Suppression of Adrenal Renin in Dahl Salt-Sensitive Rats

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SUMMARY We previously showed that adrenal renin is highest in the rat zona glomerulosa (ZG) and that low sodium or high potassium and nephrectomy increase adrenal ZG renin and aldosterone. Dahl salt-sensitive rats (S) have been shown to have lower plasma renin activity and plasma aldosterone and higher plasma 18-hydroxy-11-deoxycorticosterone than Dahl salt-resistant rats (R). In this study we assess the possible role of adrenal ZG renin in the suppression of aldosterone in S rats. Adrenal ZG renin was significantly decreased in S as compared with R rats even at 6 weeks of age, when both S and R rats are still normotensive (S = 7.2 ± 0.2, R = 18.0 ± 1.6 ng angiotensin I/mg protein/hr). Adrenal ZG aldosterone was also significantly lower in S than in R rats (S = 21.1 ± 4.3, R = 39.5 ± 3.6 ng/mg protein). Furthermore, the rise in adrenal ZG renin and aldosterone after nephrectomy in S rats was significantly less than that in R rats. To determine if the suppressed adrenal ZG renin of S rats is due to volume expansion, we studied the effect of a sodium-deficient diet on adrenal ZG renin in S and R rats. After 2 weeks of a sodium-deficient diet S rats had significantly lower basal adrenal ZG renin than did R rats (S = 7.6 ± 0.4, R = 21.7 ± 1.9 ng angiotensin I/mg protein/hr) and a marked blunting of the adrenal ZG renin response to nephrectomy (S = 13.6 ± 1.1, DR = 167 ± 16.1 ng angiotensin I/mg protein/hr). Our previous report of blunting of the nephrectomy response by indomethacin suggests a role for prostaglandins in the regulation of adrenal renin. Adrenal ZG prostaglandin E₂ in S rats was also significantly lower than in R rats (S = 0.22 ± 0.05, R = 0.40 ± 0.05 ng/mg protein). These data indicate that the low adrenal ZG renin in S rats is associated with low plasma aldosterone and that the low adrenal ZG renin level of S rats is not due to volume expansion and probably represents a genetic abnormality of the salt-sensitive strain or the low adrenal ZG prostaglandin E₂ level, or both. (Hypertension 8: 1149-1153, 1986)

KEY WORDS • Dahl rats • extrarenal renin • adrenal renin • aldosterone • prostaglandin E₂ • sodium-deficient diet • genetic hypertension • nephrectomy

Renin has been reported in the adrenal gland by several investigators. Recently, Naruse and Inagami and co-workers further characterized this enzyme and showed that it was inactivated by specific antirenin antibody. We had previously found the highest concentration of adrenal renin in the rat zona glomerulosa and showed that low sodium or high potassium diet and nephrectomy increased both adrenal zona glomerulosa renin and aldosterone. There was positive correlation between adrenal zona glomerulosa renin and adrenal aldosterone concentration, suggesting that adrenal renin may be a local hormone in the regulation of aldosterone production. Furthermore, indomethacin blunts the adrenal renin response to nephrectomy, suggesting a role of prostaglandins in the response of adrenal renin to nephrectomy.

In 1962, Dahl et al. selectively bred rats for susceptibility (S) or resistance (R) to the hypertensive effect of a high salt (NaCl) diet. Since then, it has been shown that S rats have lower plasma renin activity, lower plasma aldosterone, and higher 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) than R rats. The present study was performed to assess the possible role of adrenal renin in the suppression of aldosterone in S rats.

Materials and Methods

Inbred female Dahl salt-sensitive (S/JR) and inbred female Dahl salt-resistant (R/JR) rats from our colonies were used. These inbred strains will be referred to in the text by their generic terms, S and R. Groups of rats were age-matched for each experiment. The rats were maintained on a normal salt diet (0.9% NaCl).
The rats were killed by decapitation at 6 weeks and 14 weeks of age. Since nephrectomy is the most potent stimulator of adrenal renin and following nephrectomy the adrenals would not be contaminated by plasma renin, bilateral nephrectomy was performed in some rats at 14 weeks of age under pentobarbital anesthesia (30 mg/kg) 20 hours before death. To study the effect of sodium on adrenal renin, 2-week-old rats were maintained on either an 8% NaCl diet or a sodium-deficient diet (Teklad, Madison, WI, USA) until death at 4 weeks of age. Moreover, 6-week-old rats that had received either 2 weeks of a 1% NaCl diet or a sodium-deficient diet were bilaterally nephrectomized under pentobarbital anesthesia 20 hours before death.

The rats were killed by decapitation, and the adrenal glands were flushed free of blood with normal saline through the aorta. The adrenal glands were then removed, and the capsules were separated from the fascicula medullary portions by a previously described technique. In previous experiments using this technique, we showed that the degree of contamination of capsular cells with fascicula medullary cells was approximately 5%. The separated capsular portion from each adrenal was homogenized for 30 seconds with a Teflon-glass homogenizer in tris(hydroxymethyl) aminomethane (Tris) acetate buffer, pH 7.4, at 4°C and centrifuged for 30 minutes at 1900 g, 4°C. The adrenal capsular supernatant was aspirated, and renin activity was measured by radioimmunoassay using a New England Nuclear kit (Boston, MA, USA). In brief, the supernatant (50 μl) was incubated with 5 μl of 6.6% 8-hydroxyquinoline, 5 μl of 3.3% dimeracrol, 25 μl of 4% ethylendiaminetetraacetic acid, and 90 μl of Tris lysozyme buffer (pH 7.4) using 75 μl of nephrectomized rat plasma as substrate. The amount of substrate used for the incubation is less than 3%. After incubation for 1 hour at 37°C, a 50-μl aliquot was taken for radioimmunoassay of generated angiotensin I (ANG I). Aldosterone in the supernatant of the homogenate of capsular cells was measured after extraction with methylene chloride by radioimmunoassay, as reported previously. In some experiments, prostaglandin E2 in the capsular supernatant was also measured by radioimmunoassay using a 125I-labeled radioimmunoassay kit from New England Nuclear after extraction using columns of octadeysilsil silica (Sep-Pak, Water Associates, Milford, MA, USA). Protein concentration of the extract was measured by the method of Lowry et al.

The results of the experiments are expressed as the mean ± SE, with n as the number of experimental rats. Statistical analysis was made using the unpaired t test and 2 × 2 factorial analysis of variance using an Olivetti P6060 computer (Dallas, TX, USA). The significance was defined as a p value of less than 0.05.

**Results**

Figure 1 shows the adrenal renin and aldosterone concentration in S and R rats at 6 and 14 weeks of age on a normal sodium diet. Adrenal renin in S rats was significantly lower than that in R rats even at 6 weeks of age, when both S and R rats are still normotensive (S = 7.22 ± 0.22, R = 17.98 ± 1.56 ng ANG I/mg protein/hr). At 14 weeks of age, when S rats are mildly hypertensive with blood pressures significantly different from R rats, S rats had significantly lower adrenal renin compared with R rats (S = 6.32 ± 0.34, R = 22.16 ± 1.97 ng ANG I/mg protein/hr). Adrenal aldosterone was also significantly lower in S as compared with R rats at both ages (S = 21.10 ± 4.29, R = 39.50 ± 3.54 ng/mg protein at 6 weeks of age; S = 20.68 ± 5.82, R = 50.92 ± 7.65 ng/mg protein at 14 weeks of age).

Figure 2 shows the effect of nephrectomy on adrenal renin and aldosterone in S and R rats at 14 weeks of age on a normal sodium diet. Nephrectomy had little effect on adrenal renin in S rats (from 2.35 ± 0.11 to 7.57 ± 1.48 ng ANG I/mg protein/hr). In R rats, however, nephrectomy strikingly increased adrenal renin about 10-fold within 20 hours (from 15.63 ± 2.83 to 116.02 ± 14.57 ng ANG I/mg protein/hr). The S rats had significantly less response to nephrectomy than R rats (p < 0.001). The adrenal aldosterone rise after nephrectomy in S rats was also significantly less than in R rats (p < 0.05).

**Figure 1.** Adrenal renin and aldosterone in Dahl S and R rats at 6 and 14 weeks of age on a normal sodium diet. The bar represents the mean ± SE. AI = angiotensin I.
Figure 3 shows the effect of an 8% NaCl diet or a sodium-deficient diet on adrenal renin in S and R rats at 4 weeks of age. The 8% NaCl diet suppressed adrenal renin in both strains. After 2 weeks of the sodium-deficient diet, however, adrenal renin in S rats was still significantly lower than that in R rats (S = 7.61 ± 0.42, R = 21.7 ± 1.86 mg ANG I/mg protein/hr). The adrenal weight of the rats receiving 8% NaCl was 10.67 ± 0.57 mg per adrenal for the S rats and 11.4 ± 0.26 mg per adrenal for the R rats. In the rats on the sodium-deficient diet, the adrenal weight was 12.4 ± 0.76 and 12.6 ± 0.43 mg per adrenal for the S and R rats, respectively.

Figure 4 shows the effect of nephrectomy on adrenal renin after either a 1% NaCl diet or a sodium-deficient diet in 6-week-old S and R rats. Nephrectomy markedly increased adrenal renin in R rats, while S rats had marked blunting of the adrenal renin response to nephrectomy (S = 13.63 ± 1.05, R = 167.04 ± 16.10 ng ANG I/mg protein/hr).

Since indomethacin blunts the adrenal renin and prostaglandin E₂ response to nephrectomy, we assayed the content of prostaglandin E₂ in the adrenal capsular portion in S and R rats at 14 weeks of age. Figure 5 shows adrenal zona glomerulosa prostaglandin E₂ in S and R rats at 6 and 14 weeks of age. In the same fashion as adrenal renin and aldosterone, adrenal prostaglandin E₂ in S rats was also significantly lower than in R rats at both ages.

Discussion

The presence of a renin-like enzyme in the adrenal gland was described by Ryan in 1967 and subsequently has been reported by several other investigators. Recently, our laboratory showed that adrenal renin concentration is highest in zona glomerulosa as compared with zona fasciculata and medulla. We also showed that low sodium or high potassium diet and nephrectomy increase adrenal zona glomerulosa renin and aldosterone and that a high sodium diet suppresses adrenal renin and aldosterone. There was a strong positive correlation between the adrenal zona glomerulosa renin and adrenal zona glomerulosa aldosterone, suggesting that adrenal renin may play a role in regulating aldosterone production. It has been shown that the S rats have lower plasma renin activity, lower plasma aldosterone, and higher 18-OH-DOC than R rats. In this study, adrenal zona glomerulosa renin and aldosterone were significantly decreased in S as compared with R rats, even at 6 weeks of age when both S and R rats are still normotensive. Furthermore, the adrenal zona glomerulosa renin and aldosterone rise after nephrectomy was significantly less in S than in R rats. These data suggest that the low aldosterone levels in S rats may be related to low adrenal zona glomerulosa renin.

Since S rats have higher 18-OH-DOC secretion and higher plasma levels of 18-OH-DOC concentration and because a high sodium diet decreases adrenal
HYPERTENSION

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FIGURE 4. Adrenal renin response to nephrectomy (Nepex) after a 1% NaCl or a sodium-deficient diet in 6-week-old Dahl S and R rats. The bar represents the mean ± SE. AI = angiotensin I.

FIGURE 5. Adrenal prostaglandin E₂ in Dahl S and R rats at 6 and 14 weeks of age on a normal salt diet. The bar represents the mean ± SE.

renin, one possible mechanism for the low adrenal renin in S rats is suppression by an increased plasma volume as a result of high 18-OH-DOC levels. To explore this possibility, we studied the effects of a high sodium diet or a sodium-deficient diet on adrenal renin concentration in S and R rats. The high sodium diet suppressed adrenal renin in both groups of rats. After a sodium-deficient diet, however, adrenal renin was still significantly lower in S than in R rats and the response to nephrectomy was markedly blunted. These results suggest that the suppressed adrenal renin in S rats is not due to volume expansion.

There is evidence that prostaglandins play a role in the control of renal renin release. Prostaglandins have been reported to stimulate renin release in vivo and in vitro. We have shown previously that nephrectomy increases adrenal prostaglandin E₂ and adrenal renin while indomethacin lowers the adrenal prostaglandin E₂ response to nephrectomy, suggesting that prostaglandins may play a role in the adrenal renin response to nephrectomy. Since it has been shown that S rats have lower urinary prostaglandin E₂ than R rats, we assayed the content of prostaglandin E₂ in the adrenal zona glomerulosa portion. Adrenal prostaglandin E₂ in S rats was significantly lower than that in R rats at 6 and 14 weeks of age, suggesting a role for prostaglandins in the regulation of adrenal renin.

In conclusion, these data indicate that the S rats have lower levels of adrenal renin, adrenal aldosterone, and adrenal prostaglandin E₂ than R rats. These abnormalities are not corrected by a sodium-deficient diet, suggesting that these defects are not due to volume expansion. Whether the low adrenal renin is secondary to the low adrenal prostaglandin E₂ remains to be established.

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