EFFECTS OF RENAL SYMPATHECTOMY ON SODIUM AND WATER EXCRETION IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—In stroke-prone spontaneously hypertensive rats (SHRSP), urinary excretion of sodium and water and glomerular filtration rate (GFR) are markedly decreased by acute normalization of the renal perfusion pressure using an aortic clamping technique. To examine the mechanism for the decreased sodium and water excretion, SHRSP rats were subjected to bilateral renal denervation. Sodium and water excretion and GFR in SHRSP with aortic clamping were significantly restored by the renal denervation. The restoration was more prominent in the urine flow and GFR. When renal perfusion pressure was normalized by administration of sympatholytic drugs in another group of SHRSP, sodium and water excretion were decreased. However, the extent of the decrease in urine flow but not in sodium excretion was significantly less than that in SHRSP with aortic constriction. GFR was not changed by administration of the sympatholytic drugs. Renal denervation lowered the blood pressure in SHRSP. These results suggest that renal sympathetic nerve activity is greatly involved in the reduced water excretion and partly involved in the reduced sodium excretion in SHRSP rats.

There is considerable evidence to support the hypothesis that the sympathetic nervous system influences renal sodium excretion independent of changes in systemic or renal hemodynamics (1). In rats, adrenergic nerve endings are found on the cells of the afferent and efferent arterioles and renal tubules (2). Direct electrical renal nerve stimulation decreased renal sodium excretion in the absence of detectable changes in glomerular filtration rate and renal blood flow (3). This effect could be blocked by adrenolytic drugs (4, 5). When renal denervation was produced in anesthetized rats by application of phenol to the renal artery, urinary sodium excretion increased approximately five- to six-fold without changes in renal hemodynamics (6). Similar results have been obtained by chronic renal denervation (7).

In spontaneously hypertensive rats (SHR) enhanced sympathetic nerve activity has been demonstrated by direct measurement of sympathetic nerve activity (8–10). However, renal sodium and water excretion in SHR was normal in the hypertensive stage (11, 12). This is true of the stroke-prone strain of SHR (12) established by Okamoto et al. (13). However, a reduced ability of SHR and stroke-prone SHR (SHRSP) to excrete sodium and water could be demonstrated by reducing the renal perfusion pressure to a level similar to that of control Wistar Kyoto rats (12, 14). The purpose of the present experiments was to investigate
whether renal sympathetic activity contributes to the decreases in renal sodium and water excretion in SHRSP that are observed when the renal perfusion pressure is normalized. Two procedures were employed: chronic renal denervation and pharmacological reduction of renal perfusion pressure using sympatholytic drugs.

MATERIALS AND METHODS

Male stroke-prone spontaneously hypertensive rats (SHRSP) 8 to 9 weeks of age were used. They were housed in a room at constant temperature (24±1°C), humidity (55±5%), and illumination (7:00 a.m. to 7:00 p.m.). The rats, fasted 20 hr prior to the experiments but allowed free access to drinking water, were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and maintained at a rectal temperature between 37 to 38°C by infrared lamps.

Renal denervation and aortic constriction: A total of 40 SHRSP were subjected to bilateral renal denervation (n=20) or sham operation (n=20) one week before the clearance experiments. Under ether anesthesia, renal denervation was accomplished through a ventral incision by stripping the renal adventitia and painting the renal artery with 10% phenol (wt./vol.) in absolute ethanol. The sham operation consisted of a ventral incision and isolation of the kidneys from adjacent tissues. Animals of the two groups were divided into two subgroups with or without aortic constriction.

On the day of the experiment, immediately after the induction of pentobarbital anesthesia, the femoral artery and vein and bladder were cannulated with polyethylene tubing (PE-50) or soft tubing (Technicon). Femoral arterial pressure was used as an estimation of renal perfusion pressure. An adjustable constrictor clamp was placed around the abdominal aorta just above the renal arteries. In the groups with aortic constriction, the clamp was constricted to maintain the mean arterial pressure at approx. 100 mmHg, the average pressure of normal rats (12). In the other groups, the same operation procedure was performed, but the clamp was not constricted.

Reduction of renal perfusion pressure by sympatholytic drugs: Mechanical reduction of renal perfusion pressure (aortic constriction) resulted in marked decreases in sodium and water excretion in SHRSP. Therefore, in this experiment, the effects of pharmacological reduction in renal perfusion pressure on sodium and water excretion were examined in SHRSP. Phentolamine mesylate (5 mg/kg) and hexamethonium bromide (2.5 mg/kg) were intravenously administered because an individual injection of the drugs failed to maintain a stable renal perfusion pressure at approx. 100 mmHg. A small additional dose of hexamethonium was injected into some animals whose renal perfusion pressure did not decrease below 105 mmHg for the clearance period.

Analytical procedures: Glomerular filtration rate (GFR) was measured by the clearance of inulin, by the method reported previously (12). In brief, an intravenous priming dose of inulin (20 mg/kg) was given to each rat at a rate of 0.4 ml/100 g body wt.; and inulin, 0.6 mg in 0.02 ml/kg per min, was added to the sustaining infusion of saline. Urine was collected continuously after the start of the infusion, and a urine sample taken after 30–60 min was used for the inulin assay. Blood was sampled from the femoral arterial cannula at the beginning and end of the clearance period. Blood and urine samples taken before the inulin administration were used for blank samples. Inulin was measured in the plasma and urine by the method of Vurek and Pegram (15). GFR was calculated as the urine-to-plasma inulin concentration ratio times the urine flow rate measured in μl
Sodium was measured in plasma and urine by flame photometry (Corning, 455). Mean femoral arterial pressure was continuously monitored by a pressure transducer and polygraph (San-ei). Renal norepinephrine concentration in intact and denervated kidneys from SHRSP was determined by a liquid chromatography technique employing an electro-chemical detector (16). The kidneys were weighed and frozen by liquid nitrogen immediately after the experiments.

Data were expressed as the mean±S.E. Analysis of variance (wholly significant difference method) was used for statistical analysis. Statistical significance was taken as P<0.05.

RESULTS

Effects of renal denervation on sodium and water excretion in SHRSP: Body wt., kidney wt., and renal norepinephrine contents in sham-operated (control) and denervated SHRSP with or without aortic constriction (AC) are shown in Table 1. Body wt. ranged between 173–179 g after the fasting. Kidney wt. was almost identical in all groups, though the weight was slightly but significantly larger in the control SHRSP with AC (SHRSP-AC). Renal norepinephrine content was markedly decreased in the denervated groups; the contents in the denervated groups were 3.8 and 4.1% of that in the control SHRSP group. The norepinephrine content in control SHRSP-AC was slightly higher than that in control SHRSP.

Renal perfusion pressure and renal function in each group are summarized in

![Graphs showing renal perfusion pressure and renal function](image)

**Table 1.** Body weight, kidney weight, and renal norepinephrine content in each group of stroke-prone spontaneously hypertensive rat (SHRSP)

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated SHRSP</th>
<th>Sham-operated SHRSP-AC</th>
<th>Denervated SHRSP</th>
<th>Denervated SHRSP-AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>178±2</td>
<td>179±5</td>
<td>173±3</td>
<td>176±5</td>
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<tr>
<td>Kidney wt.</td>
<td>0.93±0.01</td>
<td>0.98±0.02*</td>
<td>0.95±0.01</td>
<td>0.95±0.02</td>
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<tr>
<td>(g/100 g body wt.)</td>
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<tr>
<td>Norepinephrine</td>
<td>130±5</td>
<td>149±7*</td>
<td>4.9±0.4**</td>
<td>5.3±0.5**</td>
</tr>
<tr>
<td>(ng/g kidney wt.)</td>
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Mean±S.E., n=10. SHRSP-AC: SHRSP with aortic constriction. Body wt.: weight after 20 hr fasting. *P<0.05 and **P<0.001 vs sham-operated SHRSP.
Basal values of femoral arterial pressure were 149±3 and 145±4 mmHg in control SHRSP and SHRSP-AC, respectively, and 119±5 and 118±3 mmHg in denervated SHRSP and SHRSP-AC, respectively. Thus, the arterial pressure in SHRSP was significantly reduced by chronic renal denervation. Femoral arterial pressure during the clearance period (renal perfusion pressure) was slightly (about 10 mmHg) decreased in control and denervated SHRSP. The level in denervated SHRSP was significantly (P<0.001) higher than that in denervated SHRSP-AC. In the SHRSP-AC groups, the renal perfusion pressure was about 97 mmHg; significantly lower than that in control SHRSP. In control SHRSP-AC, urine volume and sodium excretion were markedly decreased by the reduction of renal perfusion pressure. Renal denervation itself did not alter urine flow and sodium excretion in SHRSP: no significant differences were observed between control and denervated SHRSP groups. The decreases in urine volume and sodium excretion observed in control SHRSP-AC were partially but significantly restored by renal denervation: urine volume and sodium excretion in denervated SHRSP-AC were significantly greater than those in control SHRSP-AC. The restoration was more prominent in urine volume than in sodium excretion. GFR was markedly decreased in control SHRSP-AC, but not in denervated SHRSP-AC, by the reduction in renal perfusion pressure, indicating that renal denervation inhibits the decrease in GFR because of aortic constriction. Plasma sodium concentration tended to be increased in the two SHRSP-AC groups.

Effects of pharmacological reduction in renal perfusion pressure on sodium and water excretion in SHRSP: To further investigate the role of renal sympathetic nerve activity in sodium and water excretion in SHRSP, renal perfusion pressure in SHRSP was reduced by the administration of ganglion-blocking and adrenergic alpha-receptor blocking agents. In Table 2, the data are compared with those obtained in SHRSP whose renal perfusion pressure is reduced by phentolamine and hexamethonium.

<table>
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<tr>
<th>Table 2. Renal perfusion pressure, urinary volume, sodium excretion, and several variables in control, aorta-constricted and drug-treated SHRSP</th>
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<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>SHRSP</td>
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<tr>
<td>Basal arterial pressure (mmHg)</td>
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<tr>
<td>Renal perfusion pressure (mmHg)</td>
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<tr>
<td>Urinary volume (ml/min/g)</td>
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<td>Sodium excretion (meq/min/g)</td>
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<td>GFR (ml/min/g)</td>
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<tr>
<td>Plasma sodium concentration (meq/l)</td>
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<tr>
<td>Body wt. (g)</td>
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<tr>
<td>Kidney wt. (g/100 g body wt.)</td>
</tr>
</tbody>
</table>

Mean±S.E. n=10. Urinary volume, GFR, and sodium excretion are expressed as the value per gram of kidney wet wt. Body wt.: weight after 20 hr fasting. *P<0.05 and **P<0.001 vs controls. +P<0.05 vs the aorta-constricted group. Drug-treated SHRSP: SHRSP treated with phentolamine and hexamethonium.
aortic constriction. Basal femoral arterial pressure in the control, aorta-constricted, and drug-treated SHRSP (n=10 in each) was 144–148 mmHg. The arterial pressure (renal perfusion pressure) in the constricted and treated SHRSP groups was reduced to about 100 mmHg, significantly lower than that in the control SHRSP group.

Urine volume and sodium excretion were markedly decreased in the two groups of SHRSP with the reduction in renal perfusion pressure. However, urine flow in the drug-treated SHRSP was slightly but significantly greater than that in SHRSP-AC, although renal perfusion pressure in the two groups was similarly reduced. Sodium excretion in the drug-treated SHRSP and SHRSP-AC was decreased to the same level, which was significantly lower than that in the control SHRSP. GFR was also significantly reduced in SHRSP-AC but not in the drug-treated SHRSP. Plasma sodium concentration was almost the same in the three groups.

DISCUSSION

In a previous study (12), we observed that in anesthetized SHRSP (9 weeks of age), arterial pressure and renal vascular resistance were markedly elevated; but urine flow and sodium excretion were similar to those in control Wistar Kyoto rats. However, when renal perfusion pressure in SHRSP was normalized by aortic constriction, sodium and water excretion were markedly decreased (12); this was confirmed in the present study. Moreover, it was found recently that the ability of SHRSP to excrete sodium and water was significantly reduced in the prehypertensive stage (17). These findings suggest that the ability of SHRSP kidneys to excrete sodium and water may be essentially reduced by factors such as altered hemodynamics and sympathetic activity.

Renal denervation in SHRSP inhibited the decreases in urine flow and sodium excretion that were demonstrated when renal perfusion pressure was reduced to a level similar to that in normal Wistar Kyoto rats. The inhibition was very prominent for urine flow and slight for sodium excretion. On the other hand, when renal perfusion pressure in SHRSP was reduced to normal by the administration of sympatholytic drugs, the decreases in urine flow but not in sodium excretion were significantly lessened compared with that in SHRSP-AC. These results suggest that sympathetic nerve activity is involved at least in part in the decreases in sodium and water excretion in SHRSP with aortic constriction.

GFR was decreased in intact SHRSP by the reduction in renal perfusion pressure. The decrease in GFR was markedly inhibited by both renal denervation and by administration of sympatholytic drugs. The inhibition seems to be associated with a change in renal blood flow because the decrease in GFR in SHRSP-AC was accompanied by a decrease in renal blood flow (12). Thus, changes in GFR and urine flow were almost completely restored by renal sympathectomy; and the decrease in sodium excretion was partly restored by renal denervation, but not by the administration of antiadrenergic drugs. On the basis of these results it is speculated that the decreases in urine flow and GFR in SHRSP-AC are largely mediated by an increased sympathetic activity and presumably the resultant decrease in renal blood flow. Moreover, an involvement of other factors such as circulating catecholamines (18) should be considered for sodium reabsorption in SHRSP-AC.

Bello-Reuss et al. (6, 19) reported that in anesthetized rats renal nerve stimulation produced an increase in sodium reabsorption which occurred in the proximal convoluted tubule in the absence of changes in GFR or renal blood flow and that renal denervation induced a marked increase in urinary sodium
and water excretion independent of changes in GFR or renal blood flow. In contrast to the latter results, renal denervation per se did not alter urinary sodium and water excretion in SHRSP and renal sympathectomy increased GFR in SHRSP-AC. Thus, although the detailed mechanisms are unknown, the role of the renal nerve in renal sodium handling and GFR seems to be somewhat different between SHRSP and normal rats.

In the present study, an unexpected result was observed: renal denervation markedly decreased basal arterial pressure in SHRSP one week after the surgical operation. This finding suggests a possible involvement of renal sympathetic nerves in the pathogenesis of hypertension in SHRSP. Winternitz et al. (20) have also reported that the renal sympathetic nerves contribute to the development, but not the maintenance of hypertension in SHR.

In summary, the present data suggest that renal sympathetic nerve activity is related at least in part to the decreases in sodium and water excretion demonstrated in SHRSP whose renal perfusion pressure is normalized. Moreover, the data provide additional evidence for the participation of renal sympathetic nerves in the genesis of hypertension in SHRSP.

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REFERENCES

