Active, Inactive and Total Renin Concentrations in Plasma of Hypertensive Patients

TOSHIKAZU GOTO, KEISHI ABE, KAZUO TSUNODA, MASAHIDE SEINO, MINORU YASUJIMA, YUTAKA IMAI, SATORU CHIBA, MAKITO SATO, TOSHIKARI HARUYAMA, KEN OMATA, KO SATO, JIRO TAJIMA, MASAYA TANNO, KEI KUDO and KAORU YOSHINAGA

The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980

To assess the role of inactive renin in hypertensive patients, active, inactive and total renin concentrations (ARC, IRC and TRC) were measured in 37 patients with hypertension of various etiologies. Inactive renin was activated by trypsin and renin concentration was measured using an excess of sheep substrate. Mean values of ARC, IRC, TRC and active renin ratio (AR ratio = ARC/TRC) were higher in 6 cases of renovascular hypertension, and lower in 6 cases of primary aldosteronism and 1 case of idiopathic hyperaldosteronism, when compared with 59 cases of normal subjects. Between ARC and IRC, a slightly positive correlation was observed. Moreover, between ARC and TRC as well as between ARC and AR ratio, close positive correlations were observed. Exceptionally, in a case of juxtaglomerular cell tumor, AR ratio was low in spite of the extremely high value of ARC. Our data suggest that the increase in circulating active renin is due to both the enhancement of the release of renin from the kidney and the increase in the activation of inactive renin, and vice versa.

Human plasma contains two types of renin, active and inactive (Skinner et al. 1975; Sealey et al. 1977 a; Weinberger et al. 1977). The latter can be converted in vitro to active form by acidification (Skinner et al. 1975), low temperature (Osmond et al. 1973; Sealey and Laragh 1975), treatments with trypsin and other proteolytic enzymes (Day et al. 1975; Sealey et al. 1979). Inactive renin has been proposed to be either a proenzyme of normal active renin (Sealey et al. 1977 a; Hsueh et al. 1981), or renin bound by its inhibitor protein (Leckie and McGhee 1980). But the nature of inactive renin is still obscure.
the present study, to assess the physiological role of inactive renin, we measured
inactive renin concentration in the patients with various types of hypertension by
a newly developed assay method (Goto et al. 1984).

MATERIALS AND METHODS

Subjects

Thirty-seven patients (24 men and 13 women aged from 16 to 71 with a mean of 40)
were studied. They consisted of 18 cases of essential hypertension (EH) (12 men and 6
women aged from 16 to 71 with a mean of 40), 6 of renovascular hypertension (RVH) (5 men
and a woman aged from 23 to 64 with a mean of 42), 6 of primary aldosteronism (PA) (3 men
and 3 women aged from 27 to 50 with a mean of 38), one of idiopathic hyperaldosteronism
(IHA) (a woman aged 29 years), 3 of hypertension due to renal parenchymal disease (RPD)
(a man and 2 women aged from 33 to 48 with a mean of 41), 2 of malignant hypertension
(MHT) (2 men aged 19 and 24 years), and one of juxtaglomerular cell tumor (JGT) (a man
aged 71 years).

As a control, 59 normal subjects (34 men and 25 women aged from 18 to 84 with a mean
of 49) were also studied. Details in normal subjects will be reported elsewhere.

Preparation of plasma

Peripheral venous blood sampling was performed from fasted subjects at 8:00 a.m.
after 1 hr of recumbent position. Blood was drawn into a heparinized syringe, and the
plasma was separated immediately by centrifugation at 3,000 rpm for 15 min and stored
at -20°C until the assay.

Activation of inactive renin

Plasma (100 μl) was mixed with 10 μl of trypsin solution (8 mg/ml in 1 mmol HCl,
stored frozen) and incubated for 5 min at 4°C. Thereafter, 10 μl of soyabean trypsin
inhibitor (40 mg/ml) was added to stop the reaction (Goto et al. 1984).

Active renin concentration, total renin concentration, and inactive renin concentration

Active (ARC) and total renin concentrations (TRC) were measured by determining the
rate of angiotensin I (AI) formation when plasma or activated plasma was incubated with
an excess of sheep substrate. Inactive renin concentration (IRC) was defined as the
difference between TRC and ARC. For the enzyme reaction, 100 μl of plasma or 100 μl of
activated plasma was incubated at 37°C for 4 h with 500 μl of a premixed solution consisting
of sheep substrate (0.5 μg AI equivalents dissolved in 400 μl of 100 mmol phosphate buffer,
PH 6.5), EDTA-Na₂ (50 μl of a 250 mmol/1 solution, PH 6.5), and PMSF (50 μl of 200
mmol/1, in ethyl alcohol). Abnormally high renin samples, as the plasmas obtained from
a patient with a renin-producing tumor, were assayed after dilution with a 1% bovine serum
albumin solution. The reaction was terminated by cooling. Plasma proteins were
precipitated by adding 500 μl of 250 g/l polyethylene glycol dissolved in 80% ethyl alcohol
and removed by centrifugation at 5,000 rpm for 15 min. The amount of AI generated and
recovered in the slightly turbid supernatant was estimated by radioimmunoassay (Goto et
al. 1984). Renin concentration was expressed as ng AI/ml/hr. Active renin ratio (AR
ratio) was calculated as follows : ARC/TRC × 100(percent). Values were given as mean ±
S.E. The significance of differences between mean values were evaluated by Student’s
t-test. The level of significance was taken as 0.05.
RESULTS

**ARC, IRC, TRC and AR ratio**

ARC was higher but not significantly in patients with RVH and significantly lower in PA and IHA, when compared with the normal subjects. The values in EH were wide ranged. A patient with JGT showed extremely high ARC (Table 1, Fig. 1).

IRC was also insignificantly higher in RVH, and significantly lower in PA and IHA. The values in EH were widely varied, but the mean value was not different from that of normal subjects. Two patients with MHT showed high values. A patient with JGT showed extremely high IRC (Table 1, Fig. 2).

**Table 1.** Active, inactive, total renin concentration (ARC, IRC, TRC) and active renin ratio (AR ratio) in hypertensive and normal subjects

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>ARC</th>
<th>IRC</th>
<th>TRC</th>
<th>AR ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>(18)</td>
<td>4.2±0.9</td>
<td>22.3±0.3</td>
<td>26.4±3.3</td>
<td>17.2±3.9</td>
</tr>
<tr>
<td>RVH</td>
<td>(6)</td>
<td>24.5±9.7</td>
<td>36.8±5.7</td>
<td>61.2±13.9</td>
<td>35.5±5.9†</td>
</tr>
<tr>
<td>PA &amp; IHA</td>
<td>(7)</td>
<td>0.8±0.3‡</td>
<td>12.8±3.1†</td>
<td>13.6±3.4†</td>
<td>5.4±1.5*</td>
</tr>
<tr>
<td>RPD</td>
<td>(3)</td>
<td>4.5±2.4</td>
<td>22.9±5.8</td>
<td>27.3±8.2</td>
<td>14.2±4.2</td>
</tr>
<tr>
<td>MHT</td>
<td>(2)</td>
<td>5.4 &amp; 6.2</td>
<td>47.8 &amp; 57.7</td>
<td>54.0 &amp; 63.1</td>
<td>8.6 &amp; 11.5</td>
</tr>
<tr>
<td>JGT</td>
<td>(1)</td>
<td>474.6</td>
<td>3616.8</td>
<td>4091.4</td>
<td>11.6</td>
</tr>
<tr>
<td>Normal</td>
<td>(59)</td>
<td>2.6±0.3</td>
<td>24.3±1.3</td>
<td>26.9±1.4</td>
<td>12.5±1.1</td>
</tr>
</tbody>
</table>

n, number of subjects; EH, essential hypertension; PA, primary aldosteronism; IHA, idiopathic hyperaldosteronism; RPD, renal parenchymal disease; MHT, malignant hypertension; JGT, juxtaglomerular cell tumor. Renin concentration and AR ratio is expressed as ngAI/ml/hr and percent, respectively. *p < 0.05, † p < 0.01, ‡ p < 0.001, when compared with the values of normal subjects.

Fig. 1. Active renin concentration in hypertensive and normal subjects. Otherwise, as in Table 1.
As to TRC, the similar tendency with that in IRC was observed. High values in RVH or MHT, and low values in PA and IHA were obtained. The values in EH were also widely ranged (Table 1, Fig. 3).

As to AR ratio, the patients with RVH showed higher values, and those with PA and IHA showed lower values, when compared with normal subjects. The values in EH were widely ranged. In MHT and JGT, AR ratio was low (Table 1, Fig. 4).

Fig. 2. Inactive renin concentration in hypertensive and normal subjects. Otherwise, as in Table 1.

Fig. 3. Total renin concentration in hypertensive and normal subjects. Otherwise, as in Table 1.
Relationship between ARC, IRC, TRC and AR ratio

Between ARC and IRC, there was a slight but statistically significant relationship ($R = 0.42; p < 0.05$) (Fig. 5). Between ARC and TRC, or between ARC and AR ratio, a close positive relationship ($r = 0.80; p < 0.001$, $r = 0.71; p < 0.001$, respectively) was observed (Fig. 6,7). In evaluating a correlation coefficient, a case with JGT was omitted because of the particularity of the disease and the extremely high value in renin concentration.
DISCUSSION

The measurements of plasma inactive renin reported by many authors are widely scattered (Derkx et al. 1976, 1978; Leckie et al. 1977; Sealey et al. 1977 a,b; 1980; 1981; Weinberger et al. 1977; Kappelgaard et al. 1978; Millar et al. 1980). Where does this inconsistency come from? Some authors reported that inactive renin cannot be completely activated by cryoactivation (Linjin et al. 1979; Siegel et al. 1980). In the literature, AR ratio in human plasma is higher with cryoactivation than that with acid activation or trypsin activation. More-
over, when renin content is expressed as ‘activity’, instead of ‘concentration’, that is, when plasma or activated plasma was incubated with no added extra substrate, the substrate may be insufficient for the activated, increased renin. In that instance, TRC and IRC might be underestimated, resulting in falsely high AR ratio. The estimation method used in this study is completely free from these drawbacks as reported in detail elsewhere (Goto et al. 1984). Therefore, the data described above is thought to be reasonable. In the present study, there was a slight relationship between ARC and IRC in various hypertensive patients. However, between ARC and TRC, or between ARC and AR ratio, a close positive relationship was observed. That is, the higher the ARC, the higher the TRC or AR ratio, and the opposite also holds, i.e. the lower the ARC, the lower the TRC or AR ratio. The same kind of results have been reported by other authors (Derkx et al. 1978; Sealey et al. 1980).

The origin of inactive renin in plasma is still obscure. An extrarenal source of plasma inactive renin cannot be entirely ignored, since low to normal IRC are present in the plasma of nephrectomized subjects (Weinberger et al. 1977; Cooper et al. 1977; Leckie et al. 1977; Sealey et al. 1977a, b; Derkx et al. 1978). It seems reasonable, however, to assume that a major proportion of inactive renin in the circulation originates from the kidney. Because, after bilateral nephrectomy, IRC in circulation decreases rapidly, though the speed is slower than ARC (Derkx et al. 1978; Richards et al. 1979).

It is still unknown in what form (active, inactive or both) renin is released from the kidney. Inactive renin may be released from the kidney directly (Derkx et al. 1976; Birkenhäger et al. 1978; Millar et al. 1978; Derkx et al. 1983), or active renin may be converted to inactive form in the circulation (Vandongen et al. 1977; Aoi et al. 1979, 1981). But in any case, it seems reasonable to assume that TRC, i.e. ARC plus IRC, express the total amount of renin released from the kidney into the circulation. Therefore our data show that in the state in which circulating active renin is increased, the release of renin from the kidney and the activation of inactive renin are promoted together, and the opposite also holds. There may be some mechanism which increases the renin secretion and the activation of inactive renin at the same time.

In a case with JGT, AR ratio was low in spite of the extremely high ARC. In JGT, the release of inactive renin from the tumor may be highly increased, or some mechanism which inhibits the activation of inactive renin may be working.

Acknowledgments

We are grateful to Miss Mayumi Nakayama and Miss Keiko Shibukawa for their assistance in performing the experiments. This study was supported by the Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (58570370).
References


36-37, Suppl. I, 10–16.


