatile signals. In this mode, the frequency response of the resetting voltage integrator is that of the interfaced preamplifier (Beckman 481B), 0-200 Hz maximum. In vitro testing of the system's frequency response was carried out by applying unipolar square wave pulses (10 V, 1 msec) at varying frequency to the bipolar platinum electrode. The relationship between resetting voltage integrator spikes per minute and stimulation frequency was linear over the range of 0-100 Hz, with a correlation coefficient, r, of 0.995. Carotid arterial pressure was measured continuously with an electronic transducer (Statham P 23 Db) and was recorded with the output of the resetting voltage integrator on a direct writing strip chart recorder (Beckman Type R 411).

Following a 45-minute stabilization period, the control (H) period consisted of two consecutive 30-minute urine collections and recording mean arterial pressure (MAP). Arterial blood samples, 50 to 100 µl, were obtained at 30-minute intervals. At the end of the control period, each animal received 0.9% NaCl in a volume equal to 10% of body weight administered intravenously over a 30-minute period. Thereafter, the infusion rate was reduced to match the total urinary flow rate. Renal nerve activity was recorded during the 10 minutes before and continuously during the 30-minutes of volume expansion. When total urinary flow rate had stabilized, the 60-minute experimental volume expansion (VE) was begun, and the control period measurements were repeated.

Urine was collected under mineral oil in tared containers and volume was determined gravimetrically. Inulin was measured in urine and plasma by the method of Vurek and Pegram. Sodium was measured in urine and plasma by flame photometry. Kidney glomerular filtration rate (GFR) was estimated as inulin clearance, C\textsubscript{IN} = U\textsubscript{IN}/P\textsubscript{IN} × V, where U\textsubscript{IN} and P\textsubscript{IN} are urine and plasma inulin concentrations and V is urine flow rate.

The data in the text and figures are presented as the mean ± standard error (SE). The Student's t test was used for statistical analysis of data within and between each group.18

**Results**

Figure 1 shows an efferent renal nerve recording from an SHR. The efferent renal nerve activity occurred as grouped discharges of action potentials of approximately 50-125 µV. These observations are similar to those presented by Judy and colleagues8 (figs. 2 and 3).

![Rat Renal Nerve Activity](image-url)
Figure 2 shows the effect of an intravenous bolus of 5 µg norepinephrine on MAP and efferent renal nerve activity as the output of the resetting voltage integrator. The MAP increases sharply and there is a concomitant abrupt decrease in efferent renal nerve activity as reflected by the reduction in the frequency of resets by the voltage integrator. As MAP returns toward control levels, efferent renal nerve activity also returns toward control levels. In addition, efferent renal nerve activity was markedly reduced or abolished by administration of hexamethonium. These observations validate our methods for measurement of efferent renal sympathetic nerve activity and demonstrate our ability to record changes in efferent renal sympathetic nerve activity in response to physiological interventions such as stimulation of the high pressure arterial baroreceptors.

Figure 3 shows the hemodynamic and clearance data from 10 WKY and 10 SHR. MAP was greater in SHR than WKY during both control and volume expansion. Urinary flow rate, sodium excretion, and fractional excretion of sodium were higher in SHR than in WKY during both control (p < 0.025 for all) and volume expansion (p < 0.005 for all). The increments in urinary flow rate (42.8 ± 8.0 vs 17.5 ± 3.1 µl/min/100 g, p < 0.01), urinary sodium excretion (15.2 ± 2.3 vs 8.1 ± 1.5 µEq/min, p < 0.025) and fractional excretion of sodium (14.8 ± 1.2 vs 7.8 ± 1.5%, p < 0.005) produced by saline volume expansion were greater in SHR than WKY. The clearance of inulin was similar in SHR and WKY during both control and volume expansion, with the SHR values tending to be lower.

Figures 4 and 5 show the efferent renal nerve activity data. During the control hydropenic period, efferent renal nerve activity was similar in WKY (12.0 ± 0.4 Hz) and SHR (12.2 ± 0.5 Hz). The change in efferent renal nerve activity during the first 30 minutes of volume expansion was similar in WKY and SHR; at 30 minutes, efferent renal nerve activity was 5.0 ± 0.6 Hz or 41.0 ± 4.9% of control in WKY and was 4.0 ± 0.8 Hz or 33.0 ± 6.7% of control in SHR.

Discussion

These studies demonstrate that SHRs have an exaggerated diuresis and natriuresis during both control and saline volume expansion when compared to normotensive WKY, in confirmation of our previous findings. In addition, anesthetized WKY and SHR do not have different levels of efferent renal nerve activity during control conditions and showed identical decreases in efferent renal nerve activity during saline volume expansion. Therefore, the exaggerated diuresis and natriuresis observed in SHR as compared to WKY cannot be attributed to differences in reflex withdrawal of renal sympathetic neural tone.

Our previous studies using an identical protocol in SHR rats of identical age and sex demonstrated that absolute and fractional water and sodium excretion were greater in SHR than in WKY during both control and volume expansion experimental periods.
Micropuncture studies showed that, although fractional and absolute water and sodium reabsorption were similar along the proximal convolution in SHR and WKY, fractional and absolute reabsorption in Henle's loop was less in SHR than in WKY. Thus, the exaggerated diuresis and natriuresis of SHR is caused, in part, by a decreased reabsorption in the loop of Henle.

**Figure 4.** Efferent renal nerve recording from SHR and WKY during control and volume expansion periods.

Although the ability of changes in efferent renal sympathetic nerve activity to directly produce parallel changes in tubular sodium and water reabsorption is well established, this mechanism cannot explain the exaggerated diuresis and natriuresis of SHR as compared to WKY inasmuch as measurements of efferent renal sympathetic nerve activity revealed no differences in the reflex withdrawal between WKY and SHR, during volume expansion.

It has been postulated that the SHR has an increased activity of the sympathetic nervous system that contributes to the observed increases in total peripheral and regional vascular resistance as part of the hypertensive state. Many of these studies have utilized indirect techniques such as surgical denervation or pharmacological inhibitors to indicate increased sympathetic nervous system activity in SHR. Other investigators measuring certain aspects of catecholamine metabolism have suggested that sympathetic nervous system activity is normal or even reduced in SHR.

With regard to the kidney, direct recordings of efferent renal sympathetic nerve activity have been reported by Judy and colleagues and Coote and Sato. Both groups of investigators made recordings from multifiber preparations of renal nerves in SHR. Judy and colleagues found basal levels of efferent RSNA to be higher in SHR than WKY at 16, 24, and 40 weeks of age. They also found that SHRs less than 16 weeks old maintained their ability to inhibit efferent RSNA via increased baroreceptor stimulation (induced hypertension) with the same sensitivity as in normotensive Wistar rats but progressively lost it with advancing age; data on age-matched WKY rats studied similarly were not reported.

In another study, Judy and colleagues showed that SHRs are more sensitive than WKY to the inhibition of efferent renal sympathetic nerve activity by L-dopa. Coote and Sato found that the threshold pressure required to produce a silence in efferent renal sympathetic nerve activity was higher in SHR than in normotensive Wistar rats; in addition, comparable increases of MAP above the threshold pressure produced a shorter duration of inhibition of efferent RSNA in SHR than in normotensive Wistar rats. Data on age-matched WKY rats studied similarly were not reported. Therefore, studies employing direct renal nerve activity recording techniques in age-matched SHR and genetic control normotensive WKY rats indicate that basal levels of efferent renal sympathetic nerve activity are higher in SHR.

We are in agreement with Coote and Sato that it is exceedingly difficult to employ multifiber renal nerve activity recordings to compare actual renal nerve discharge rates between two groups of rats. Although we used the technique of multifiber renal nerve activity recordings as did Judy and colleagues, we find that age-matched 16-week-old SHR and WKY have similar levels of efferent renal sympathetic nerve activity whereas Judy and colleagues find higher levels in SHR than in WKY at 16 weeks. It is possible that differences in the source of animals, level of
anesthesia, or method of processing the renal nerve activity could account for these differences. It is likely that single-fiber renal nerve activity recordings will be required to understand these differences.

Although statements concerning differences in actual renal nerve discharge rates between two different groups of rats are fraught with difficulty when based on multifiber renal nerve activity recordings, the response within each rat to an intervention designed to reflexly alter actual renal nerve discharge rates would be faithfully reflected by multifiber renal nerve activity recordings, and such data will permit valid comparisons to be made between two different groups of rats. Such an intervention was made by Judy and colleagues[4, 10] and Coote and Sato.[11] However, two of the three studies used normotensive Wistar rats rather than the appropriate genetic control WKY rats. The one study[10] that did compare SHR to WKY utilized a complex pharmacological intervention, L-dopa after peripheral L-amino acid decarboxylase inhibition with carbidopa, to produce central nervous system depression of sympathetic nerve activity.

Because we were interested in evaluating a possible relationship between the exaggerated diuretic and natriuretic response of SHR to isotonic saline volume expansion[1] and the known role of efferent renal sympathetic nerve activity in the regulation of renal tubular sodium and water reabsorption,[2, 3] we used isotonic saline volume expansion to reflexly alter efferent renal sympathetic nerve activity. Schad and Sellers[12] demonstrated that isotonic volume expansion to 5%-15% (average, 11%) of body weight reduced efferent renal sympathetic nerve activity by 25%-85% (average 48%); the efferent renal sympathetic nerve activity decreased progressively during the course of the expansion period and the minimum level was observed approximately 10-15 minutes following completion of the load. They attributed this response to stimulation of type B left atrial receptors by a rising left atrial pressure,[13] which is known to increase with expansion of the blood volume,[14, 15] the inhibitory effect of stimulation of type B left atrial receptors on sympathetic efferent outflow correlates with the discharge rate of type B left atrial receptors.[16] When we subjected SHR and WKY rats to 10% isotonic saline volume expansion, efferent renal sympathetic nerve activity was observed to decrease in a progressive fashion throughout the 30 minutes of expansion. The percent decreases of efferent renal sympathetic nerve activity were not different between WKY and SHR. Therefore, in response to the identical intervention designed to produce low-pressure baroreceptor reflex alterations in efferent renal sympathetic nerve activity, WKY and SHR behaved similarly.

Insofar as this reflex mechanism is representative of the many mechanisms involved in the reflex control of efferent renal sympathetic nerve activity, it is clear that the reflex withdrawal of efferent renal sympathetic nerve activity by stimulation of low pressure baroreceptors is not different between WKY and SHR. Since the decrease in efferent renal sympathetic nerve activity following isotonic saline volume expansion was identical in anesthetized SHR and WKY, the exaggerated diuresis and natriuresis observed in SHR as compared to WKY cannot be attributed to differences in withdrawal of renal sympathetic neural tone. It remains possible that basal efferent renal sympathetic nerve activity may differ in conscious WKY and SHR and that this difference might allow for different degrees of attenuation during isotonic saline volume expansion.

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