Adrenergic Mechanisms Do Not Contribute to Salt-Induced Vasoconstriction in Stroke-Prone Spontaneously Hypertensive Rat

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SUMMARY The study was done to determine if neurogenic mechanisms participate in salt-induced vasoconstriction in stroke-prone spontaneously hypertensive rats (SHR-SP) in the established stage of hypertension. Either high (8% NaCl) or normal (0.3% NaCl) salt diet was given for 5 weeks to 8-week-old SHR-SP. High salt intake increased mean arterial pressure (MAP) and hindquarter vascular resistance (VR) in SHR-SP in the established stage of hypertension ($p < 0.01$). However, hindquarter sympathetic vascular tone assessed by the difference in hindquarter VR before and after sympathetic denervation was not increased in SHR-SP on high salt diet more than that in rats on normal salt diet. The reduction of MAP by alpha-adrenergic blockade produced by a supramaximal dose of intravenous phentolamine in conscious rats was not greater in rats on high salt diet than that in rats on normal salt diet. These results suggest that adrenergic tone was not increased in rats on high salt diet. In addition, the increase in arterial pressure during high salt diet was not altered by destroying noradrenergic neurons by chronic treatment with 6-OHDA, 75 to 100 mg/kg intraperitoneally given every week. Hindquarter vascular responses to direct sympathetic nerve stimulation and tyramine were markedly reduced, and responses to norepinephrine were augmented in rats treated with 6-OHDA, which suggested that sympathetic denervation of the blood vessels had been achieved. These results suggest that high salt diet produces vasoconstriction in SHR-SP in the established stage of hypertension but adrenergic mechanisms do not contribute importantly to salt-induced vasoconstriction.

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Key Words • Hindquarters vascular resistance • 6-hydroxydopamine • high salt intake

High salt intake has been reported to alter neurogenic mechanisms in several models of hypertension. De Champlain et al.1-3 demonstrated that altered neurogenic mechanisms contribute importantly to the development of DOCA/salt hypertension in rats. Recent studies suggest that high salt intake augments neurogenic vasoconstriction in borderline hypertension in humans4 and in the Dahl strain of genetically hypertensive rats.$^5$, $^6$ High salt intake aggravates hypertension$^7$, $^8$ and increases vascular resistance$^11$, $^12$ in spontaneously hypertensive rats (SHR). Aggravation of hypertension during high salt intake is particularly prominent in stroke-prone SHR (SHR-SP).$^7$ However, the mechanisms by which high salt intake increases vascular resistance in SHR or SHR-SP are not known.

To determine if neurogenic mechanisms contribute to the increase in vascular resistance during high salt intake in SHR-SP in the established stage of hypertension, we examined: 1) whether neurogenic vascular tone was higher in SHR-SP on high salt diet than in those on normal salt diet after 5 weeks of dietary treatment; and 2) whether chronic treatment with 6-hydroxydopamine (6-OHDA) would prevent salt-induced elevation of arterial pressure in SHR-SP.

Methods

Two groups of SHR-SP were fed standard 0.3% NaCl chow from weaning until about 8 weeks of age. Subsequently, rats in one group ($n = 28$) were fed chow containing 8% NaCl while those in the other group ($n = 31$) continued feeding on 0.3% NaCl chow. Potassium chloride content was 0.8% for both diets.
Systolic arterial pressure was measured weekly by tail plethysmography using a programmed electrophysymomanometer (UEDA Electronic Works, Ltd., USM-105R, Tokyo, Japan).

Hindquarter Preparation for Measuring Vascular Resistance

The control of hindquarter vascular resistance was examined in the perfused hindquarters in anesthetized rats with sodium pentobarbital, 50 mg/kg given intraperitoneally. The preparation for hindquarter perfusion in rats has been described previously. The abdominal aorta was exposed through a midline incision, and ligated distal to the renal arteries; two cannulas were inserted. Blood from the proximal aorta was pumped at a constant flow into the distal aorta perfusing the vascularly isolated hindquarters, using a perfusion pump (Watson Mathew Limited, MHRE 100, England). Hindquarter perfusion pressure was measured via a side arm in the perfusion tubing downstream from the pump. In tabulating perfusion pressure at a given flow, we subtracted the pressure resulting from the resistance of the perfusion tubing. Resistance of the tubing was determined at the end of each study.

To determine vascular resistance, we obtained a pressure-flow curve. Perfusion pressure at a constant flow represents vascular resistance. Perfusion pressure was recorded at hindquarter blood flows of 3, 4, and 5 ml/min.

Assessment of Sympathetic Vascular Tone

The hindquarters were denervated by cutting the lumbar sympathetic chains. Efficacy of denervation was tested by demonstrating that the response to stimulation of the proximal end of sympathetic chains at 10 Hz was reduced by more than 90%. We assessed sympathetic vascular tone from the difference in vascular resistance before and after denervation.

In addition, we studied the effects of intravenous phenolamine on arterial pressure in rats on high salt diet and those on normal salt diet. The effects of phenolamine on arterial pressure were examined in previously cannulated conscious rats. Two cannulas were inserted in an artery and a jugular vein under light anesthesia with ether. The experiments were done with rats contained in a small chamber after they recovered fully from anesthesia. Phenolamine, 1 mg/kg, was given intravenously by a bolus injection while arterial pressure was continuously recorded. Arterial pressure was stable for at least 15 minutes before an injection of phenolamine. At the end, an additional dose of phenolamine, 0.5 mg/kg, was given, and it was confirmed that there was no further reduction of arterial pressure.

Treatment with 6-OHDA

A group of SHR-SP (n = 9) about 8 weeks old were given 6-OHDA, 75 to 100 mg/kg, intraperitoneally every week for 5 weeks, and another group of rats (n = 12) were given vehicle. Treatment with 6-OHDA or vehicle was begun at the time when they were put on high salt diet. Water was provided for drinking ad libitum. Systolic arterial pressure was measured once every week for at least 2 weeks before the beginning of treatment with 6-OHDA or vehicle and once every week during the period of treatment. The measurements of systolic arterial pressure were obtained 5 to 7 days after the last injection of 6-OHDA or vehicle to avoid acute and transient effects of 6-OHDA on arterial pressure.

The amounts of chow consumed in each cage, in which four rats were housed, was measured every week, and the average consumption was calculated in grams per day per rat. The body weight of each rat was measured before and after treatment with 6-OHDA or vehicle. In addition, six rats from each group treated with 6-OHDA or vehicle were housed individually in metabolic cages for 5 days during the fourth week of treatment, and chow consumption, water intake, urine output, urinary sodium, and potassium excretion were measured daily.

After 5 weeks of treatment with 6-OHDA or vehicle, we studied the effect of bilateral adrenalectomy on arterial pressure in both groups of rats under anesthesia with sodium pentobarbital, 50 mg/kg, given intraperitoneally. The effect of adrenalectomy on arterial pressure was examined because previous studies suggest that 6-OHDA does not deplete catecholamines in the adrenal medulla and that increased synthesis of adrenal catecholamines may compensate and minimize the effect of chemical sympathectomy by 6-OHDA. A carotid artery was cannulated for continuous recording of systemic arterial pressure. To prevent bleeding, the glands were removed after the vessels connected to the glands were completely tied off. Arterial pressure at 45 minutes after removal of the adrenal glands was compared with that before adrenalectomy.

To test the effectiveness of chemical sympathectomy with 6-OHDA, we then examined the vascular responses to direct sympathetic nerve stimulation and to local injections of norepinephrine and tyramine in the perfused hindquarters. The method for hindquarter perfusion was described previously.

The hindquarter vascular response to sympathetic nerve stimulation was examined by electrically stimulating the lumbar sympathetic chains at L4 using a bipolar stainless steel electrode. The chains were stimulated for 10 seconds at supramaximal voltage (10 V), 3 msec duration and at 10 and 30 Hz. The hindquarters vascular responses to norepinephrine and tyramine were examined by injecting norepinephrine (30 and 300 ng base) and tyramine (10 μg) into the perfusion tubing upstream from the pump in 0.01 ml of 5% dextrose in water. Injection of vehicle alone had no effect. Responses to nerve stimulation, norepinephrine, and tyramine were examined at a flow of 3.0 ml/min.

Data are expressed as averages ± SEM. Statistical analysis was done using the t test, paired t test, and analysis of variance. A p value of < 0.05 was considered statistically significant.
Results

During 7 weeks of observation, systolic arterial pressure in SHR-SP on normal salt diet (n = 31) increased steadily from 196 ± 3 mm Hg to 214 ± 3 mm Hg (mean ± SEM). Systolic arterial pressure in SHR-SP on high salt diet (n = 28) increased from 193 ± 3 to 239 ± 5 mm Hg over the same period. The increase in systolic pressure in SHR-SP on high salt diet was significantly greater than that in SHR-SP on normal salt diet (p < 0.01).

Adrenergic Vascular Tone During High Salt Diet

Before sympathetic denervation, the hindquarters pressure-flow curve in SHR-SP on high salt diet was shifted toward the pressure axis as compared to that in SHR-SP on normal salt diet (p < 0.01), which indicated that hindquarter vascular resistance was increased in SHR-SP on high salt diet (figure 1). Lumbar sympathetic denervation did not significantly alter the hindquarter pressure-flow curve in either group, and thus hindquarter vascular resistance remained elevated after sympathetic denervation in SHR-SP on high salt diet, more than that in SHR-SP on normal salt diet (p < 0.01).

The reduction of mean arterial pressure (MAP) by intravenous phentolamine in SHR-SP on high salt diet (n = 4) was not different from that in SHR-SP on normal salt diet (n = 4) (102 ± 15 mm Hg for high salt vs 90 ± 8 mm Hg for normal salt).

Effects of 6-OHDA on Arterial Pressure

The increase in systolic arterial pressure produced by high salt intake in SHR-SP treated with 6-OHDA (196 ± 10 to 238 ± 8 mm Hg) was not different from that in SHR-SP on high salt diet treated with vehicle (195 ± 4 to 246 ± mm Hg) or that in SHR-SP on high salt diet with no treatment (fig. 2). Systolic arterial pressures in three groups of SHR-SP on high salt diet were significantly higher after the third week of high salt intake than in SHR-SP on normal salt diet (p < 0.01).

In rats under anesthesia treated with 6-OHDA, MAP was 194 ± 10 mm Hg, not different from that in rats treated with vehicle, 185 ± 10 mm Hg. Bilateral adrenalectomy significantly decreased the MAP by 19 ± 4 mm Hg in rats treated with 6-OHDA (p < 0.01). In rats treated with vehicle, systolic arterial pressure was lowered by adrenalectomy but not significantly, (13 ± 7 mm Hg). However, the MAP after adrenalectomy was not different between rats treated with 6-OHDA and those treated with vehicle. Furthermore, hindquarter vascular resistance after adrenalectomy was not different between rats treated with 6-OHDA (33 ± 5 mm Hg/ml/min) and those treated with vehicle (36 ± 4 mm Hg/ml/min).

As shown in figure 3, 6-OHDA significantly reduced responses to direct sympathetic nerve stimulation and to tyramine (p < 0.01). Responses to norepinephrine were significantly greater in rats treated with 6-OHDA than those treated with vehicle (p < 0.01).

Table 1 summarizes chow consumption, water intake, urine output, and urinary excretion of sodium and potassium in SHR-SP treated with 6-OHDA or vehicle while they were housed in metabolic cages. None of these values were different between two groups. The average body weights in rats treated with 6-OHDA before and after treatment (before = 270 ±

<table>
<thead>
<tr>
<th>Chow consumption (g/day/rat)</th>
<th>6-OHDA (n = 6)</th>
<th>Vehicle (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (ml/day/rat)</td>
<td>78 ± 6</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>Urine output (ml/day/rat)</td>
<td>57 ± 5</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Urinary Na (mEq/day/rat)</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
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<tr>
<td>Urinary K (mEq/day/rat)</td>
<td>2.8 ± 0.2</td>
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FIGURE 2 Effect of high salt intake on systolic arterial pressure in SHR-SP. Note that systolic arterial pressures in three groups of SHR-SP on high salt diet were significantly higher than that in SHR-SP on normal salt diet after the third week of high salt intake (p < 0.01).

21 g; after = 257 ± 16 g; mean ± se) were not different from those in rats treated with vehicle before and after treatment (before = 257 ± 16 g; after = 243 ± 14 g).

Discussion

Our results suggest that adrenergic mechanisms do not contribute importantly to salt-induced vasoconstriction in SHR-SP in the established stage of hypertension. This conclusion is based on findings that chronic treatment with 6-OHDA did not affect the arterial pressure elevation induced by high salt intake in SHR-SP and that the increase in arterial pressure or in hindquarter vascular resistance produced by high salt intake was not caused by augmented adrenergic mechanisms.

Previous studies have indicated that several compensatory mechanisms may develop after the treatment with 6-OHDA and minimize the effect of sympathectomy on arterial pressure. It is, therefore, possible that failure to alter the salt-induced elevation of arterial pressure by destroying noradrenergic neurons with 6-OHDA in SHR-SP might

FIGURE 3. Hindquarter vascular responses to direct sympathetic nerve stimulation (SNS) and to local administration of norepinephrine (NE) and tyramine (10 μg). Open bars represent responses in rats treated with vehicle and shaded bars, those treated with 6-OHDA.
have been due to the development of compensatory mechanisms. Compensatory mechanisms that could be important following chronic treatment with 6-OHDA are plasma volume expansion, the development of postsynaptic hypersensitivity to catecholamines, and an activation of the adrenal medulla. It appears, however, that the development of compensatory mechanisms does not fully explain the failure of 6-OHDA to alter the salt-induced elevation of arterial pressure in SHR-SP. Although bilateral adrenalectomy significantly lowered arterial pressure in SHR-SP treated with 6-OHDA but not in SHR-SP treated with vehicle, arterial pressures as well as hindquarter vascular resistances after bilateral adrenalectomy were not different between rats treated with 6-OHDA and those treated with vehicle. Postsynaptic hypersensitivity to norepinephrine was present in the hindquarters of rats treated with 6-OHDA (fig. 3). However a significantly greater response to intraarterially administered norepinephrine was found in rats treated with 6-OHDA only at an injection of a large dose of norepinephrine (fig. 3). As one considers the circulating level of catecholamine in these rats, it appears unlikely that denervation hypersensitivity to catecholamines by itself was sufficient to compensate for nearly complete loss of sympathetic nerve function. We did not measure plasma volume in these rats. However, the increase in plasma volume in animals treated with 6-OHDA does not seem to compensate fully for the reduction of arterial pressure caused by sympathetic denervation. In the studies by Porlier and coworkers, there was a significant reduction of arterial pressure despite plasma volume expansion. The results of our study also suggest that the failure was not due to an alteration of salt consumption or urinary sodium excretion since treatment with 6-OHDA did not affect chow consumption or urinary sodium excretion in these rats.

Previous studies have suggested that sympathetic nerves in the blood vessels are relatively resistant to 6-OHDA and may rapidly regenerate after treatment. However, adequacy of chemical sympathectomy in the blood vessels induced by 6-OHDA was indicated by the findings that pressure responsiveness to sympathetic nerve stimulation and to tyramine was markedly reduced in rats treated with 6-OHDA (fig. 3).

Based on these consideration, we interpret the result of our study with 6-OHDA to suggest that adrenergic mechanisms are not important in salt-induced vasoconstriction in SHR-SP in the established phase of hypertension. This conclusion based on the study of chronic treatment with 6-OHDA is supported by the previous demonstration that neural mechanisms are important in salt-induced hypertension in rats of the Dahl strain. It is interesting to find that, although high salt intake increases arterial pressure and vascular resistance in these two strains of genetically hypertensive rats, the mechanisms for the increases seem to be different. However, the difference in the mechanisms of salt-induced vasoconstriction in these two strains might be related to the phase of hypertension when high salt diet was given. The study was done in the developmental phase of hypertension in the Dahl strains whereas in this study the rats of SHR-SP were in the established phase of hypertension at the beginning of the high salt diet. It has been shown that the sympathetic nervous system is no longer important in maintaining elevated blood pressure in the established phase of hypertension in SHR. It is possible that neural mechanisms play an important role in salt-induced vasoconstriction in the early phase of hypertension in SHR-SP.

Systolic arterial pressure in rats treated with 6-OHDA on high salt diet was lower after 1 week of treatment than that in rats treated with vehicle on high salt diet or in rats on high salt diet alone (fig. 2). However, this does not imply that adrenergic tone was increased in rats on high salt diet. Salt-induced elevation of arterial pressure was not apparent after 1 week of high salt diet (fig. 2). Adequacy of chemical sympathectomy in the blood vessels is relatively resistant to 6-OHDA and may rapidly regenerate after treatment.

The results in SHR-SP in our study are in contrast to the previous demonstration that neural mechanisms are important in salt-induced hypertension in rats of the Dahl strain. It is interesting to find that, although high salt intake increases arterial pressure and vascular resistance in these two strains of genetically hypertensive rats, the mechanisms for the increases seem to be different. However, the difference in the mechanisms of salt-induced vasoconstriction in these two strains might be related to the phase of hypertension when high salt diet was given. The study was done in the developmental phase of hypertension in the Dahl strains whereas in this study the rats of SHR-SP were in the established phase of hypertension at the beginning of the high salt diet. It has been shown that the sympathetic nervous system is no longer important in maintaining elevated blood pressure in the established phase of hypertension in SHR. It is possible that neural mechanisms play an important role in salt-induced vasoconstriction in the early phase of hypertension in SHR-SP.

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