Renin-angiotensin system in spontaneously hypertensive rats

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SHIONO, KUMIKO, AND HIROFUMI SOKABE. Renin-angiotensin system in spontaneously hypertensive rats. Am. J. Physiol. 231(4): 1295-1299. 1976. — Plasma and kidney renin activities (PRA, KRA) were determined in male spontaneously hypertensive rats (SHR), the inbred strain of F27-30. Blood samples of 0.5 ml for PRA determination were obtained through a cannula inserted into the abdominal aorta without anesthesia to minimize renin release from the kidney. The PRA was lower in SHR than in normal Donryu rats of age 5, 10, 20, and 30 wk. The KRA of SHR at age 10, 20, and 30 wk was also lower, confirming a previous report. At 5 wk of age, KRA was slightly higher than that of normal controls. At 50 wk of age, PRA and KRA were significantly lower in SHR (F25) from a random-bred colony than in normal Donryu rats. It is suggested that the renin-angiotensin system is suppressed in SHR as a compensatory reaction against blood pressure elevation.

plasma renin activity; kidney renin activity; submandibular renin activity

SOKABE (26) reported a decrease of renin activity in the kidney of the spontaneously hypertensive rat (SHR) of F8-9 several weeks after the blood pressure had risen and suggested that the decrease was a compensatory reaction to the blood pressure elevation. Plasma renin activity (PRA) may better reflect the activity of the renin-angiotensin system. Several groups of investigators found PRA in SHR to be either decreased (9, 14), increased (7, 23), or unchanged (4, 8, 16). This discrepancy may be due to difficulty in obtaining the blood sample without inducing renin release in the rat (3, 16, 17, 19, 22, 24). Since previous reports (3, 16, 17, 19, 22) used only normotensive rats, we have determined effects of blood withdrawal and anesthesia on blood pressure and PRA in SHR. We also have reexamined PRA values in SHR of various ages, taking care to minimize any extra renin release caused by blood sampling. Kidney renin activity (KRA) was also determined in parallel.

METHODS

Materials. Male SHRs, the inbred strain (20) of F27-30, from the colony of the Department of Pharmacology, Jichi Medical School, derived from the Saikyu Research Center F25 strain, were used. In preliminary studies SHRs (F23-24) of either sex from a random-bred colony at the Iyakushigen Institute for Medical Research were also used. Normal rats of the Donryu strain were used as controls. They were fed a rat chow (NaCl 3.1 mg/g) and tap water ad libitum.

Mean blood pressure was determined through a polyethylene cannula inserted into the abdominal aorta without anesthesia (17, 27). Tail blood pressure was measured by the plethysmographic method.

Blood and tissue samples. Two blood samples of 0.5 ml each were obtained without anesthesia through the cannula inserted into the abdominal aorta a day before. The ammonium salt of ethylenediaminetetraacetic acid (EDTA) (1.5 mg/ml blood) was used as anticoagulant. The blood sample was cooled to 0°C and centrifuged 1,700 g for 20 min at 0°C. The plasma was separated and kept frozen at -20°C. The kidney or submandibular gland was removed after exsanguination under ether anesthesia and kept frozen at -20°C.

Determination of renin activity. Plasma renin activity was determined by a modification of the method of Imai and Sokabe (12), based on the method of Boucher et al. (2). Five-tenths of a milliliter of Dowex 50W-X2 (NH4+) resin, 0.2 ml of plasma from the first 0.5-ml blood sample, and 0.1 ml of 0.2 M AcONH4 buffer were incubated at 37°C, pH 5.5, for 24 h by stirring in a 2-ml siliconized glass test tube, with a stirring device (Taiyo Concentrator TC-8) used to determine angiotensin-forming activity, designated as X. The medium contained 19.6 meq EDTA-2Na/liter. Kidney extract was made by adding 10 ml water/tissue and was dialyzed against a solution of 11.8 meq EDTA-2Na/liter at 0°C for 18 h. One milliliter of Dowex resin, 0.1 ml of plasma from the second 0.5-ml sample, 1.0 ml of 0.2 M AcONH4 buffer, and 0.5 ml of acid treated (pH 3.0, 0°C for 40 min) kidney extract were incubated at 37°C, pH 7.4, for 40 min by shaking (130/min) to obtain angiotensinogen concentration in plasma, designated as M. X and M media were kept frozen after incubation; applied to a column with 0.5 or 1 ml of resin (pH 6.0); washed with 5 or 10 ml of 0.2 M AcONH4 buffer (pH 6.0), 7.5 or 15 ml of 0.1 ml acetic acid/ml, and 15 or 30 ml of water; and eluted with 5 or 10 ml of 0.1 M diethylamine and of 0.5 N NH4OH, respectively. The eluate was evaporated in a 50-ml flask with a rotary evaporator (Ishii KM 1). Reevaporation in 2 ml of 0.8 ml ethanol/ml was repeated 3 times. The final residues were dissolved in 0.9 and 1.2 ml of 9 mg NaCl/ml containing 10 ml Tween 20/ml, respectively, for X and M determinations. Angiotensin I thus formed was bioassayed by its pressor activity in a rat anesthetized with pentobarbital sodium (50 mg/kg ip), and treated
with pentolinium bitartrate (5 mg/kg iv), with one-
aparticular acid-5-isoleucine angiotensin I as the standard. X and M were expressed in nanograms per milliliter of plasma. The first-order reaction constant K was calculated by the following equation

\[ K = 2.30 \left( \frac{2 - [\log_{10} 100(M - X)/M]}{t} \right) \]

The PRA was expressed both in angiotensin-forming activity (nanograms per milliliter per hour) and in the reaction constant \(10^{-2} K\), since angiotensin formation in vitro by these procedures is determined by the amounts of renin and angiotensinogen present. Eight 0.2-ml aliquots of a pooled rat plasma incubated for X formed 150 ± 6.3 ng/ml (mean ± SE) of angiotensin I, showing good reproducibility.

Kidney and submandibular gland renin activities were determined by a modification of the method of Nishimura and Sokabe (18). Tissue extract was made and dialyzed as described above. For the submandibular gland a solution of 11.8 meq EDTA-ZNa/liter was used instead of water to inhibit kininogen. Each 0.5 ml of pooled rat plasma, sodium phosphate buffer (0.15 M NaH2PO4, 0.15 M Na2HPO4, 20 ng thimerosal/ml), and acid-treated extract were incubated at 37°C, pH 7.4, for 10–20 min to determine angiotensin-forming activity of the extract. Kidney extract was diluted 8 times with a solution of 9 mg NaCl/ml. The extract of normal rat kidney was incubated by the same procedure for 80 and 160 min to obtain angiotensinogen concentration in the plasma used. The KRA was expressed both in the reaction constant \(10^{-2} K\) and total amount in the kidney \(10^{-2} K \cdot g/100 \text{ g body wt.}\)

The Student t test was used for statistical analysis.

RESULTS

Effects of blood withdrawal and anesthesia on blood pressure and plasma renin activity. Blood was withdrawn through the aortic cannula successively in amounts of 0.5, 0.5, and 4.0 ml. Blood pressure and PRA were determined at each step. Blood withdrawal up to 1.0 ml did not affect blood pressure significantly in normal rats of Donryu strain or SHR at 5 and 20 wk of age. When a total of 5 ml of blood was collected, blood pressure decreased by 62.3 ± 7.4 mmHg (mean ± SE, n = 8) from the level of 119 ± 2.5 in 5-wk-old Donryu rats, 83.3 ± 11.5 from 126 ± 2.4 in 5-wk-old SHR, 18.5 ± 5.7 from 125 ± 3.8 in 20-wk-old Donryu rats, and 72.8 ± 13.1 from 180 ± 5.0 in 20-wk-old SHR (P < 0.001, 0.001, 0.005, and 0.001, respectively). Blood withdrawal up to 1.0 ml did not affect PRA in Donryu rats or 20-wk-old SHR. The PRA after withdrawal of 5 ml blood increased more than twice as much as those after withdrawal of 1 ml blood in these rats. At 5 wk of age PRA increased even after withdrawal of 1 ml blood. Values are shown in Table 1.

Pentobarbital anesthesia (40 mg/kg ip) decreased blood pressure by 30.0 ± 8.8 and 65.8 ± 10.8 mmHg from the levels of 132 ± 1.8 and 187 ± 5.5 in 20-wk-old Donryu rats and SHR (n = 6, \( P < 0.005 \) and 0.001, respectively), confirming previous findings (17). The PRA was doubled by anesthesia (Table 1).

**Table 1. Effects of blood withdrawal and anesthesia on PRA in SHR**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Age, wk</th>
<th>Blood withdrawal, ml</th>
<th>PRA ng/ml per h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Donryu</td>
<td>( \alpha )</td>
<td>5</td>
<td>1.51 ± 0.51</td>
<td>2.67 ± 0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>2.34 ± 0.52</td>
<td>2.60 ± 0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1.89 ± 0.77</td>
<td>1.40 ± 0.71</td>
</tr>
<tr>
<td>SHR</td>
<td>( \alpha )</td>
<td>5</td>
<td>0.90 ± 0.25</td>
<td>2.09 ± 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>1.28 ± 0.32</td>
<td>1.46 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.94 ± 0.19</td>
<td>1.88 ± 0.31*</td>
</tr>
</tbody>
</table>

*Anesthetized with pentobarbital (40 mg/kg ip).*

Values are means ± SE, n = 6. \( P \) values indicate statistically significant differences compared to preceding figures on same line.

**Plasma, kidney, and submandibular gland renin activity.** Body weight and mean blood pressure of rats in the final series are shown in Fig. 1. The PRA determined in 0.5 ml blood samples obtained without anesthesia, and expressed in terms of angiotensin formation or the first-order reaction constant, were lower in the SHR than in normal Donryu rats at age 5, 10, 20, and 30 wk (Fig 2). The KRA expressed per gram of tissue \( (K) \) or as total amounts in the kidney were lower in the SHR than in Donryu rats at age 10, 20, and 30 wk (Fig 3), confirming a previous report (26). At 5 wk of age, KRA in the SHR was slightly higher than that of normal control.

At 50 wk of age PRA and KRA were significantly lower in the SHR than in the controls. Plasma renin activities \( (10^{-2} K) \) were 7.21 ± 1.56 \( (n = 7) \) and 16.3 ± 2.8 \( (n = 5) \) in female SHRs of F25 from a colony of the Iyakushigen Institute for Medical Research and Normal female rats of Donryu strain, respectively. Kidney renin activities \( (10^{-2} K) \) were 76.4 ± 7.7 \( (n = 8) \) and 155 ± 16 \( (n = 6) \), respectively.

Renin activity in the submandibular gland was deter-
The present study indicated that PRA in the inbred SHR strain was lower than in the normal Donryu strain at 5-30 wk of age. The method of obtaining the blood
after 10 wk, suggesting that the decrease is a result of blood pressure elevation as a compensatory reaction (26). Higher KRA values in the SHR at 5 wk of age than in the control may be the results of suppressed release of renin from the kidney. When the suppressing stimuli have been continued, production and storage may decrease after a latent period.

Variation of KRA with age in the SHR differed in three series of determinations (24, 26). This may be due to differences in severity of hypertensive disease, especially the course of blood pressure elevation. The differences among colonies and generations may partly explain the various results on renin-angiotensin system in the SHR (4, 7–9, 14, 16, 23).

In normal Donryu rats KRA decreased with age also. We compared KRA with the juxtaglomerular granululation index (JGI) (10, 11) at 7, 17, and 50 wk of age and found that JGI did not decrease with age. It is a semi-quantitative index for granulation of the juxtaglomerular cells per glomerulus. Number of glomeruli per gram of tissue may decrease with age.

The submandibular gland of SHRs and Donryu rats contained about 20% of renin in the normal kidney at 50 wk of age, as reported by de Jong et al. (6). Since the renin activity increased with age but did not differ between SHRs and Donryu rats, it was not able to influence the PRA value.

In other rat strains with spontaneous hypertension, PRA values were low: New Zealand strain with genetic hypertension, at 6–28 wk of age (15); Milan hypertensive strain, at 3–7 wk of age (1); and hypertension-prone rats developed from a single Sprague-Dawley line, at 5–13 wk (13). These results agree with those of the present study.

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Part of this study was performed in the Department of Pharmacology, Toho University School of Medicine, Tokyo.

Preliminary reports were given at the 8th and 10th Annual Scientific Meetings of the Council for SHR in Nagasaki, 1972 (24) and Osaka-fu, 1974 (23).

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REFERENCES


12. Imai, M., and H. Sokabe. A method for the determination of

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TABLE 2. Renin activity in submandibular glands in SHR

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Age, wk</th>
<th>No. of Rats</th>
<th>Renin in Submandibu- lar Glands, $10^{-3} K$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donryu</td>
<td>♂</td>
<td>7</td>
<td>10</td>
<td>0.99 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>7</td>
<td>10</td>
<td>1.2 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>17</td>
<td>9</td>
<td>16.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>10</td>
<td>10</td>
<td>10.1 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>50</td>
<td>7</td>
<td>54.4 ± 16.7</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>50</td>
<td>8</td>
<td>37.2 ± 13.3</td>
</tr>
</tbody>
</table>

Values are means ± SE.

samples is critical for PRA in the rat. The samples must be less than 0.5 ml and obtained without anesthesia to minimize renin release. The reported high PRA values in the SHR after light ether anesthesia (23) or decapitation without anesthesia (7) seems to be caused by the extra renin release. In SHRs at 5 wk of age PRA was already suppressed. Since blood pressure tended to rise at this age, PRA may decrease as a compensatory reaction against blood pressure elevation (26). This is explained by the renal arteriolar baroreceptor influence on renin release (5).

There are no data on increased aldosterone secretion (9), increased sodium load at the macula densa site, or decreased sympathetic activity to explain the suppressed PRA in the SHR. If we accept the concept that the SHR is genetically the purer form of essential hypertension in the rat, it may be stated from the present study that essential hypertension becomes "low renin" along with progress of the disease (21).

We used the Donryu strain as the control because of its availability and stability of blood pressure. Judged from the results on PRA (24) and KRA (26), activity of the renin-angiotensin system is similar in the Donryu and Wistar-Kyoto strain, from which SHR had been derived. Plasma renin activity increased from 5 to 20 wk of age in SHR, and from 5 to 10 wk in Donryu rats, and then decreased with age. The meaning of this variation with age is unknown.

Kidney renin activity decreased markedly in SHR