Genetic Influence on the Renin-Angiotensin System

LOW RENIN ACTIVITIES IN HYPERTENSION-PRONE RATS

By Junichi Iwai, Lewis K. Dahl, and Knud D. Knudsen

ABSTRACT

Two strains of rats with opposite, genetically determined predispositions to hypertension were compared. Rats from the hypertension-prone strain had significantly lower plasma and kidney renin activities than did rats from the hypertension-resistant strain. Although renin activities were modified by NaCl intake and blood pressure, significant differences between the two strains were present with all four experimental regimens used: low-NaCl diet, high-NaCl diet, unilateral renal artery constriction, and unilateral renal artery constriction plus contralateral nephrectomy. Therefore, we concluded that renin activities along with blood pressure were modified by genetic influences in these two strains of rats.

KEY WORDS

- genetic hypertension
- salt hypertension
- heredity
- NaCl
- renal hypertension
- kidney renin
- plasma renin

Our work on two strains of rats with opposite, genetically determined predispositions to hypertension may help resolve the role of the renin-angiotensin system in the pathogenesis of human and experimental hypertension. An earlier study (1) showed that hypertension-prone rats had lower renin activity in their kidneys as judged by juxtaglomerular granulation than did hypertension-resistant rats. In another study (2), plasma from the hypertension-prone strain inhibited the activity of hog renin. Thus, both studies indirectly suggested that renin activity was lower in the hypertension-prone strain. The present report, an extension of this earlier work, was based on a detailed analysis of renin activities in rats from both strains subjected to four different treatments. Under the same experimental conditions, the hypertension-prone rats had significantly lower renin activities in both plasma and kidneys than did the hypertension-resistant rats.

Methods

Rats from two Brookhaven strains with opposite, genetically determined predispositions to hypertension were used in this study: rats from the sensitive or S strain were predisposed for hypertension and those from the resistant or R strain were predisposed against hypertension. These two strains were originally developed from a single Sprague-Dawley line: one strain rapidly developed hypertension from high NaCl intake, but the other strain had little or no response to the same diet (3, 4). Subsequently, it was demonstrated that these genetically determined responses were not limited to salt hypertension; the responses also occurred when other common techniques were used to induce experimental hypertension (5-7). The genetic background therefore appeared to be critical in determining whether hypertension would develop after exposure to a variety of noxious stimuli thought to be "causal" in hypertension (8).

Details on care, food, and technique of blood pressure measurement have been described in earlier papers (8, 9). Special chows containing either 0.3% NaCl (low-NaCl chow) or 8% NaCl (high-NaCl chow) were fed ad libitum as specified in each experiment. Tap water (0.5-0.7 mEq Na/liter) was used throughout. The renin activity level in plasma and kidney was measured by the micromethod of Boucher et al. (10), and renin substrate was prepared according to their technique. The procedure for bioassay has been previously described (2).

This study was made on 408 male and female rats divided into four groups. The rats were 5 weeks old at the start of the experiments and had been fed low-NaCl chow since birth. Siblings were randomly distributed among the four experimental groups to ensure genetic variance (11). The general procedures common to all four groups will be detailed here, but the differences applicable to a specific group will be summarized later under the appropriate group heading. Twelve rats from each group (6 from each strain) were killed and studied at weekly intervals for 8 weeks. In both group III and group IV an additional 12 rats were studied after only 3 days on the experimental regimen. Eight weeks was a suitable end point, since both salt hypertension and renal hypertension reach a plateau in the S rats before that time (5, 7, 9). Each week, under light ether anesthesia, blood pressure was measured in the rats to be killed; 0.3 ml of blood for determination of plasma renin activity was then obtained directly from the jugular vein by venipuncture. In group III, one or
both kidneys were removed through a lumbar incision for measurement of kidney renin activity. In rats with intact renal arteries there was no difference in the renin activity of the two kidneys; therefore, the measurement for a single kidney was an accurate index for both. The data for the final statistical analysis of blood pressure and plasma and kidney renin activities were based on the average values obtained over the 8 weeks of study. Tests for significance were made with Student's t-test, and analysis of variance was made with special computer programs. Only values of \( P < 0.05 \) were considered significant; all values of \( P < 0.01 \) were assigned that nominal value.

Group I included 48 R rats and 48 S rats with intact kidneys. These rats were maintained on low-NaCl chow. This group was the control group against which the responses in groups II, III, and IV were compared.

Group II included 48 R rats and 48 S rats with intact kidneys. These rats were maintained on high-NaCl chow from the beginning of the experiment.

Group III included 54 R rats and 54 S rats that were maintained on low-NaCl chow. At the beginning of the experiment, under ether anesthesia, the left renal artery was constricted according to the technique of Wilson and Byrom (12); the right kidney and its circulation were untouched. In this group only kidney renin activity was measured in both kidneys, since constricting one renal artery produces normal or increased renin activity in the ipsilateral kidney and markedly decreased renin activity in the contralateral kidney (13).

Group IV included 54 R rats and 54 S rats that were maintained on low-NaCl chow. At the beginning of the experiment, the right kidney was removed and the left renal artery was constricted by the method used in group III.

**Results**

In group I, the two strains differed significantly in the three components investigated (Fig. 1 and Table 1). The blood pressure of both strains remained within our normal range (9), but the pressure in the S rats was higher (\( P < 0.01 \)) than that in the R rats (average 107.8 ± 1.90 and 96.6 ± 1.36 mm Hg, respectively). Blood pressure of the S rats rose mildly (\( P < 0.05 \)) during the study, but that of the R rats did not. Both plasma renin activity (PRA) and kidney renin activity (KRA) in the intact kidney were higher in the R rats (\( P < 0.01 \)); none of the renin activities changed (\( P > 0.05 \)) during the study. The constant level of renin activities in both R and S strains, i.e., their failure to show a progressive increase during the experiment, suggests that the low-NaCl chow was not a sodium-depleting diet. This observation was not surprising, since the sodium content of the diet exceeded the minimal requirements for rapidly growing rats (14). Also this observation was empirically evident, because rats on this chow grew normally.

**GROUP II**

In group II, the two strains differed significantly (\( P < 0.01 \)) in the three components compared. The most noticeable difference between them was in blood pressure. S rats rapidly developed hypertension on the high-NaCl diet, and their blood pressure averaged about 180 mm Hg during the final 3 weeks of the experiment. However, the blood pressure of R rats did not differ significantly (\( P > 0.05 \)) from that of the control R rats on low-NaCl chow. Both PRA and KRA again were higher in the R rats than they were in the S rats (\( P < 0.01 \)). Compared with the respective data for the controls, PRA and KRA were decreased in all group II rats (\( P < 0.01 \)).

The failure to detect a fall in PRA in the S rats during the 8 weeks on high-NaCl chow was probably due to the very low initial levels. By the end of the first week on high-NaCl chow, PRA was...
already lower ($P < 0.01$) than the mean level for S rats on low-NaCl chow. The renal renin activity in S rats and R rats and the PRA in R rats declined during the experiment ($P < 0.01$).

**GROUP III**

In group III, KRA was measured in both the intact kidney and the constricted kidney (Fig. 2 and Table 1); blood pressure and PRA were also measured. The response of the two strains differed ($P < 0.01$) with respect to all four variables. Blood pressure rose rapidly in the S rats to about 160 mm Hg from the sixth week on, this pressure was significantly higher ($P < 0.01$) than that of the control S rats. In the R rats, blood pressure also increased compared with that in the control R rats. Although the incremental effect of unilateral renal artery constriction was statistically significant ($P < 0.01$), it was clinically mild. Peak pressures averaged only about 180 mm Hg in group III R rats during the last 3 weeks of the study, which is within our normal range (9). PRA in the R rats was not only much higher than that in corresponding S rats but averaged at least twice ($P < 0.01$) the level in any other rats in this study. PRA in the S rats did not differ ($P > 0.05$) from that of their controls. KRA in the intact kidney of R rats remained well above that of S rats ($P < 0.01$), but the activity of both R rats and S rats was lower ($P < 0.01$) than that of their respective controls. Indeed, the intact kidneys of the S rats had the lowest ($P < 0.01$) KRA observed in any kidneys in this study. In contrast, the constricted kidneys of both strains, had the highest ($P < 0.01$) KRA observed in all four groups, and, as usual, the response of the R rats was higher ($P < 0.01$) than that of the S rats.

**GROUP IV**

In group IV, R rats and S rats again differed significantly in the three variables measured. However, in striking contrast with the other three groups blood pressure in the R rats rose sharply, leveling off after the third week at approximately 170 mm Hg compared with about 180 mm Hg in the S rats during this same period ($P < 0.05$). As in the other three groups, PRA was higher in the R rats than it was in the S rats ($P < 0.01$). Compared with the control levels of the respective strains, PRA...
in R rats remained unchanged and it decreased in S rats \((P < 0.01)\). KRA in the constricted kidneys was higher in R rats than it was in S rats \((P < 0.01)\), but it was unchanged \((P > 0.05)\) compared with the respective controls.

Intergroup comparisons of the variables are given in Table 2.

**INFERENCES**

A genetic influence on blood pressure was clearly evident because, after similar treatment regimens, the S rats had consistently higher average blood pressures than did the R rats. PRA and KRA were uniformly lower in S rats than they were in comparable R rats, suggesting that renin activity was also under genetic influences. However, there was no consistent correlation between renin activity levels and blood pressure levels. For example, the R rats with the lowest blood pressure (group I) and those with the highest blood pressure (group IV) had almost identical PRA levels. Also, the average blood pressure response of group I R rats and group II R rats did not differ significantly, although PRA was sharply depressed in group II compared with that in group I. Finally, group II S rats had average blood pressures that did not differ significantly from those in group III, but PRA levels were significantly higher. KRA levels in intact kidneys were lower in group III S rats than they were in group II S rats.

The customarily observed resistance of R rats to hypertension was markedly attenuated in group IV. The average blood pressure of group IV R rats \((165.8 \text{ mm Hg})\) during the entire 8 weeks of study was somewhat lower than that of the group IV S rats \((172.7 \text{ mm Hg})\). However, in relation to their respective controls, the absolute rise in R rats was slightly greater than that in S rats \((69.2 \text{ and } 64.9 \text{ mm Hg}, \text{ respectively})\). In direct comparisons, dietary NaCl appeared to be more potent in modifying renin activity than was blood pressure. For instance, the R rats in group II had PRA and KRA levels significantly lower than those in group I, although blood pressures were similar. The average blood pressure of R rats in group IV was much higher than that of R rats in group I, but the PRA levels were similar. Finally, the S rats from group IV had average blood pressures and PRA levels significantly higher than those for the S rats in group II.

### Table 2

**Statistical Comparisons of the Mean Values for Blood Pressure and Renin Activity within Each Strain**

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Symbols: > means \(P < 0.01\); = means \(P > 0.05\). BP = systolic blood pressure (mm Hg), PRA = plasma renin activity (ng/0.1 ml plasma), and KRA = kidney renin activity (ng/mg wet weight). Intact = intact kidney, and constricted = kidney with renal artery constriction.
Comparison

The unique observations in this study were not the lack of consistent correlation between blood pressure and renin activity, the greater potency of dietary NaCl compared with blood pressure on modifying renin activity, the increase in renin activity in a kidney with its renal artery constricted and the decrease in renin activity in the contralateral intact kidney, or the normal renin activity in a kidney with its renal artery constricted and the contralateral kidney removed. These observations have been made earlier by others (13, 15-18). Rather, this study demonstrated that renin activity, like blood pressure in the two strains, was under genetic influence. It is unlikely that the consistently lower renin activity in the S rats was due only to their generally higher blood pressure. Examination of Figures 1 and 2 shows that when blood pressure in the two strains was virtually identical (e.g., group I weeks 1 and 2, group II week 1, group IV weeks 3-7), the total exchangeable sodium (Na), the total exchangeable sodium (22Na), and the total carcass sodium are similar in the two strains (19). Given free choice, S rats consume less NaCl than do R rats (20). Gitlow et al. (unpublished observations) have observed that S rats and R rats metabolize 3H-norepinephrine similarly on either low- or high-NaCl intakes. As one index of sympathetic nerve activity, this observation suggests that differences in sympathetic nerve activity are not responsible for differences in renin release in the two strains (18).

Aldosterone and deoxycorticosterone (DOC) secretion rates are the same in the two strains (21), and aldosterone secretion drops sharply and equally in both strains in response to salt (22). Adrenal steroidogenesis, nonetheless, is not identical in the two strains. Both in vitro and in vivo studies (21) indicate that S rats have a twofold greater ability than do R rats to 18-hydroxylate DOC to form the mineralocorticoid 18-hydroxy-deoxycorticosterone. This increment in 18-hydroxylation in S rats is offset by an equal decrement in 11β-hydroxylation of DOC to form corticosterone. Normotensive Wistar rats share this ability (21), but they do not share the low renin activity (23); therefore, it is doubtful that these differences in steroid production, at least alone, account for the low renin activity in S rats.

The well-recognized sensitivity of renin activity levels to sodium (18) suggests that some subtle difference in the renal handling of this ion might be present in the two strains. Earlier studies have shown that, in spite of significant differences in average blood pressures, glomerular filtration rate and renal plasma flow are comparable in the two strains (24) as are the natriuretic and diuretic responses to an acute load of hypertonic saline (25). Considering the work cited earlier (19, 20) the present study provided no evidence to suggest that sodium was handled differently by members of the two strains. However, Ben-Ishay and Knudsen (unpublished observations) have observed that in rats of both strains maintained on low-NaCl diets from weaning, the S rats respond to an oral isotonic saline load with an increased diuresis-natriuresis compared with the R rats. This phenomenon is true for two different groups studied at 8 and at 16 weeks of age, respectively. Such observations do not explain the low renin levels, but for the first time evidence is available that indicates that renal sodium control differs in the two strains of rats.

Abundant information suggests that renin-release is controlled at least in part by changes in sodium load to the macula densa; the macula densa theory has been reviewed by others (17, 18, 26-28). In view of these findings, it is tempting to speculate that something related to the tubular concentration of sodium, the rate of sodium flow past the macula densa, or the intracellular sodium concentration of the macula densa cells is involved. Since no conclusive evidence is yet available, further speculation probably would not be rewarding.

Two other strains of rats with genetically controlled hypertension are available for comparison (29, 30). In Smirk's New Zealand strain (31) plasma and renal renin levels are similar to those of controls rats at 60 days of age (the youngest tested), renal renin is lower in the hypertensive rats at 90 days, and both plasma and renal renin are lower than they are in controls at 120 days. These rats thereby differ from our S rats in that our rats had low renin activity levels throughout the period of study, i.e., before, during, and after the development of hypertension and as young or adult rats. The spontaneously hypertensive (SH) rats of Okamoto and Aoki (30) behave more like the New Zealand strain than do our rats. Sokabe (32) has reported that renal renin is low when only older SH rats with hypertension are studied but that young rats from this strain have normal levels (33). Koletsky et
al. (34) have observed that the juxtaglomerular indexes are lower in SH rats than they are in controls but that the renal vein plasma activity is not changed. This disparity is difficult to understand since, in general, juxtaglomerular granulation has been found to be a fairly reliable index of renin secretion (18, p 120). We observed earlier that S rats had a lower juxtaglomerular index than did R rats when both were maintained on a low-NaCl diet (1). de Jong et al. (23) have recently reported that plasma renin activity is similar at 8 weeks of age in SH rats and in normotensive controls but that from 12 through 35 weeks of age, the SH rats have significantly higher plasma renin activity levels. Renal renin decreased only in older (35 weeks) SH rats. Baer et al. (35), by contrast, have stated that plasma renin levels measured by either bioassay or radioimmunoassay techniques are low in SH rats. Since no data are given, direct comparison with our S rats cannot be made. It is difficult to understand, however, why different investigators should report low, normal, and high plasma renin activity levels in rats from the same strain apparently on similar regimens. It is possible that the reported differences in renin activity are due to differences in the techniques used for blood sampling: decapitation (22), renal vein venipuncture (32, 34), and jugular vein venipuncture (35).

Rapp selectively bred mice (36) and rats (37) for high and low juxtaglomerular granularity. Among the mice, by the fourth generation separation into high and low substrains had occurred. It is pertinent to the present study that those selected for a high juxtaglomerular index had a slightly but significantly lower average blood pressure than those bred for a low juxtaglomerular index. Similar findings on blood pressure and juxtaglomerular index were made in the rats by the third generation; in addition, serum and renal renin were higher in the rats bred for a high index and vice versa. Rapp's observations (36, 37) seem relevant to ours. In both laboratories a reciprocal relationship between blood pressure and juxtaglomerular index or renin activity was generally observed: rats with the higher average juxtaglomerular index and renin activities had lower average blood pressures and vice versa. We bred for differences in blood pressure and found that we had indirectly selected for differences in juxtaglomerular index and renin activity; Rapp bred for differences in juxtaglomerular index and found that he had indirectly selected for differences in blood pressure.

We find it difficult to rationalize that this finding is a coincidence without relevance to the pathogenesis of hypertension. Because of the numerous anomalies between blood pressure and renin activity, many students of hypertension have expressed grave doubts concerning the role of the renin-angiotensin system in chronic hypertension. Without being able to explain these anomalies, we suspect that in the pathogenesis of most hypertension—renal, renovascular and nonrenal—the renin-angiotensin system ultimately will be found to be intimately involved.

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References


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