Hypertension-Producing Factor in Serum of Hypertensive Dahl Salt-Sensitive Rats
YASUNOBU HIRATA, LOUIS TOBIAN, GEZA SIMON, AND JUNICHI IWAI

SUMMARY To investigate whether serum in hypertensive Dahl salt-sensitive rats (S rats) contains a hypertensinogenic substance, we examined the effects of repeated injections of serum from such S rats on blood pressure (BP) and pressor responses. Serum was collected from either hypertensive or normotensive S rats (fed an 8% or 0.11% NaCl diet, respectively) and injected into uninephrectomized recipient S rats for 2 weeks (0.45 ml, twice a day, i.v.). Serum from hypertensive rats injected for 14 days significantly increased BP by 14 mm Hg (143 vs 129, p < 0.05), pressor responses to angiotensin II (ANGII) by 45% (p < 0.005), pressor responses to norepinephrine (NE) by 38% (p < 0.025), and Na concentration in the aortic wall of recipient rats by 5.9% (p < 0.05), compared to the effects of the injection of serum from normotensive S rats. These results imply that hypertensive S serum contains a hypertensinogenic substance and that this serum factor produces a mild hypertension in the recipient rats and also contributes importantly to the hypertension in donor S rats. Dahl salt-resistant rats (R rats) on either 8% or 0.11% NaCI had normal BP. Their sera produced no differences in BP or in pressor responses in recipient rats. Hence 8% NaCl, which produced no hypertension, also induced no hypertensinogenic serum factors in R rats. We sought to determine whether nephrectomy would alter these humoral factors. The BP averaged 139 mm Hg in rats receiving normotensive sham-nephrectomized S serum vs 154 in those receiving hypertensive sham-nephrectomized S serum, 15 mm Hg higher (p < 0.05). ANGII and NE pressor responses were uniformly higher in rats receiving hypertensive sham-nephrectomized S serum by 32% (p < 0.05) and by 36% (p < 0.025), respectively. In contrast, among nephrectomized rats there were no significant differences between rats receiving hypertensive S serum vs normotensive S serum. Removal of kidneys appeared to eliminate the hypertensinogenic factors, which suggests that they either come from the kidney or are strongly influenced by the kidney. (Hypertension 6: 709-716, 1984)

KEY WORDS • blood pressure • pressor response • angiotensin II • norepinephrine • tissue sodium • nephrectomy • NaCl hypertension

SLOWLY acting hypertensinogenic humoral factors have been reported in various kinds of experimental and human essential hypertension. Hypertension in Dahl salt-sensitive rats (S rats) also seems to be at least partly due to an uncommon humoral factor. Dahl and his coworkers1 showed that salt-resistant rats (R rats) became hypertensive when they were united in parabiosis to S rats on a high NaCl diet. A previous study in this laboratory2 also demonstrated that perfusion of blood from hypertensive S rats increased the vascular resistance of isolated hindquarters from R rats. It was also demonstrated in this laborato-

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ry3 that some circulating humoral factors in S rats halved the rate of sodium excretion by an isolated kidney from normal Sprague-Dawley rats. Although the characteristics of these humoral factors remain unclear, much current research involves humoral factors that may act as inhibitors of ouabain-sensitive Na+,K+-ATPase. Dahl et al.4 speculated that some humoral factor in S rats might possess both natriuretic effects and hypertensinogenic effects. Extending this theory, Haddy and Overbeck5 have demonstrated that the vascular Na+,K+-ATPase activity was suppressed in certain types of volume-overload hypertension by a circulating Na+,K+-ATPase inhibitor. However, they have not been able to find this type of inhibitor in hypertensive Dahl S rats. In fact, the cardiovascular Na+,K+-ATPase activity is actually exaggerated in hypertensive S rats.6,7 These uncertainties led us to test for hypertensinogenic humoral factors involved in the hypertension of S rats.

If a humoral factor does exist in hypertensive S rats, its origin is uncertain. The humoral factor in S rats is seemingly related to the kidney. Knudsen et al.9 found
that R rats did not become hypertensive when they were united in parabiosis to nephrectomized S rats. This suggested a relationship between the hypertensinogenic substance and the kidneys. Further, it was shown in this laboratory\(^9\) that total nephrectomy greatly diminished the vasoconstricting effect of hypertensive S blood.

We are reporting three studies. First, we examined the effects of repeated injections of serum from S rats on the blood pressure (BP) and the pressor responses in recipient rats to clarify whether the blood in hypertensive S rats contains a hypertensinogenic humoral factor. Second, we compared the effects of serum from R rats on a high NaCl diet with that from R rats on a low NaCl diet. We also compared serum from S and R rats when both were fed a high NaCl diet and with only the S rats becoming hypertensive. Finally, we also investigated the effect of nephrectomy on the humoral factor.

**Methods and Materials**

**Experiment 1: Injection of Serum from Hypertensive and Normotensive S Rats into Recipient Rats**

**Donor Rats and Serum Preparation**

All the Dahl S and R rats were from the Brookhaven Laboratory. The Dahl S rats became hypertensive when fed high NaCl diets but remained normotensive on very low NaCl diets. Dahl R rats remained normotensive on either diet. Blood was collected from S rats that had been fed either a 0.11% or an 8% NaCl diet for 12 weeks after weaning. The 0.11% NaCl diet contained 33% low-sodium Purina chow (0.3% NaCl), 1.3% safflower oil, 13.3% casein, 48% ground corn, 2.7% Na-deficient mineral mix, 0.7% vitamix mix, and 0.7% cellulose. The 8% NaCl diet was made by adding NaCl crystals to the 0.11% NaCl diet. The average systolic BP of these donor rats on a 0.11% and on an 8% NaCl diet was 140 mm Hg (n = 96) and 196 mm Hg (n = 89), respectively, measured by the microphonic tailcuff method of Friedman and Freed\(^1\) without anesthesia. Blood was taken from the abdominal aorta in rats under light ether anesthesia, and spun at 4°C at 3000 g for 10 minutes. Separated serum was pooled and stored in many small 1 ml tubes at −70°C.

**Recipient Rats**

Male S rats weighing about 275 g were used as recipient rats. They had been nephrectomized and fed regular Purina chow (1.0% NaCl) since weaning. The recipient rats were nephrectomized in order to slightly sensitize them to hypertensinogenic serum factors by reducing somewhat the total antihypertensive action of normal kidneys. Dahl S rats were utilized both as donor rats and as recipient rats to minimize genetic differences and immune reactions. Catheters made of PE20 polyethylene tubing with short PE10 tips were inserted into the right jugular veins of these rats for a 2-week period. Systolic BP was measured by the tail microphonic method of Friedman and Freed without anesthesia on two occasions before venous cannulation. Serum frozen at −70°C was then thawed to room temperature just before each use and was injected twice a day for 2 weeks through microfilters with a 0.22 μm mesh. The volume of injected serum was 0.45 ml each time. Collection, storage, and injection of serum was done aseptically. The normotensive recipient rats were injected with serum from either normotensive S rats or hypertensive S rats on a round-robin basis.

**Pressor Response Test**

On the day after the last serum injection (the 28th injection), we measured the mean arterial pressure (MAP) and pressor responses to angiotensin II (ANGII) and norepinephrine (NE) were measured. From a catheter inserted into the left femoral artery of a recipient rat under Inactin anesthesia (100 mg/kg), the MAP was monitored with a Statham P23 ID transducer and a Grass recorder. After an equilibration period, the MAP was noted and then pressor responses to bolus injections of ANGII (5, 10, 30, and 90 ng/kg, i.v.) and then NE (50, 100, 300, and 600 ng/kg, i.v.) were elicited in each recipient rat. The ANGII and NE were dissolved in 5% dextrose at each concentration and were stored at −70°C. The injected volume was 7.5 μl/300 g of rat. The magnitude of pressor responses was expressed as the maximal increase in MAP (peak height above the basal MAP). The BP had returned to baseline before each succeeding injection. The order of injections was the same for every rat.

We used direct intraarterial measurement of MAP in rats under inactin anesthesia during the final BP measurements after the 2 weeks of serum injections. This was found to provide the most precise estimation of BP. Initially we tried indwelling intraarterial catheters in unanesthetized rats but the BP was constantly changing in response to external noise and events. The BP measurement under anesthesia was the type of measurement preferred by Dahl himself in the early development of his rat strains.

After the pressor response test, 1 ml of blood was taken from the arterial catheter for measuring electrolytes. Then the thoracic aorta and heart were quickly dissected out, lightly blotted to remove loose connective tissue and blood, put into preweighed quartz tubes with tight rubber stoppers, and then weighed to obtain wet weight. The fresh samples were dried at 100°C for 48 hours and were reweighed for dry weight. Water content of the tissue was considered to be the difference between wet and dry weight. Dry samples were then bathed in 0.75 N nitric acid at 40°C for 48 hours with gentle shaking. The Na and K concentrations in the nitric acid extract were determined by flame photometry, Na, K, blood urea nitrogen (BUN), and total protein in the pooled serum of donor rats were also measured.

**Experiment 2: Injection of Serum from R Rats on High and Low NaCl Diets for Comparison with S Serum**

After Experiment 1, it was not clear whether hypertensinogenic factors were related to hypertension in the donor rats or to a high NaCl diet in the donor rats. To
clarify this issue, we made two separate comparisons. We collected serum from three different groups of donor rats: 1) hypertensive S rats on a 8% NaCl diet for 12 weeks after weaning (average BP 204 mm Hg, n = 39); 2) normotensive R rats on a 8% NaCl diet for 12 weeks after weaning (BP 134 mm Hg, n = 38); and 3) normotensive S rats on a 0.11% NaCl diet for 12 weeks after weaning (BP 132 mm Hg, n = 42). Using the same methods as in Experiment 1, we injected 0.45 ml of serum into three groups of uninephrectomized S rats twice a day for 14 days and then determined MAP and pressor responses to ANGII and NE in the recipient rats on a round-robin basis.

**Experiment 3: Injection of Serum from Nephrectomized and Sham-Nephrectomized S Rats**

Since other circulating humoral agents in hypertensive rats have had a relationship to the kidney, we conducted the following experiment. We collected serum from four kinds of S rats: 1) totally nephrectomized S rats that were on an 8% NaCl diet prior to nephrectomy and on a 0.11% NaCl diet after nephrectomy (average BP 202 mm Hg, n = 66); 2) totally nephrectomized S rats that were on a 0.11% NaCl diet both before and after nephrectomy (BP 142 mm Hg, n = 72); 3) sham-nephrectomized S rats that were on an 8% NaCl diet prior to sham nephrectomy and on a 0.11% NaCl diet after sham nephrectomy (average BP 196 mm Hg, n = 74); and 4) sham-nephrectomized S rats that were on a 0.11% NaCl diet both before and after sham nephrectomy (BP 140 mm Hg, n = 72). The serum collected was that of the last two weekly tail cuff blood pressures before nephrectomy or sham nephrectomy. Water and food were provided ad libitum between the surgery and the blood collection.

After nephrectomy, the rats were fed a low-salt diet containing 0.11% NaCl to prevent a large unphysiological increase in body Na after renal Na excretion had ceased with the nephrectomy. However, even on the 0.11% NaCl diets, body Na increased somewhat in the nephrectomized rats. The sham-nephrectomized rats had a similar feeding protocol, since the postoperative state is often accompanied by reduced renal Na excretion. The groups being compared always had the same diet after either nephrectomy or sham nephrectomy. The serum handling, the injection of serum into recipient rats, and the determination of MAP and pressor responses in the recipient rats were carried out in the manner described in Experiment 1.

**Statistics**

All statistical comparisons were made by using three different tests: Student’s t test involving the standard error of the difference between means; the Wilcoxon rank sum test; and the analysis of variance test. In every case in which the Student t test resulted in a p < 0.05, this same probability occurred in both the Wilcoxon test and in the analysis of variance (ANOVA).
significantly higher MAP by 19 mm Hg than the HR group (p < 0.05) (Figure 1). This occurred in spite of comparable systolic BP in the two groups before serum injection. Pressor responses to both AII and NE were also significantly enhanced by averages of 29% and 43% in the HS group, compared to the HR group (Figures 4 and 5) (p < 0.05).

In the second comparison, we analyzed serum from donor R rats eating an 8% high NaCl diet (HR) and serum from donor R rats eating a 0.11% low NaCl diet (LR). The NaCl intake was vastly different in these two groups, but the BP was similarly normotensive in both groups: 134 mm Hg in HR vs 132 mm Hg in LR (N.S.). The MAP was closely similar in the HR recipient group and the LR recipient group on the day following 2 weeks of serum injections: 123 vs 121 mm Hg, respectively (N.S.) (Figure 1). Furthermore, the pressor responses to AII and NE in the same two groups were also closely similar (N.S.) (Figures 4 and 5). These two comparisons indicated that the hyperten-
A sinogenic serum factor occurs in the presence of NaCl-induced hypertension and does not occur during high NaCl diets that fail to bring about hypertension.

The Na, K, and BUN in the pooled serum of donor rats were similar in these three groups.

**Experiment 3**

Body weight before and after serum injections and systolic BP by the tail cuff method before serum injections were closely similar in the four groups of recipient rats, with the systolic BP at about 146 mm Hg. It seemed appropriate to compare nephrectomized rats with nephrectomized rats and to compare sham-nephrectomized rats with other sham-nephrectomized rats.

When the two groups of recipient rats that received serum from either sham-nephrectomized hypertensive S rats (HS-sham) or from sham-nephrectomized normotensive S rats (NS-sham) were compared, the HS-
TABLE 1. Sodium, Potassium, and Water Concentration in the Aortic Wall, Heart, and Plasma of Recipient Rats in Experiment 1

<table>
<thead>
<tr>
<th></th>
<th>HS group</th>
<th>NS group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta Sodium</td>
<td>106.6 ± 1.6*</td>
<td>100.7 ± 2.1</td>
</tr>
<tr>
<td>Potassium</td>
<td>56.6 ± 1.2</td>
<td>56.1 ± 1.4</td>
</tr>
<tr>
<td>Water (%)</td>
<td>73.1 ± 1.0</td>
<td>74.0 ± 0.8</td>
</tr>
<tr>
<td>Heart Sodium</td>
<td>49.7 ± 0.6</td>
<td>50.6 ± 1.1</td>
</tr>
<tr>
<td>Potassium</td>
<td>105.2 ± 0.9</td>
<td>101.7 ± 1.7</td>
</tr>
<tr>
<td>Water (%)</td>
<td>78.0 ± 0.2</td>
<td>79.1 ± 0.6</td>
</tr>
<tr>
<td>Plasma Sodium</td>
<td>143.9 ± 1.1</td>
<td>145.4 ± 1.2</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
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Values are means ± SE. HS group = recipient rat group that received serum from hypertensive S rats; NS group = recipient rat group that received serum from normotensive S rats.

*p < 0.05, compared to NS group.

Discussion

In the present study, we found significantly higher BP and greater pressor responses to ANGII and NE in recipient S rats that had repeatedly received serum from hypertensive S rats than in rats that had received serum from normotensive S rats. Although higher NE and vasopressin concentrations have been reported in the plasma of S rats on a high NaCl diet, neither NE nor vasopressin is long-acting. The effects induced by the serum factor in this study were observed 16 hours following the last injection. Since pressor responses to both NE and ANGII were enhanced to a roughly similar degree in the present study (NE = +38%, ANGII = +45%), it would not seem to be a receptor specific phenomenon.

The observation of Jones and Hart may have a bearing on hyperreactivity in the early phase of hypertension. They showed that aortic K turnover and estimated intracellular Na concentration increased before the development of DOCA-salt hypertension, and suggested that such alterations might reflect an increased membrane leakiness and a reduced membrane-stabilizing activity of Ca. In the current experiment, the aortic Na concentration in HS rats was 5.9% higher than that in NS rats in spite of similar plasma Na concentration. Hence, it is conceivable that the serum factor might enhance vascular reactivity partly by altering ion permeability in vascular smooth muscle cells.

There is ample evidence showing that vascular hyperreactivity occurs prior to the elevation of BP in two-kidney, one clip (2K1C) Goldblatt hypertension, one-kidney, one clip (1K1C) hypertension, DOCA-salt hypertension, and figure 8 ligature hypertension. It was also shown that vascular hyperreactivity preceded the onset of hypertension in S rats. These observations strongly suggest that the heightened pressor responses induced by the hypertensinogenic serum factor are an early manifestation of systemic hypertension in the current studies. The increase in Na concentration of the aortic wall is also a characteristic of essential hypertension.

The critical humoral factor in the serum of hypertensive S rats must be fairly potent. Each time 0.45 ml of serum was injected into a recipient rat, the various factors in this serum were diluted about 142-fold by the extracellular fluid. Hence, the recipient rat had a concentration of these factors which was only 0.7% of that present in the donor rats. Despite this great dilution of the hypertensinogenic factors in the recipient rats just after injection and despite the metabolic degradation that must occur, the small remaining amount of hypertensinogenic factors was still powerful enough to produce a mild hypertension, increased pressor responses, and increased Na in aortic wall in the recipient rats. In view of the considerable power of greatly diluted hypertensinogenic factors, when these same hypertensinogenic factors exist undiluted in the plasma of NaCl-fed S rats, surely they must exert a considerable hypertension-promoting effect in the donor hypertensive S rats.

The results of Experiment 1 could be interpreted either that high NaCl feeding per se or hypertension per se could induce the appearance of the hypertensinogenic factor. However, serum from R rats on a high NaCl diet did not exert any effects at all on the BP and pressor responses in recipient rats in Experiment 2. This result clearly indicates that the hypertensinogenic serum factor will not appear in a NaCl-fed rat if the NaCl-feeding does not produce hypertension in that rat.

In another part of Experiment 2, both S rats and R rats were fed a diet containing 8% NaCl, so that a high NaCl intake was similar in the two groups. On this diet the S rats became hypertensive and the R rats remained normotensive, which provided two groups with contrasting BP but similar NaCl intake. The serum from...
the NaCl-fed hypertensive S rats increased the BP and the pressor responses in recipient rats whereas the serum from NaCl-fed normotensive R rats did not increase BP and pressor responses. This study again strongly pointed to hypertension as the critical factor associated with the appearance of the hypertensinogenic serum factor rather than a high NaCl intake. Evidently, high NaCl feeding without hypertension does not bring out the hypertensinogenic factor.

In Experiment 3, we again confirmed that rats receiving serum from hypertensive S rats with sham nephrectomy showed significantly higher BP and greater pressor responses than those given serum from normotensive S rats with sham nephrectomy. The differences in BP, pressor responses to NE between rats receiving hypertensive S serum and normotensive S serum were 15 mm Hg, 32%, and 36%, respectively, very much like the results of Experiment 1. However, total nephrectomy in both these groups almost abolished these differences, which indicates that a functioning kidney in the S rat is a crucial organ with regard to the appearance of the hypertensinogenic factor. It is likely that the hypertensinogenic serum factor is either produced by the kidneys or, alternatively, is produced in some non-renal locus that is greatly influenced by the presence or absence of renal tissue. It is conceivable that the hypertensinogenic factor is secreted from a non-renal location and is degraded by the kidney in low NaCl S rats, but is not degraded by the kidney of high NaCl S rats. If this were the case, then total nephrectomy in both S groups would result in equal amounts of hypertensinogenic factor, since the excised kidney from low NaCl S rats would no longer be able to degrade the hypertensinogenic factor. However, if this were the case, one would expect to find more hypertensinogenic factor in low NaCl normotensive S rats with nephrectomy than in low NaCl normotensive S rats with sham nephrectomy. In our study this did not occur. The BP in recipient rats averaged 141 vs 139 mm Hg (NS) in these two respective groups. These results make it unlikely that the nephrectomy abolishes the difference in the hypertensinogenic factor between HS and NS through the mechanism of a decrease in degradation by the kidney in the nephrectomized NS rats.

The BP and NE pressor responses in recipient rats receiving nephrectomized, hypertensive S serum were slightly higher than in those receiving nephrectomized normotensive S serum. It is possible that the hypertensinogenic serum factor has a long half-life. Serum was collected 20 hours after nephrectomy, and these 20 hours may not be long enough for the serum factor to disappear completely from the blood. If this were the case, more hypertensinogenic factor would still be remaining in the plasma of the nephrectomized HS rats than in the nephrectomized NS rats. This could account for the slightly greater BP and NE pressor responses in recipients receiving nephrectomized HS serum vs nephrectomized NS serum.

With these serum injections, could the effects on the recipients be caused by the absence of a vasodepressor substance in the serum from the hypertensive S rats? Since the recipient rats were normotensive, presumably they had enough endogenous "vasodepressor factor" to keep their BP normal. If the serum from the hypertensive S rats did indeed lack the normal amount of vasodepressor substance, it should not really have resulted in a rise in BP since the recipient rat had plenty of vasodepressor factor. A more likely explanation would be that the serum from hypertensive S rats contains a hypertensinogenic factor, with or without a normal amount of vasodepressor factor. This hypertensinogenic factor would then confer a mild hypertension on the recipient rat.

The hypertensinogenic factor revealed here could be the same one found by Dahl et al. 1, 4, 9 Dahl's factor was thought to be released only by S rats on a high NaCl diet. It could cross over to an R rat that was a parabiotic partner and raise the BP of that R rat. Even on a low NaCl diet, an S kidney with a Goldblatt clamp could release the hypertensinogenic substance, whereas a similar R kidney could not. Moreover, a nephrectomized S rat might develop renoprival hypertension on a moderate NaCl intake but it could not be transmitted to its R rat parabiotic partner. 9 In fact, when nine intact S and R parabiotic partners were both on a high NaCl diet, all the R partners rapidly developed significant hypertension prior to their S mates. When the S partner had both kidneys removed, only three of seven S partners became hypertensive at all. Furthermore, nephrectomized S rats or R rats on a very low Na intake will both develop hypertension if they are parabiotically connected to an intact S rat but will not if either one is connected to an R rat. 9 Altogether, these studies suggest that the S rat does secrete a hypertensinogenic substance which does not appear in the absence of renal tissue. This agrees very well with the characteristics of our substance, with the parabiotic union allowing a much greater transfer of it to a recipient than our twice-a-day serum injections.

The hypertensinogenic factor from S rats in this study is not likely to be the same as the antinatriuretic factor of S rats found in a previous study, 3 since the Na-retaining factor was found in normotensive S rats on a low NaCl diet. The hypertensinogenic factor in this study might be related to a circulating humoral pressor agent found only in hypertensive salt-fed S rats, 2 particularly since the vasoconstrictive factor is abolished after bilateral nephrectomy. 10

In conclusion, hypertensive S rats on a high NaCl diet produce a humoral hypertensinogenic factor. This serum factor elevated BP, increased pressor responses, and increased Na concentration in the aortic wall of recipients receiving this serum. Normotensive S rats on a low NaCl diet did not have the humoral hypertensinogenic factor. Normotensive R rats on either a high or a low NaCl diet did not have the hypertensinogenic factor. These results strongly imply that the serum factor may play a significant role in the development of hypertension in salt-fed S rats. Furthermore, the kidneys in S rats appear to participate in the production or activation of the hypertensinogenic serum factor.
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