Plasma Active and Inactive Renin in Patients with Diabetes Mellitus

SATORU FUJII1, NOBUO SHIMOJO1, MASAHISA WADA1
AND YOHIHIKO FUNAE2

1Second Department of Internal Medicine and
2Laboratory of Chemistry Osaka City
University Medical School, Osaka 545, Japan

Synopsis

Plasma active and acid activated inactive renin was measured in healthy subjects and in patients with diabetes mellitus. The angiotensin I generated from the incubation of non-acidified plasma with pig renin substrate was expressed as plasma renin concentration (PRC) and that from acidified plasma was expressed as total renin concentration (TRC). The inactive renin concentration (IRC) was calculated as TRC minus PRC. With regard to plasma renin activity (PRA) and PRC, no significant difference was found between normal and diabetic groups. TRC and IRC in diabetics with no clinical sign of microangiopathy were 22.8±1.7 and 15.2±1.3 ng/ml/h (mean±SE), and these values were not significantly different from those in the healthy subjects (20.5±1.5 and 13.2±1.5 ng/ml/h). However, TRC and IRC in diabetics with retinopathy and clinical nephropathy was 38.8±3.4 and 30.7±3.2 ng/ml/h, and these values were significantly higher than those in the above two groups, respectively. Moreover, TRC and IRC in diabetics with retinopathy and no clinical nephropathy was 33.8±5.7 and 24.9±5.5 ng/ml/h, and these values were significantly higher than those in the control group. IRC was not significantly correlated with fasting blood sugar and mean blood pressure levels, however, a significant correlation was found between IRC and BUN, and IRC and P.S.P. excretion in 15 minutes.

These findings suggest that increased inactive renin in diabetes mellitus may be related to the progression of the renal lesions associated with diabetic microangiopathy.

Since renin was demonstrated to exist in active and inactive forms in kidney extracts of pigs (Boyd, 1974) and rabbits (Leckie, 1973; Leckie and McConnell, 1975), the inactive form of renin, which could be activated by acidification, was investigated in human plasma. Skinner et al. (1975) and Leckie and McConnell (1975) reported the presence of a similar inactive form of renin in normal human plasma. Moreover, Day et al. (1975) described an inactive form of renin in the plasma of patients with Wilms’ tumor, and in diabetic patients and hypertensive patients with proteinuria. On the other hand, the altered function of the renin-angiotensin system has been reported in patients with diabetes mellitus, especially in those with microvascular and/or electrolyte complications (Christlieb, 1976), however, the pathophysiological roles of those inactive renin in this system have not been elucidated.

The present studies were performed to assay plasma active and inactive renin in patients with diabetes mellitus and to observe whether or not the amount varied in plasma from patients with no complications and those with microangiopathy.

Received May 31, 1979.
Materials and Methods

Patients

Then healthy Japanese subjects (aged 24 to 75 years) and 34 patients with diabetes mellitus (aged 33 to 70 years) from the outpatient clinic at Osaka City University Hospital were studied. The 34 diabetics were separated into three groups; fourteen diabetics with no diabetic retinopathy and no proteinuria (Group I), ten with retinopathy (Scott I-IIb) and proteinuria less than 30 mg/dl (Group II), and ten with retinopathy (Scott IIb-V) and clinical nephropathy as defined by persistent proteinuria at least 300 mg/dl (Group III). Each subject studied was on a random sodium intake while off all antihypertensive medication for at least one week, and plasma electrolytes, 24 hour urinary electrolytes and creatinine on the day before the study were measured so that renin concentrations could be related to sodium status.

Activation method of inactive renin

After an overnight fast, each subject rested supine for at least one hour. Blood was drawn into iced EDTA tubes, centrifuged at 4°C, 2000 rpm, 20 min, and the plasma was aspirated. Activation of inactive renin was performed by the following procedures described by Weinberger et al. (1977); 0.5 ml of plasma was dialyzed for 24 hr at 4°C against 0.05 M glycine-HCl buffer, pH 3.3, and then each sample was dialyzed against 0.05 M sodium phosphate buffer, containing 0.1 M NaCl, pH 7.2, for an additional 24 hr. Plasma renin activity (PRA) was measured by radioimmunoassay using a commercial kit (CERE-SORIN, Italy). 2, 3-Dimercapto propanol (BAL) and 8-hydroxyquinoline were added to all studies as angiotensinase inhibitors. Plasma renin concentration (PRC) was measured by adding 0.2 ml of plasma sample which was dialyzed against sodium phosphate buffer, pH 7.2, at 4°C for 48 hr, to pig renin substrate (Pentex) (1400 ng angiotension I equivalent per ml of incubation medium) and incubating at 37°C for two hours. Total renin concentration (TRC) was measured by adding a sample of acidified plasma to renin substrate and incubating as described for PRC. The amount of angiotensin I thus generated in each plasma sample was quantified by radioimmunoassay and corrected by using substrate blanks, and expressed as ng/ml per hour.

The angiotensin I generated from the incubation of nonacidified plasma with substrate was expressed as PRC, which reflects active renin; that from acidified plasma was expressed as TRC, which includes both active and inactive forms of renin. The inactive renin (IR) ratio was calculated as follows; IR ratio = (TRC - PRC)/TRC. Student's t-test was used to evaluate the statistical significance.

Results and Discussion

Table 1 shows the summary of clinical features in patients with diabetes mellitus. Group III diabetics showed relatively hypoproteinemia (6.5 ± 0.2 g/dl) and moderate azotemia (BUN: 43.2 ± 6.5 mg/dl). Fig. 1 shows the mean values for PRA, PRC, TRC, IRC and IR ratio in ten healthy subjects and 34 patients with diabetes mellitus. In healthy subjects PRA, PRC, TRC and IRC were 1.9 ± 0.4 (mean ± S.E.), 7.3 ± 0.4, 20.5 ± 1.5 and 13.2 ± 1.5 ng/ml/h, respectively. Thus approximately two thirds of the total renin in plasma is normally inactive. These results are quite similar to those reported by Skinner et al. (1975) and Weinberger et al. (1977). PRA and PRC level was not significantly different between the healthy and diabetic groups. The mean values of TRC, IRC and IR ratio were 22.8 ± 1.7 ng/ml/h, 15.2 ± 1.3 ng/ml/h and 0.6 ± 0.1 ng/ml/h in Group I diabetics with no clinical sign of microangiopathy, and these values were not significantly different from those in the healthy group. Mean TRC and IRC were 38.8 ± 3.4 ng/ml/h and 30.7 ± 3.2 ng/ml/h in diabetics with retinopathy and clinical nephropathy, as shown in Fig. 1. These values were significantly higher than those observed in Group I diabetic patients (p < 0.01, respectively). Day et al. (1975) demonstrated that plasma from diabetic subjects with nephropathy contained inactive "big" renin. While, about three quarters of plasma total renin concentration, in the present study, was inactive in diabetics with clinical nephropathy.

TRC and IRC in the Group II diabetics were 33.8 ± 5.7 and 24.9 ± 5.5 ng/ml/h, and these values were significantly higher than those in the control group, respectively (p < 0.05). It has been well known that the glomerulosclerotic change is often complicated with diabetic retinopathy even, no proteinuria. Group II diabetic patients with
Table 1. Clinical features in healthy subjects and patients with diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>38.9±3.8</td>
<td>50.0±3.2</td>
<td>62.0±3.5</td>
<td>58.1±2.7</td>
</tr>
<tr>
<td>Duration (Years)</td>
<td>4.7±1.2</td>
<td>12.2±2.0</td>
<td>18.7±1.9</td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>82.6±4.5</td>
<td>174.8±187.6</td>
<td>180.7±133.3</td>
<td>185.2±17.5</td>
</tr>
<tr>
<td>Mean B. P. (mmHg)</td>
<td>106.8±6.0</td>
<td>102.4±3.2</td>
<td>119.7±5.5</td>
<td>126.7±3.7</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>15.8±1.0</td>
<td>16.6±1.3</td>
<td>20.9±2.6</td>
<td>43.2±6.5</td>
</tr>
<tr>
<td>P. S. P. in min. (%)</td>
<td>38.2±2.1</td>
<td>35.0±2.5</td>
<td>27.7±3.6</td>
<td>6.2±1.4</td>
</tr>
<tr>
<td>Serum Protein (g/dl)</td>
<td>7.4±0.2</td>
<td>7.3±0.2</td>
<td>7.5±0.3</td>
<td>6.5±0.2</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.

retinopathy and a relatively long course of illness (12.2±2.0 years) had almost the same levels of mean blood pressure, as were found in Group III, as shown in Table 1.

IRC was not significantly correlated with fasting blood sugar (r=0.29) and mean blood pressure levels (r=0.29), however, a significant correlation between IRC and BUN, and between IRC and P.S.P. excretion in fifteen minutes was observed in the 34 diabetics (r=0.62, p<0.005, and r= -0.60, p<0.01, respectively). These data suggest that increased inactive renin may be related to the progression of lesions in the juxtaglomerular apparatus. However, further studies on active and inactive renin in kidney tissue from diabetic patients are required.

On the other hand, hypoproteinemia and/or increased concentration of serum α1 and α2-globulin was found in most diabetics with relatively high IRC values. The altered serum protein metabolism, which is often found in diabetic patients with microvascular complication (Cleve, 1968), may thus
be related to the increased inactive renin in those patients. Although the mechanisms of conversion of inactive to active renin in vivo remain to be determined, activation by endogenous neutral protease including kal-likrein is of particular interest (Atlas et al., 1978; Sealey et al., 1978; Osmond et al., 1978).

Markedly low PRA associated with high IRC was observed in some diabetics in Group III, in the present study. Christlieb (1973) reported that long-term diabetics with microvascular complication no longer respond to stimuli normally sufficient for renin secretion, and suggested that if “big” renin (intrinsic enzymatic activity is low) replaced normal, active renin in plasma, those patients might reveal a low plasma renin activity. Recently, Day et al. (1975) found that the inactive form of renin in the plasma of proteinuric patients, including those with diabetic nephropathy had a molecular weight of 60,000 and that the renin could be activated by acidification without conversion to the native lower-molecular weight renin, and could not be identified in normal human plasma. On the other hand Body (1977) reported that inactive renin which could be converted to an enzymologically active form by acidification to pH 3.3 by dialysis was found in normal subjects and in patients with essential hypertension, and that the inactive renin had a molecular weight 43,000. In the present study, the inactive form of renin was found in both healthy subjects and diabetic patients. However, the results of our studies do not clarify the mechanism of acid activation and molecular weight definition, since the present study was designed to examine only the biological activity (angiotensin I generation) of human plasma before and after acid activation in normals and diabetics. Further investigation is necessary to elucidate the relation between these findings and the previous reports of inactive renin.

Acknowledgement

We wish to thank Dr. Kenjiro Yamamoto, Prof. of pharmacology, for his valuable advice. We also wish to thank M. Ohara, Kyoto University for assistance with the manuscript.

References