RENIN-ANGIOTENSIN SYSTEM IN HUMAN HYPERTENSION
DUE TO SEGMENTAL RENAL ISCHEMIA*

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Dynamics of renin-angiotensin (RA) system under various conditions was
studied in 8 hypertensive patients due to segmental renal ischemia. The
ischemia in the kidney of these patients was localized lesion, involving only
10–30% of the affected kidney. Increased level of peripheral vein renin
activity (PVRA) at basal condition was found in half of them. Irrespective
of their resting PVRA, they responded to various renin secreting stimuli with
more remarkable enhancement of PVRA than control group. Significantly
higher renal vein renin activity was estimated on the affected side compared
with that of the unaffected. Localized increase in renal renin content was
proven in the ischemic area of the kidney. From these results, it may be
concluded that RA system is etiologically related to the pathogenesis in this
type of hypertension and that careful renin studies are practically useful to
diagnose a small, but functionally significant, ischemic process in the kidney.

It has been well known that the segmental
renal ischemia (SRI) is responsible for
the development of hypertension in animal
and man. Although the pathogenesis in this
type of hypertension has not been fully eluci-
dated, it is a widespread hypothesis that this type
of hypertension might be mediated through a cer-
tain humoral factor liberated from ischemic renal
tissue. In experimental animals with partial in-
farction of the kidney, increased production and
release of renin from the affected kidney were
suggested by some authors, whereas normal or decrease in renin level was reported by
other investigators. Little is known about
the etiological participation of renin-angiotensin
(RA) system in these hypertensive patients with
SRI.

The purpose of this study is to clarify the
contribution of such a small ischemic area of the
kidney to develop hypertension in man, with
special reference to the etiological role played by
the RA system.

**METHOD**

*Plasma Renin Activity (PRA)*

Prior to PRA determination, patients were
maintained on unrestricted diet without any
special medication for at least two weeks. Blood
samples were drawn into heparinized syringe
from antecubital vein of the patients in recumbent
position in the morning after overnight fast.
The effect of salt intake on PRA was examined
under restriction (30–40 mEq/day) for 3 days or
loading (650–750 mEq/day) of salt for 7 days.

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**Key Words:** Renovascular Hypertension
Segmental Renal Ischemia
Renin
Plasma Renin Activity
Renal Renin Content
Furosemide

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Japanese Society of Nephrology, November, 1971, in Tokyo, Japan.*
Peripheral vein renin activity (PVRA) was estimated before and after these treatments. Changes in PVRA caused by an intravenous injection of furosemide (1.0 mg/kg body weight) and maintaining thereafter upright position for two hours were examined. Response of PVRA to intravenous administration of hydralazine (0.3 mg/kg body weight) was also studied. Furosemide test and hydralazine test were performed after breakfast between 9.00 and 12.00 a.m., in order to avoid the diurnal variation in renin secretion. Renal and inferior caval venous blood samples were obtained through the transfemoral retrograde Seldinger catheter under television fluoroscopic control.

Plasma renin activity was estimated by the method previously reported and our normal range of PVRA were 3.0 – 17.0 ng/ml in the basal condition described above, mean ± standard deviation being 7.9 ± 4.7 ng/ml.

Renin Content in Renal Tissue

Cohn's fraction IV-4 of human plasma protein has been known rich in angiotensinogen and suitable to be used as a renin-substrate for the estimation of PRA. To 0.1 ml of 5% solution of fraction IV-4 in physiological saline (42 mg protein/ml) was mixed with 0.06 Goldblatt units of human renin in 1.0 ml of 1/15M sodium phosphate buffer. To this mixture were added 0.15 ml of 8.3% EDTA-2Na in distilled water, 0.03 ml of 5% disopropyl fluorophosphate (DFP) in isopropyl alcohol, 0.3 ml of 1M acetate buffer (pH 5.5), and 1.5 ml of physiological saline. The resulting solution was adjusted to pH 5.5 with 1N HCl, and agitated in an incubator at 37.5°C. After 15, 30, 60, 120 and 180 min. incubation, angiotensin produced was purified with Dowex 50 as described elsewhere and then bioassayed on rat blood pressure. As shown in Fig. 1, angiotensin formation proceeded rapidly, reaching its maximum (8.6 ng angiotensin II/mg protein of fraction IV-4) within 60 min. To detect any endogenous renin activity in this protein fraction, 3.0 ml of 5% solution were incubated and bioassayed in the same way as usual patient's plasma. Angiotensin-like pressor activity was generated at a rate of 0.02 ng angiotensin II/mg protein/hour. This was so weak an activity that it could be safely neglected in the estimation of renin content in renal tissue as described below.

The kidneys removed by surgery were immediately frozen with dry-ice and stored in a refrigerator at -15°C. Prior to the renin determination, freezing and thawing at room temperature were repeated twice. After removal of the capsule, cortical slices including full width of cortex were cut out from both ischemic and non-ischemic areas. Each slice was weighed, minced with a razor and homogenized as completely as possible with cold physiological saline (10 ml/g tissue) in Potter-Elvehjem's homogenizer cooled in an ice-water bath, and then centrifuged at 1,500 g for 15 min. in a refrigerating centrifuge. An aliquot (0.1–0.5 ml) of the supernatant was mixed with 2.0 ml of 5% solution of fraction IV-4, 0.3 ml of 1M acetate buffer (pH 5.5), 0.15 ml of 8.3% EDTA-2Na, and 0.03 ml of 5% DFP. This mixture was adjusted to pH 5.5 with 1N HCl and allowed to stand at room temperature for 30 min. to inhibit angiotensinase. Then it was shaken in a water bath at 37.5°C for 120 min. After the incubation, angiotensin produced was purified with Dowex 50 and bioassayed as described above. To study the time course of angiotensin production, some specimens were checked at 0, 60, 120 and 180 min. of incubation. With an excess of substrate, as used here, angiotensin formation was proved to increase linearly with time, and to be paralleled to the amount of renin present. The recovery rate of 50–100 ng of synthetic Val angiotensin II added at the start of the incubation was 42.0–52.0% (average 47.3%) after 120 min.

**Patients**

The present study consists of 8 patients (7 men and a woman) with segmental renal ische-
Their ages ranged from 12 to 35 with an average of 23. Clinical data of them are summarized in Table I. Seventy six patients with benign essential hypertension served as controls.

Their blood pressure measured on admission ranged from 150/84 to 230/160 mmHg. Urine analysis, serum electrolytes, blood urea nitrogen, phenolsulfonphthalein test and creatinine clearance were normal in all. Intravenous pyelography revealed significant difference between the two kidneys with regard to renal sizes, appearance time of contrast medium or concentration of it in 3 (case 1, 2, 5) out of 8. In case 5, a cylindrical calcification was found at the right kidney, suggesting a calcified intrarenal aneurysm. 131I-Hippuran renography was normal in 6 out of 8. 203Hg-Neohydrin renoscintigraphy showed a suspicious area (uptake slightly decreased ?) in the lower segment of the right kidney in case 2. Split renal function study gave a positive result in only one (case 6, TRFR 0.44) out of 4. The abdominal aortography and selective renal angiography revealed various lesions in intrarenal branches: segmental artery occlusion or stenosis in 2 (case 2, 6), a small intrarenal aneurysm with segmental atrophy of renal mass or localized defect in nephrogram in 4 (case 3, 4, 5, 7) (Fig. 2), and bilateral multiple aneurysms with cortical atrophy in one (case 8). None of them had cardiac decompensation or other complications which might influence the renin secretion.

The affected kidney was removed surgically in 6 patients (case 1, 2, 4, 5, 6, 7). Blood pressure went down gradually to the normal level after the operation in all. The postoperative average diastolic blood pressure was 89, 86, 83 and 75 % of the preoperative level on the 5th, 10th, 20th and 30th day after surgery, respectively (Fig. 3). Normalization of blood pressure was attained by the 20--30th postoperative day. The remaining 2 (case 3, 8) have not been operated, and are being followed up under medical treatment.

**PLASMA RENIN ACTIVITY (PRA)**

PRAs in the patients with SRI are summarized in Table II. PVRA in basal condition ranged 5.0

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Fig.2. Selective renal angiography in case 7. In upper segment of the right kidney, a small aneurysm with localized defect of nephrogram is clearly visualized.

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<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Blood pressure on admission (mmHg)</th>
<th>Optic fundi (KW-grade)</th>
<th>Renal lesions (angiographic &amp; histologic findings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>180/116</td>
<td>III</td>
<td>R. Lower polar infarction after nephrolithotomy</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>M</td>
<td>224/128</td>
<td>III</td>
<td>R. Segmental infarction (Injury in Rugby football game)</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>M</td>
<td>160/84</td>
<td>0</td>
<td>R. Intrarenal aneurysm with segmental cortical atrophy</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>M</td>
<td>158/94</td>
<td>II</td>
<td>L. Intrarenal aneurysm with segmental infarction</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>F</td>
<td>180/120</td>
<td>I</td>
<td>R. Intrarenal calcified aneurysm with segmental infarction</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>M</td>
<td>230/100</td>
<td>0</td>
<td>L. Renal arterial branch stenosis with segmental ischemia</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>M</td>
<td>208/150</td>
<td>III</td>
<td>R. Intrarenal aneurysm with upper polar (apical) ischemia</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>M</td>
<td>230/160</td>
<td>Optic atrophy</td>
<td>Bil. Multiple intrarenal aneurysms with cortical atrophy</td>
</tr>
</tbody>
</table>

R: Right, L: Left, KW-grade: Keith-Wagener's classification, TRFR: Tubular rejection fraction ratio.

Fig.3. Postoperative course of the blood pressure in 5 patients with segmental renal ischemia.

120 ng/ml with an average of 24.2±21.2 (SD) ng/ml. These were significantly higher than those in normal subjects or patients with essential hypertension (P<0.01). Increased PVRA was found in 4 (50%).

Renal vein renin activity (RVRA) on the affected side was higher than on the unaffected. Case 8, who had multiple aneurysms bilaterally, showed remarkably high RVRA on both sides. In case of unilateral SRI, the ratio of RVRA between the two kidneys was 2.22±0.68 (N=6), which was significantly greater than 1.28±0.25 (N=21) in controls (P<0.01).

Remarkable increase in PVRA was observed after i.v. furosemide and upright position in all subjects, even in patients who had normal resting

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### WITH SEGMENTAL RENAL ISCHEMIA

<table>
<thead>
<tr>
<th>Intravenous pyelography</th>
<th>Renography</th>
<th>Renoscintigraphy</th>
<th>Split renal function study (TRFR)</th>
<th>Renal functions</th>
<th>PSP</th>
<th>BUN (15 min.)</th>
<th>Ccr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localized defect in R. nephrogram pattern</td>
<td>R. Ischemic</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>8.0</td>
<td>...</td>
</tr>
<tr>
<td>Size; R&lt;L</td>
<td>Inconclusive</td>
<td>Uptake decreased in R. Lower segment</td>
<td>1.10</td>
<td>13</td>
<td>25</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>...</td>
<td>22</td>
<td>...</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>0.96</td>
<td>12</td>
<td>47</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>R. Cylindrical calcification</td>
<td>Normal</td>
<td>...</td>
<td>0.79</td>
<td>10</td>
<td>...</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>0.44</td>
<td>9.0</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Size; L&gt;R</td>
<td>...</td>
<td>16</td>
<td>31</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

PSP: Phenolsulfonphthalein test, Ccr.: Creatinine clearance

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**Fig. 4.** Effects of various renin secreting stimuli on peripheral vein renin activity in case 7.

**PVRA.** The increase was from 19.9±15.4 to 43.3±16.6 (SD) ng/ml, while those in controls were from 5.2±2.2 to 13.0±7.7 (SD) ng/ml. The net increase above resting level was 30.3±19.9 (SD) ng/ml in SRI, which was significantly greater (P<0.001) than 4.4±4.1 (SD) ng/ml in controls.

Fig. 4 shows the influence of various stimuli on PVRA obtained in case 7. Significant increase or decrease in PVRA was confirmed in response to low or high salt intake, respectively. Exaggerated elevation of PVRA was observed after i.v. hydralazine or furosemide and upright position.

Postoperatively, PVRA returned to normal within 2–3 weeks. The higher responsiveness of PVRA to renin secreting stimuli also became normalized after surgery.

**AFFECTED KIDNEY**

When the removed kidneys were cut, ischemic...
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Site of lesions</th>
<th>Preoperative level (ng/ml plasma)</th>
<th>Postoperative level* (ng/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peripheral venous blood</td>
<td>Renal venous blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basal level</td>
<td>Furosemide &amp; upright position</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1</td>
<td>Right</td>
<td>40</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>Right</td>
<td>18, 26, 48, 74, 16</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Right</td>
<td>5, 11, 6</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Left</td>
<td>8, 10</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>Right</td>
<td>8, 5, 20, 8, 19</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Left</td>
<td>23, 12</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Right</td>
<td>120, 35, 18, 39, 20, 15, 11</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Bilateral</td>
<td>50, 90</td>
<td>...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean + SD</th>
<th>Affected side</th>
<th>Unaffected side</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>24.2 ± 21.2</td>
<td>19.9 ± 15.4</td>
<td>43.3 ± 16.6</td>
<td>(30.3 ± 19.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls (EH)**</th>
<th>Right</th>
<th>Left</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7 ± 5.2</td>
<td>5.2 ± 2.2</td>
<td>13.0 ± 7.7</td>
<td>(4.4 ± 4.1)</td>
</tr>
</tbody>
</table>

** The values were determined on 2–3 postoperative weeks.  
** The number of subjects studied is indicated in parenthesis.
areas appeared pale (case 1, 6, 7) or brownish-red (case 2, 4) in color, and were easily discerned from normal tissue surrounding them. The extents of ischemia were 10–30% of the affected kidney, estimated roughly from the findings of arteriograms or inspection of cut surface of the
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Kidney tissue for enzyme source</th>
<th>Renin concentration (ng Angiotensin II/g tissue/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1:</td>
<td>Ischemic area</td>
<td>3,600</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>2,500</td>
</tr>
<tr>
<td></td>
<td>Adjacent tissue to ischemic area</td>
<td>1,300</td>
</tr>
<tr>
<td></td>
<td>Non-ischemic area</td>
<td>210</td>
</tr>
<tr>
<td>Case 7:</td>
<td>Ischemia area</td>
<td>3,600</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>2,000</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>970</td>
</tr>
<tr>
<td></td>
<td>Adjacent tissue to Ischemic area</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Non-ischemic area</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>84</td>
</tr>
</tbody>
</table>

Renin concentrations in two kidneys (case 1, 7) are shown in Table III and Fig.7. The results were expressed in terms of ng angiotensin II per g renal tissue per hour. The renin content in the ischemic area in case 1 was 2,500 – 3,600 ng, and 970 – 3,600 ng in case 7. These were conspicuously higher than those in non-ischemic areas; 210 ng in case 1 and 84 – 114 ng in case 7. Renin contents in the boundary zone between ischemic and non-ischemic areas were still high in case 1 (1,300 ng), but not elevated in case 7 (118 – 180 ng).

Histologically, the ischemic area was characterized by moderate or severe tubular and glomerular atrophy, tubular dilatation or necrosis, lymphocytic infiltration, and interstitial fibrosis (case 2, 4, 5, 7) (Fig. 5). Small arteries were normal, or their muscle coat was thinner than normal. Non-ischemic tissue showed hypertensive lesions such as thickening of artery wall (interlobar arteries, case 5, 6, 7), fibrinoid degeneration in afferent arterioles (case 7), and focal tubular atrophy with interstitial fibrosis (case 5, 7) (Fig. 6).

3,600 ng/g tissue/hour
2,000
970
180
128
90
90
114
102
84

Fig.7. The cut surface and renin content of the kidney with segmental renal ischemia in case 7.
DISCUSSION

It has long been discussed that a partial or segmental ischemia in the kidney can cause sustained hypertension in man and animals. But opinions are divergent still now in details. Loomis reported that experimental rats from which the kidney tissue was partially removed leaving only 25–50% of the whole organ showed a mild hypertension in 20–45% of animals, while the partial infarction produced in 25–50% of the whole kidney was followed by marked hypertension in almost all animals. However, in dogs or rabbits, it is impossible or difficult to produce the elevation of blood pressure by means of the partial reduction of renal tissue. Unilateral or segmental nephrectomy in human does not result in systemic hypertension. From the present study, it is really proved that a localized ischemia involving only 10–30% of one kidney is enough to cause human hypertension.

Loomis postulated that such hypertensive cardiovascular disease may be a consequence of the effect of vasopressor and vessel necrotizing agents liberated from the ischemic renal tissue into the systemic circulation. Masson and his associates actually found in rats with partial renal infarction that pressor activity in the kidney is invariably greater in the damaged area than in the normal. Koletsky also reported that increased RVRA is transiently demonstrated in rats with microembolic kidney, and that atrophic, but partially functioning tissue next to the necrotic infarct appears to be a possible source of the enhanced output of renin as suggested by several authors. When a partner of the parabiotic rats is rendered hypertensive by the segmental renal infarction, the other one with intact kidneys soon shows significant elevation of blood pressure, indicating a transmissible humoral factor related to this type of hypertension. Transient increase of PVRA or increased renin content in the kidney has also been reported in association with the occurrence of the renal infarction in man. On the other hand, some investigators found no elevation of vasopressor activity in the renal blood of the hypertensive rats with unilateral segmental renal infarction. Sokabe and Grollman also reported in such an experimental rat that both renal content and liberation of renin are reduced throughout the experimental periods.

In the present series, about half of the patients with SRI showed elevated PVRA in the basal condition, and almost all cases had significantly higher RVRA in the affected side of the kidney than the other. These patients responded supernormally to various renin secreting stimuli with remarkable rise in PVRA, even when the resting PVRA was normal. Furthermore, the renal renin concentration in the ischemic area was found 10–32 times higher than that in the non-ischemic. These results suggest the ischemic tissue produces and liberates renin in a pathologic amount. In accord with the previous observations in animal experiments, the present study supports the view that RA system might be etiologically related to the pathogenesis in this type of hypertension. However, in some patients (case 2, 4, 7) in our series the blood pressure response to the operation was slow, its normalization being attained as late as 20–30 days after surgery. But their resting PVRA and its response to ren secreting stimuli returned within 2–3 postoperative weeks. This delayed recovery of blood pressure might indicate that some co-existent mechanism is contributing together with the RA system to the hypertension.

It seems sometimes difficult to make a definitive diagnosis in patients suspected of having small, but functionally significant ischemic lesion in the kidney. The routine clinical examinations such as radioisotope renography, renoscinigraphy, intravenous pyelography, and split renal function study failed in 60–80% of patients studied to detect any abnormality in their kidneys (Table I), as several authors have pointed out previously. Renin studies under various conditions examined here proved more useful for the diagnosis of hypertension due to branch lesions of the renal artery.

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