Renin in Rats with Spontaneous Hypertension

By Subha Sen, Robert R. Smeby, and F. Merlin Bumpus

ABSTRACT

An age-dependent study of plasma renin activity (PRA), kidney renin activity (KRA), and plasma renin substrate was carried out in rats with spontaneous hypertension during the prehypertensive, the early hypertensive, and the established hypertensive phases of their disease. PRA and KRA were both significantly elevated before and during the initial phase of hypertension and normal or subnormal during the established phase. In normal controls, neither KRA nor PRA was significantly different during the entire growth period. Plasma renin substrate was elevated throughout the growth period in rats with hypertension. This relationship between renin and blood pressure suggests that renin may play a primary role, possibly along with other factors, in the initiation of hypertension in rats with spontaneous hypertension.

KEY WORDS

blood pressure plasma renin activity kidney renin activity kidney renin substrate kidney

Rats with spontaneous hypertension (1) provide a useful and easily available model to study hypertension. The cause of hypertension in these rats is not known, but it has been suggested that the renin-angiotensin system does not contribute to the disease. Koletsky et al. (2) showed that venous renin activity is normal or subnormal during the later stage of spontaneous hypertension, but de Jong et al. (3) showed increased plasma renin activity throughout the whole period of development of spontaneous hypertension.

Age is an important factor in the development of hypertension in these rats: they do not demonstrate stable hypertension before they are 8–10 weeks old. Although their blood pressure increases after 5 weeks, it is very labile. To determine whether the renin-angiotensin system contributes to the etiology of hypertension in these rats, this paper presents an age-dependent study of the plasma renin activity (PRA), the kidney renin activity (KRA), and the plasma renin substrate in rats with spontaneous hypertension weighing from 60 g (3–4 weeks, prehypertensive stage) to 250 g (14–16 weeks, established hypertensive stage).

Methods

All rats with spontaneous hypertension used in this study were either bred at the Cleveland Clinic or obtained from the Purina Laboratory and are of the Kyoto Wistar strain developed by Okamoto and Aoki (1). Normal Wistar rats were used as controls in each group, since normal Kyoto Wistar rats were not available at the time of this study. For each experiment, rats were divided into five groups according to their mean body weight and age as described in Table 1 and Figures 1 and 2.

Measurement of Plasma Renin Activity.—PRA was measured according to the micromethod of Boucher et al. (4) with substrate prepared from plasma of rats bilaterally nephrectomized 48 hours previously. Blood samples were collected from the tail arteries of rats under light ether anesthesia in a Vacutainer containing the tripotassium salt of ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. For each assay 0.3–0.4 ml of blood was drawn. Angiotensin present at the end of incubation was determined by bioassay in pentolinium-treated vagotomized rats. All results were expressed as the amount of angiotensin formed (in nanograms) from 0.1 ml of plasma after 15 hours of incubation (ng/0.1 ml 15 hours⁻¹).

Measurement of Kidney Renin Activity.—KRA was measured according to Boucher et al. (4) in kidney slices homogenized in cold distilled water. Tissue slices were placed in 1 ml of cold water, frozen, and then allowed to thaw at room temperature.
temperature. Freezing and thawing were repeated three times to increase the cell rupture and the renin release. The slices were then homogenized, and the homogenate was centrifuged at 6,400–12,000 g (0–5°C) for 20 minutes. The residue was washed twice with cold water, and the supernatant fluids were pooled and diluted to a known volume. Then 0.1 ml of the extract was incubated with the substrate prepared from nephrectomized rats. Angiotensin formed after 1 hour of incubation was determined by bioassay as previously described. All results were expressed as the amount of angiotensin formed (in nanograms) per milligram of tissue after 1 hour of incubation (ng/mg hour⁻¹).

Measurement of Plasma Renin Substrate.—The concentration of plasma renin substrate was determined indirectly by the incubation of plasma samples with an excess of homologous renin in the presence of diisopropylfluorophosphate (DFP), EDTA, and phosphate buffer for 90 minutes at 37°C. The incubation system used was the same as that described earlier (5). The angiotensin which formed was bioassayed according to Pickens et al. (6). Results were expressed as means ± SE of angiotensin generated in 90 minutes.

Results

PRA in 6 normal rats in group 1 (60 g) was 33.3 ng/0.1 ml 15 hours⁻¹, but in 10 rats with spontaneous hypertension it was 50.9 ± 2.1 ng/0.1 ml 15 hours⁻¹, which is significantly higher than normal (P < 0.001) (Fig. 1). In group 2 (105 g), PRA was higher in the 12 rats with spontaneous hypertension (65.5 ± 1.7 ng/0.1 ml 15 hours⁻¹) than it was in the 6 normal controls (30.5 ± 2.5 ng/0.1 ml 15 hours⁻¹) (P < 0.001). Blood pressure in both rats with spontaneous hypertension (110 ± 15 mm Hg) and normal rats (110 ± 20 mm Hg) was normal.

In group 3 (150 g), PRA in the ten rats with spontaneous hypertension was lower than it was in group 2 rats with hypertension, but it was still significantly higher (40.4 ± 2.2 ng/0.1 ml 15 hours⁻¹) than it was in the five normal control rats (29.0 ± 2.0 ng/0.1 ml 15 hours⁻¹) (P < 0.01). The average blood pressure in group 3 was 115 ± 12 mm Hg in the normotensive rats and 155 ± 20 mm Hg in the rats with spontaneous hypertension. The blood pressure of these rats with spontaneous hypertension is indicative of the early stages of hypertension.

In group 4 (200 g), PRA in the rats with spontaneous hypertension was further reduced but was not significantly different from that found in the corresponding normal control

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**Figure 1**

Plasma renin activity in rats with spontaneous hypertension (SH) and in normotensive Wistar control rats (NORMAL). PRA was determined in five groups by weight.

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rats. PRA in 15 rats with spontaneous hypertension was 29.6 ± 1.2 ng/0.1 ml 15 hours⁻¹, and it was 30.3 ± 1.9 ng/0.1 ml 15 hours⁻¹ in the 10 normal control rats. Rats with spontaneous hypertension in this group had an average blood pressure of 185 ± 15 mm Hg, and the normal control rats had an average blood pressure of 115 ± 15 mm Hg.

In group 5 (250 g), the oldest of the rats was studied. PRA in the 20 rats with spontaneous hypertension was again lower (19.6 ± 0.8 ng/0.1 ml 15 hours⁻¹) than that reported for the hypertensive rats in the other groups and also significantly lower than PRA in the 10 normal control rats in group 5 (27.6 ± 1.63 ng/0.1 ml 15 hours⁻¹) P < 0.001). The blood pressure of rats with spontaneous hypertension in group 5 averaged 200 ± 10 mm Hg and that of normotensive rats averaged 120 ± 10 mm Hg.

KRA was measured in five groups of rats (Fig. 2). KRA in rats with spontaneous hypertension in group 1 (100 g) was significantly higher (458 ± 17.3 ng/mg hour⁻¹) than it was in the normal Wistar control rats (308 ± 8.8 ng/mg hour⁻¹) (P < 0.001). KRA in rats with spontaneous hypertension in group 2 (150 g) was lower (390 ± 9.1 ng/mg hour⁻¹) than KRA in corresponding rats in group 1, but it was significantly higher than that of normal control rats in group 2 (306 ± 7.6 ng/mg hour⁻¹) (P < 0.001). In group 3 (200 g), KRA in rats with spontaneous hypertension was again lower than it was in the two previous groups (297 ± 8.0 ng/mg hour⁻¹) and also lower than KRA in corresponding normal control rats (336 ± 14 ng/mg hour⁻¹) (P < 0.05). In group 4 (250 g), KRA was again lower (286 ± 6.5 ng/mg hour⁻¹) than KRA in rats of group 3 and significantly lower than in normal controls (339 ± 9.6 ng/mg hour⁻¹) (P < 0.001). In group 5 (300 g), KRA in rats with spontaneous hypertension was reduced more and was again significantly lower (160 ± 18.5 ng/mg hour⁻¹) than it was in normal control rats (305 ± 3.9 ng/mg hour⁻¹).

Plasma renin substrate in group 1 was 668 ng/ml in rats with spontaneous hypertension and 502.2 ng/ml in the normal control rats.

Kidney renin activity in rats with spontaneous hypertension (SH) and in normotensive controls. Rats were divided into five groups by weight.

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(P < 0.01) (Table 1). In group 2, the substrate level in rats with spontaneous hypertension and in normal control rats was not different, 720 ng/ml and 658 ng/ml, respectively. In group 3, substrate level in rats with spontaneous hypertension was again slightly higher than that in corresponding normal control rats, 842 ng/ml and 654 ng/ml, respectively (P < 0.05). In groups 4 and 5 substrate levels in rats with spontaneous hypertension were also significantly higher than those in corresponding normal controls (Table 1).

Discussion

These data showed that PRA was significantly higher in rats with spontaneous hypertension at an early age, decreased to a normal level with the development of stable hypertension, and finally became lower than normal when hypertension was established. In normotensive control rats, PRA was not significantly different during the entire growth period.

de Jong et al. (3) reported that PRA in rats with spontaneous hypertension increased in the early phase of hypertension and stayed elevated during the rest of the hypertensive period. Our data showed suppression of PRA in the established stage of hypertension in agreement with the findings of Koletsky et al. (2) and of Baer et al. (7). The difference in

the results may possibly be due to differences in the method of collection of blood samples. de Jong et al. (3) collected blood by decapitation of rats, which might cause stimulation of renin release. Bozovic and Efendic (8) showed a fivefold increase in renin release from the kidneys of rats killed by decapitation in comparison with that from the kidneys of rats killed by sodium thiopentol anesthesia.

KRA seemed to run parallel to PRA in rats with spontaneous hypertension. At the early age, the KRA was significantly higher than it was in controls. KRA was reduced as the rats developed hypertension. In normal controls, KRA was not significantly different during the entire growth period. These data suggest that, during the development of hypertension, something, possibly the hypertension itself, caused the suppression of renin synthesis in the kidney, and this phenomenon was reflected in the PRA. In the 200-g group, PRA was not significantly different in rats with spontaneous hypertension and normal rats (Fig. 1), but, in the same group, KRA was lower than normal (P < 0.05), which suggests that KRA was reduced first and then PRA.

The renin substrate did not seem to be important in the pathogenesis of hypertension, although it was higher than normal. When PRA was high, the substrate concentration was high; it also remained slightly higher than normal when PRA was suppressed.

Causes of hypertension in rats with spontaneous hypertension are not understood. Aoki (9) reported that the kidney might not be involved, because 12 hours after bilateral nephrectomy blood pressure was not reduced in rats with spontaneous hypertension. These experiments, however, were conducted with 36-week-old hypertensive rats when hypertension was well established. Our data suggest that there are renal changes which seem to parallel the onset or the development of hypertension in rats with spontaneous hypertension. First, PRA and KRA are significantly elevated before and during the initial phase of hypertension. Second, it has been reported previously from this laboratory (10) that the

<p>| TABLE 1 |
| Plasma Renin Substrate in Rats with Spontaneous Hypertension and in Normotensive Rats |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Rat</th>
<th>Renin substrate (ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (100 g)</td>
<td>SH</td>
<td>668 ± 38</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>502.2 ± 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (150 g)</td>
<td>SH</td>
<td>720 ± 36.5</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>658.8 ± 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (200 g)</td>
<td>SH</td>
<td>842 ± 68</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>654 ± 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (250 g)</td>
<td>SH</td>
<td>960 ± 85</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>742.2 ± 42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (300 g)</td>
<td>SH</td>
<td>845 ± 50</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>696 ± 32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± s.e. SH = rats with spontaneous hypertension, NS = not significant. There were five control rats and five hypertensive rats in each group.

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erythropoietin titer is high in these rats just before the onset of hypertension and returns to a lower level following the establishment of hypertension, although this level is still quite high when compared with that of normal controls. The kidney is the major source of both renin and erythropoietin (11).

The role of renin as a humoral factor responsible for development of hypertension is not well determined. It can be concluded from the changes in renin values (PRA and KRA) relative to the changes in blood pressure that either renin plays a primary role along with other possible factors in the etiology of the hypertension or that the renal changes possibly result from the same physiological change which is involved in the development of the hypertensive state.

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