HIGH LEVEL OF PLASMA INACTIVE RENIN IN BARTTER’S SYNDROME

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In 10 cases of Bartter’s syndrome, plasma active and inactive renin (AR and IR) were measured by two different methods. First, plasma renin activity (PRA), and total renin activity (TRA) after activating IR with trypsin, were measured by radioimmunoassay (RIA) of angiotensin I (A1) generated from endogenous substrate. And secondly, plasma active renin concentration (ARC) and total renin concentration (TRC) were measured by RIA of A1 generated in the presence of an excess of exogenous substrate. The difference between TRA and PRA, and between TRC and ARC were designated as inactive renin activity (IRA) and inactive renin concentration (IRC), respectively. Small amounts of IRA were found only in 2 cases and no IRA in 8 cases. However, the existence of large amounts of IR in Bartter’s syndrome was revealed by the IRC determination. This suggests that the shortage of endogenous renin substrate, consumed by the markedly increased AR, may have interfered with the detection of IRA in Bartter’s syndrome, though the IR is markedly increased as well. The molecular weights of AR and IR were determined by Sephadex G-100 gel filtration in 3 cases. Both AR and IR seemed to be smaller than those of normal subjects.

In recent years, the presence of an inactive form of renin has been found in human plasma.\(^1\) It was demonstrated by in vitro activation by acidification, cold exposure or exposure to exogenous proteolytic enzymes\(^2\)–\(^5\) However, despite numerous experimental and clinical studies, the physiological role of inactive renin is still controversial.

Bartter’s syndrome represents a clinical category characterized by hyperreninemia, hyperaldosteronism with hypokalemic metabolic alkalosis, hyperplasia of juxtaglomerular cells and diminished blood pressure response to infused exogenous angiotensin II etc.\(^6\)–\(^8\) Chan et al reported some cases of this syndrome in which the existence of inactive renin could not be found.\(^9\)

In this study we demonstrate the existence of massive inactive renin and a multiplicity of molecular weights of active and inactive renins in Bartter’s syndrome.

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Key Words:
Active renin
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Molecular weight
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Renin Activity & Concentration in Bartter’s Syndrome

![Graph showing plasma renin activity (PRA) and inactive renin activity (IRA) in Bartter’s syndrome.](image)

Fig.1. Plasma renin activity (PRA) and inactive renin activity (IRA) in Bartter’s syndrome is shown in the upper panel. In 8 cases IRA was not detected (*). Active renin concentration (ARC) and inactive renin concentration (IRC) of this syndrome were shown in the lower panel. In all cases, inactive renin was detected massively.

MATERIALS AND METHODS

Materials

The patients studied had been clearly diagnosed as Bartter’s syndrome from their history, laboratory data (serum potassium level, plasma renin activity, etc.), angiotensin II infusion test and renal biopsy. Their ages varied from 6 to 53 (24.1 ± 13.3) (mean ± standard deviation) including 6 males and 4 females. They were off-medicated for at least 4 weeks before this study. Venous blood samples were taken after 1-hour supine resting position, then 1 mg/ml disodium ethylenediaminetetraacetate (EDTA-Na₂) was added to each blood sample. Plasma samples were stored at −80°C until analysed.

Gel Filtration

We used Sephadex G-100 packed in a column 95 cm long with an inner diameter of 1.6 cm, and 50 mmol/L pyrophosphate buffer (pH 7.5). One ml of plasma was applied on the column and the flow rate was 5 ml/h obtaining every one ml of effluent. The column was calibrated with blue dextran (void volume), bovine serum albumin (BSA, MW 67000), ovalbumin (OVA, MW 43000) and chymotrypsinogen A (MW 25000).

Activation of Inactive Renin

Trypsin (Sigma, Type III) (1.5 mg/ml) and benzamidine (5 mmol/L) were added to plasma samples, and the mixture was incubated at −4°C for 1 hour. Then the reaction was stopped by soy bean trypsin inhibitor (Sigma) (1.5 mg/ml). Each fraction of eluate from the column was treated with trypsin (0.5 mg/ml) at 25°C for one minute without benzamidine and the reaction was stopped by soy bean trypsin inhibitor (0.5 mg/ml).

Measurements of Renin Activity and Concentration

For the measurement of plasma renin activity, 0.05 ml of tris-HCl (2 mol/L, pH 7.4) buffer solution, 0.7 mg of 8-hydroxyquinoline (8-HQ) and 0.1 mg of dimercaprol (BAL) were added to 0.5 ml of a plasma sample. Plasma renin activity was measured by radioimmunoassay of angiotensin I generated by the reaction of plasma renin with endogenous substrate during one hour of incubation at 37°C. The activity after the activation of inactive renin was referred to as total renin activity. The difference between total renin activity and plasma renin activity was designated as inactive renin activity.

For the measurement of active renin concentration, we used a modification of Skinner’s

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**TABLE I MOLECULAR WEIGHT OF ACTIVE AND INACTIVE RENIN**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MW of AR</th>
<th>MW of IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>41000</td>
<td>47000</td>
</tr>
<tr>
<td></td>
<td>45000*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>42000</td>
<td>49000</td>
</tr>
<tr>
<td></td>
<td>45000*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45000*</td>
<td>52000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48000</td>
</tr>
</tbody>
</table>

*This is the most dominant peak in elution profile of each case.

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*Abbreviations: MW = Molecular Weight; AR = Active Renin; IR = Inactive Renin.*
Fig. 2. Renin concentration in eluates of case 2 from Sephadex G-100. The molecular weight of active renin is 41000 and 45000 daltons and that of inactive renin is about 47000 daltons. Closed circles: active renin in untreated eluates. Open circles: renin in eluates incubated with trypsin. BSA and OVA: optical density peaks of bovine serum albumin and ovalbumin.

Method 0.1 ml of plasma was incubated with 0.02 ml of tris-HCl (2 mol/L, pH 7.4), 3 mmol of diisopropylfluorophosphate (DFP) and 0.1 ml of excess sheep renin substrate (equivalent to approx. 2500 ngAI/ml) at 37°C for 20 minutes. Active renin concentration was determined by radioimmunoassay of angiotensin I generated. The total renin concentration was measured after the activation of inactive renin. The difference between the total renin concentration and the active renin concentration was designated as inactive renin concentration.

The renin concentration in the eluates from the Sephadex G-100 column was assayed before and after the activation of inactive renin. To 0.1 ml of each fraction, we added 10 mmol of EDTA-Na₂, 3 mmol of DFP, 0.1 mg of cefalexin sodium, and 0.1 ml of excess sheep substrate. The mixture was incubated at 37°C for 2 hours. Generated angiotensin I was also measured by radioimmunoassay. The difference between the values before and after the activation was defined as the active renin concentrations in each fraction.

We used a commercial kit (CIS SORIN) for the radioimmunoassay of angiotensin I, and the activity and concentration were expressed in terms of nanograms of angiotensin I liberated by one ml of plasma or eluates per hour.

RESULTS

Plasma renin activity and inactive renin activity in the patients with Bartter's syndrome were shown in Fig. 1 (upper panel). Plasma renin activity varied from 11.3 to 126.1 ngAI/ml/h (37.5 ± 35.2). In 8 cases, renin activity did not increase after trypsin treatment, so that inactive renin was not detected. In the other 2 cases, a small increase in the activity was detected after trypsin exposure.

Active and inactive renin concentrations were shown in Fig. 1 (lower panel). Large amounts of inactive renin (from 0.5 to 10 times of active renin) were found. Active and inactive renin concentration varied from 48.2 to 1200.7 ngAI/ml/h (286.6 ± 349.8) and from 116.5 to 1966.6 ngAI/ml/h (702.1 ± 649.7), respectively. There was no significant correlation between active and inactive renin concentration (r = 0.27).

In 7 normal subjects, for a comparison, active and inactive renin concentration in ambulant state varied from 4.0 to 16.9 ngAI/ml/h (8.1 ± 4.9) and from 33.3 to 93.0 ngAI/ml/h (57.3 ± 22.2), respectively.

The molecular weights of active and inactive renin estimated in 3 individual plasmas from the patients are summarized in Table 1. The elution profiles showed the peaks between about 41000
to 54000 daltons. An example of elution profiles is shown in Fig. 2.

DISCUSSION

A previous report described that very high plasma renin activity was associated with an essential absence of inactive renin and a reciprocal relationship was found between plasma inactive and active renin levels in Bartter's syndrome. We obtained similar results in the present study on active and inactive renin activities without an addition of exogenous substrate. However, the existence of large amount of inactive renin was revealed in the presence of an excess of exogenous sheep substrate. Active renin concentration and inactive renin concentration showed a tendency of positive correlation instead of reciprocal relation.

As for the reason why the inactive renin could not be detected without exogenous substrate, there is a possibility that the endogenous renin substrate was so low in this syndrome due to markedly accelerated consumption that the reaction could not proceed further in spite of the activation of inactive renin. Another possibility is that the renin substrate was destroyed further by the 1-hour trypsin treatment.

The present study demonstrated that not only active renin but also inactive renin is massively released in Bartter's syndrome in which the renin-angiotensin system is fully accelerated. The simultaneous increase in inactive renin level has also been shown when the active renin secretion is accelerated chronically with prolonged diuretic therapy, long term hemodialysis treatment, long term captopril administration, or sodium depletion.

In this study, the presence of various sizes of active and inactive renin was found in the plasma of patients with Bartter's syndrome, and these renins seem to be smaller than those of normal subjects. The occurrence of various sizes of active and inactive renin in human plasma and urine has already been reported from our laboratory. In patients with essential hypertension, the small sizes of active and inactive renin were predominant in the high renin group, and one patient had two sizes of active renin. It is possible that small and various sizes of active and inactive renins appear in the blood stream when the renin-angiotensin system is strongly accelerated.

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