Hypertension Produced by Sodium Depletion and Unilateral Nephrectomy:  A New Experimental Model

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SUMMARY Unilateral nephrectomy of sodium-restricted male Sprague-Dawley rats produced a sustained elevation in systolic blood pressure (SBP) that was reversed by sodium repletion. A chronic intraperitoneal infusion of SQ 14,225 prevented the development of hypertension in sodium-deplete unilaterally nephrectomized rats. Sodium depletion of two-kidney rats increased SBP to a lesser extent, while unilateral nephrectomy of sodium-replete animals had no effect. These results provide evidence for a new model of experimental hypertension in the rat and emphasize the importance of a renal component, as demonstrated by unilateral nephrectomy, in the maintenance of normal pressure-volume relationships. (Hypertension 2: 125-129, 1980)

KEY WORDS • renin-angiotensin system • converting enzyme inhibitor • unilateral nephrectomy • sodium restriction

WHILE investigating the pathogenesis of one-kidney Goldblatt hypertension in sodium-deplete Sprague-Dawley rats, systolic blood pressure (SBP) of the sodium-restricted control animals unexpectedly increased after unilateral nephrectomy. The present study was undertaken to document the development of this pressor response and to elucidate the mechanisms by which the pressure was elevated. To accomplish these objectives, separate groups of male Sprague-Dawley rats were 1) sodium restricted, then unilaterally nephrectomized; 2) unilaterally nephrectomized while maintained on a normal sodium diet; 3) sodium depleted and subsequently replete to provide another control group; and 4) sodium restricted and unilaterally nephrectomized while angiotensin II formation was blocked by the converting enzyme inhibitor SQ14,225 (2-D-methyl-3-mercaptopropanyl-L-proline).

Methods

Four groups of male Sprague-Dawley rats were housed individually in stainless steel metabolic cages and kept in temperature-controlled rooms that were maintained on a 12-hour light-dark cycle. Throughout the study, sodium balances, determined over 2- to 3-day periods, were calculated as the differences between the sodium intake and urinary sodium excretion. Systolic blood pressure was determined three times each week by use of the tail-cuff method. All pressures were measured between 8 and 11 a.m. and the recorded value was the average of at least three successive determinations from conscious animals that were restrained in a warmed chamber. If the SBP obtained in this manner was substantially different from that measured on the previous testing day, the animal was retested 1 hour later; this procedure was used during all phases of the study including the time that arterial pressure was rising. During an initial control period, all rats were allowed free access to tap water and to ground rat chow (containing 0.141 ± 0.005 mEq Na and 0.272 ± 0.012 mEq K per g). Once body weight had reached approximately 130 g and sodium-replete data had been obtained, one of the four protocols described below was followed.

Each of the 130 g rats in Group I (n = 8) was supplied with distilled drinking water and Teklad sodium-deficient test diet (containing 0.0047 ± 0.0004 mEq Na and 0.232 ± 0.019 mEq K per g) for 4 days before the animal was anesthetized with ether and unilaterally nephrectomized. Data were collected during an additional 12 days of sodium restriction and, finally, during a 12-day period of sodium repletion.
To study the effects of unilateral nephrectomy of rats maintained on a normal sodium intake, the 8 animals in Group II were fed the ground rat chow throughout the study. As in Group I, control measurements were obtained, the rats were unilaterally nephrectomized, and data were collected for 24 days.

The third group of rats (n = 8) was sodium restricted in order to determine the effects of the diet change in rats with two intact kidneys. These animals were subjected to the same dietary manipulations and data collection procedures previously described for the Group I rats.

In the fourth group of nine rats, the formation of angiotensin II was blocked by the converting enzyme inhibitor SQ14,225. The drug was delivered at 80 μg/hr via miniosmopumps (Alzet) that were surgically implanted intraperitoneally on the first and sixth days of sodium restriction. As in Group I, these animals were unilaterally nephrectomized 4 days after initiation of the low sodium diet. Nine days after removal of one kidney, SQ14,225 treatment was terminated and the sodium deplete rats were allowed to recover.

All data are expressed as means ± standard errors. The Student's paired t test was employed to identify values significantly different from controls within each group. Differences between groups were evaluated by the Student's group t test.

**Results**

In Group I, SBP did not change during the first 4 days of sodium depletion (days -4 to 0) (fig. 1). Within 3 days of the unilateral nephrectomy, on Day 0, SBP rose significantly from the sodium-replete control of 105 ± 3 to 126 ± 6 mm Hg (p < 0.005). Thereafter, SBP progressively increased to 143 ± 6 mm Hg by the twelfth day after nephrectomy. Upon sodium repletion, which began on Day 12, SBP promptly dropped to the control level. Although pressure tended to increase slightly during the final period of sodium repletion (Days 14 to 24), the changes were not statistically significant (p > 0.05). In contrast to the dramatic rise in SBP observed after removal of one kidney in the sodium-deplete animals, SBP did not change after nephrectomy of the sodium-replete rats in Group II (figure 1). Furthermore, SBP of Group II rats did not change significantly throughout the course of the study. Systolic pressures measured while the rats in Group I were sodium deplete were significantly higher after unilateral nephrectomy than the corresponding SBP of the sodium-replete animals.

To determine the effects of sodium depletion on SBP of rats with two intact kidneys, the third group of animals was subjected to the low sodium regimen. As shown in figure 2, SBP increased progressively from the sodium-replete control level of 107 ± 4 mm Hg to a maximum value of 127 ± 7 mm Hg by Day 12. This pressure was significantly greater than the sodium-replete control (p < 0.005), the SBP on Day 0 (p < 0.01), and the sodium replete value on Day 14 (p < 0.005). In comparison to the SBP response of the one-kidney rats to sodium depletion (Group I), pressure of the two-kidney animals was significantly lower (see figure 2).

**Figures**

**Figure 1.** Circles represent systolic blood pressure (SBP) (mean ± SE) of eight rats in Group I for a week preceding and for 24 days following unilateral nephrectomy. Closed symbols signify the measurements obtained while the animals were sodium replete; open symbols denote sodium-deplete data. The solid diamonds represent the average SBP of the 8 sodium-replete rats in Group II before and after unilateral nephrectomy. Significant differences between the two groups were determined by a group t test and are indicated by the asterisks.

**Figure 2.** The systolic blood pressure (SBP) response to sodium depletion of the one-kidney rats in Group I (circles) is compared to that of the two-kidney animals in Group II (triangles); the abscissa label "days after nephrectomy" applies only to Group I and the corresponding days apply to Group II. Closed symbols indicate periods of sodium repletion; open symbols, periods of sodium depletion. Significant differences between the two groups were found on the days marked by the asterisks.
HYPERTENSION IN SODIUM DEPLETE ONE-KIDNEY RATS/Seymour et al.

The effect of SQ14,225 treatment on the SBP response to unilateral nephrectomy of sodium-deplete rats is shown in figure 3. Inhibition of the converting enzyme abolished the pressure rise previously observed upon removal of one kidney. Once the drug was discontinued, SBP progressively increased to 129 ± 4 mm Hg, a value that was significantly greater than both the sodium-replete control and the final SBP measurement obtained during SQ14,225 treatment (p < 0.05).

The daily sodium balances of all rats were summed over three periods corresponding to 1) the 4 days immediately preceding nephrectomy in which rats in Groups I, III, and IV were sodium restricted (Days -4 to 0); 2) the 12 days after nephrectomy (Days 0 to 12); and 3) the final 12 days in which the rats in Groups I and III were returned to a normal sodium diet (Days 12 to 24). Comparison of the data from Group I and Group II rats (table 1) shows that dietary sodium restriction (Days -4 to 0 and Days 0 to 12) effectively reduced the cumulative sodium balances of Group I animals. Upon sodium repletion (Days 12 to 24 in table 1), the cumulative sodium balance of the Group I rats was the same as that of the one-kidney animals in Group II that had remained on a normal sodium intake throughout the study (p > 0.05). In both groups, the positive sodium balances are consistent with the previously reported finding that in growing rats, such as those in the present study, sodium intake exceeds sodium excretion. Following unilateral nephrectomy of Group I rats (Days 0 to 12 in table 1), the cumulative sodium balance of these animals was significantly lower than that of the two-kidney sodium-deplete rats in Group III.

The average body weights of each group are shown in figure 4. Sodium restriction slowed the rate of weight gain in Groups I and III (Days -4 through 12) and in Group IV (Days -4 through 24). The rate of weight gain was increased by sodium repletion in Groups I and III.

Discussion

Unilateral nephrectomy of male Sprague-Dawley rats receiving a low sodium diet produced a striking elevation in SBP. The elevation in blood pressure was significant within 3 days after nephrectomy, and by 12 days a marked increase in pressure was evident. In contrast, unilateral nephrectomy produced no change in SBP in sodium-replete rats, and animals with both kidneys intact subjected to dietary sodium restriction showed a slow elevation in pressure that was not

Table 1. Cumulative Sodium Balance (mEq)

<table>
<thead>
<tr>
<th>Group</th>
<th>-4 to 0*</th>
<th>0 to 12</th>
<th>12 to 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-0.583 ± 0.100 (D)</td>
<td>0.203 ± 0.045 (N)</td>
<td>12.532 ± 1.10 (N)</td>
</tr>
<tr>
<td>II</td>
<td>3.868 ± 0.516† (N)</td>
<td>6.417 ± 0.902† (N)</td>
<td>10.887 ± 0.882 (N)</td>
</tr>
<tr>
<td>III</td>
<td>-0.458 ± 0.091† (D)</td>
<td>0.578 ± 0.045† (D)</td>
<td>12.895 ± 1.337 (D)</td>
</tr>
<tr>
<td>IV</td>
<td>-1.071 ± 0.061† (D)</td>
<td>0.026 ± 0.092 (D)</td>
<td>0.592 ± 0.019† (D)</td>
</tr>
</tbody>
</table>

*At time 0, unilateral nephrectomy was done.
†Significantly different from Group I.
(D) Denotes restricted sodium intake during that period, whereas (N) represents normal sodium intake (0.141 mEq Na per g).
significant until Day 16 of sodium depletion. These findings demonstrate that the combination of sodium restriction and unilateral nephrectomy produced sustained hypertension.

The hypertension that developed during sodium restriction was readily reversed by sodium repletion of both one- and two-kidney rats (figure 2). Since the renin-angiotensin system is stimulated by sodium depletion, increased renin release was considered as a possible mechanism responsible for the hypertensive response to sodium depletion. In support of this hypothesis, continuous infusion of the converting enzyme inhibitor SQ 14,225 at 80 μg/hr completely abolished the pressor response to unilateral nephrectomy in sodium-deplete rats. Activation of the renin-angiotensin system is therefore essential for the development of this hypertensive model.

There is some basis in the literature for the proposed renin-dependent model of hypertension. In one-kidney Goldblatt hypertensive rats that had been maintained on a low sodium diet for 4 weeks after clipping, arterial pressure was reduced by acute infusion of a competitive angiotensin II antagonist. Under these experimental conditions, the blood pressure was maintained largely by elevated renin activity, which indicates that high levels of plasma renin activity can sustain hypertension. In the same study, unilaterally nephrectomized rats were fed a low sodium diet for 4 weeks; then mean blood pressure was measured intrarurally. Mean pressure of this latter group, which was approximately 125 mm Hg when the angiotensin II antagonist was infused. Apparently, pressure was being maintained by an elevation in renin activity in these sodium-deplete rats, much the same as it was in the animals studied in the present experiments. Yet another instance in which elevated plasma renin activities were associated with high blood pressures was reported by Romero et al in two-kidney rabbits with severe Goldblatt hypertension. When the animals were fed a high sodium diet, renin levels decreased and blood pressure fell. These data are consistent with our findings, which indicate that an elevated blood pressure can be supported by activation of the renin-angiotensin system.

Freeman et al have previously demonstrated that the dose and the route of administration of SQ14,225 used in the present study substantially inhibits the renin-angiotensin system. SQ14,225 infused intraperitoneally at 80 μg/hr via miniosmopumps blunted the dose-response curve to angiotensin I in normal rats. In addition, continuous infusion of SQ14,225 by the same procedure in two-kidney renal hypertensive rats completely blocked the pressor response.

Although angiotensin converting enzyme also degrades bradykinin, there is no suggestion that intraperitoneal infusion of the selected dose of SQ14,225 produced a nonspecific action in the rat. Freeman et al infused SQ14,225 at 80 μg/hr for 12 days via miniosmopumps implanted within the abdomen in normal rats. On only 1 day of the infusion period, SBP was decreased slightly. In addition, continuous infusion of SQ14,225 by the same procedure in two-kidney renal hypertensive rats completely blocked the pressor response.

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**Figure 4.** The body weights of the four groups of rats were followed throughout the course of the study. Circles represent the body weights of the one-kidney sodium-deplete rats in Group I; stars, the sodium-replete, one-kidney rats in Group II; triangles, the sodium-deplete animals in Group III; and squares, the sodium-restricted one-kidney rats treated with SQ14,225 (Group IV). Closed symbols indicate periods of sodium repletion and open symbols, periods of sodium depletion.
demonstrates the lack of a nonspecific action of the drug. As indicated, stimulation of renin release by sodium depletion and the inhibition of angiotensin II formation by SQ14,225 are well documented. Therefore, suppression of the SBP response of sodium-restricted one-kidney rats by SQ14,225 treatment can be attributed to blockade of the renin-angiotensin system.

Considering that there were no changes in SBP in the sodium-replete rats or in the SQ14,225-treated rats after unilateral nephrectomy, and that SBP also increased in two-kidney rats, the progressive rise in pressure in the one-kidney animals could not be attributed to either the reduction of renal mass alone or to nonspecific effects of the initial surgical trauma. In view of the complete suppression of the pressor response by SQ14,225, the increase in renin release due to sodium depletion appeared to be necessary for the increase in pressure in both one- and two-kidney rats. The reversal of the pressure increase by sodium repletion in both intact and unilaterally nephrectomized rats lends further credence to the suggestion that enhanced renin release was essential for the maintenance of the elevated pressure.

Although the present data are insufficient to explain the difference between the increases in SBP in the sodium restricted one-kidney and two-kidney rats, one may speculate that a reduction in renal mass in combination with hormonal changes associated with sodium depletion disturbed normal pressure-volume relationships. In addition to the well-documented increase in renin secretion that results from sodium depletion, there is evidence that unilateral nephrectomy decreases the inactivation of circulating renin and therefore may further enhance the levels of circulating renin in the one-kidney sodium-deplete rats. Alternatively, the removal of one kidney may have diminished levels of a renal antihypertensive factor and thereby facilitated the rise in SBP after unilateral nephrectomy. More precise mechanistic studies are needed before firm conclusions can be reached.

In summary, stimulation of the renin-angiotensin system by 2 weeks of restricted sodium intake significantly increased SBP in intact two-kidney animals. After 4 days of sodium depletion, unilateral nephrectomy enhanced this pressor response and produced hypertension. The following evidence suggests that the rise in SBP after sodium depletion was renin-independent: first, SBP did not increase after unilateral nephrectomy in normal renin, sodium-replete rats; and second, blockade of angiotensin II formation by the converting enzyme inhibitor SQ14,225 abolished the rise in SBP after nephrectomy of sodium-deplete animals. In view of the finding thatSQ14,225 in this dose and by this method of administration did not affect the SBP of sodium-replete rats, the drug is unlikely to act via any nonspecific pressure-lowering effect. Clearly, sodium depletion in some way disturbed normal pressure-volume relationships in Sprague-Dawley rats, a fact that must be considered in future investigations involving manipulation of sodium intake.

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References
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