Cryoactivation of plasma renin


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Summary

1. The mechanism of increased renin activity after human plasma had been kept at -5°C for 4 days (cryoactivation) was investigated.

2. The increase in renin activity of human plasma by cryoactivation was closely correlated to the increase obtained by incubation with trypsin ($r = 0.88, P < 0.001, n = 10$).

3. An inhibitor of thiol enzyme, N-ethylmaleimide did not inhibit cryoactivation.

4. Soyabean trypsin inhibitor and di-isopropylfluorophosphate (DFP) inhibited cryoactivation, suggesting that the cryoactivation may be due to the action of a trypsin-like serine enzyme.

5. In an experiment in the rat haemorrhagic shock caused parallel increments of renin activity in non-cryoactivated and cryoactivated plasma, the renin activity being about two times higher in the latter. No significant differences were found in the concentrations of renin and renin substrate between the non-cryoactivated and cryoactivated plasma samples.

6. The results may indicate that a destruction of an inhibitor of the renin-renin substrate reaction is responsible for the increase of renin activity after exposure of rat plasma to low temperature. A trypsin-like enzyme in plasma might have destroyed the inhibitor during this procedure.

Key words: cryoactivation, protease inhibitors, renin, trypsin.

Abbreviations: DFP; di-isopropylfluorophosphate; PRA, plasma renin activity; TRA, total renin activity.

Introduction

Cryoactivation of plasma renin (E.C. 3.4.99.19) was reported by Osmond, Ross & Scaiff (1973) and Sealey & Laragh (1975). The latter group attributed it to the presence of a precursor of renin, which they originally thought could be activated during frozen storage; later, however, they recognized that activation occurred in the temperature range -5°C to +5°C (Sealey, Moon & Laragh, 1976). In this study the effects of various enzyme inhibitors and trypsin on cryoactivation were investigated to obtain more information on the mechanism of cryoactivation.

Materials and methods

Blood samples were taken into tubes containing 1 mg of disodium EDTA/1 ml of blood. The plasma was stored at -20°C. A portion (0-1 ml) of Tris/HCl (1 mol/l, pH 7-4) solution was added to 1 ml of plasma. Plasma renin activity (PRA) was determined in terms of the rate of angiotensin I generation/h of incubation at 37°C. The angiotensin I was measured by radioimmunoassay. For cryoactivation plasma was kept in a cold (-5°C) bath for 4 days (Sealey et al., 1976). Total renin activity (TRA) was measured after the cryoactivation of the plasma.

Two batches of pooled plasma, from hypertensive patients with normal renin activity (NR patients) and from hypertensive patients with high renin activity (HR patients), were used.

Effects of trypsin and enzyme inhibitors

To 1 ml of plasma, 0-1 ml of trypsin (T-8253, type III, Sigma; 10 mg/ml) was added and incubated at 25°C for 5 min. Then 0-1 ml of
soyabean trypsin inhibitor (T-9003, type I-S, Sigma; 10 mg/ml) was added before the determination of renin activity. Cryoactivated plasma was treated in the same way.

Before the cryoactivation, N-ethylmaleimide (final concentration 10 mmol/l), soyabean trypsin inhibitor (final concentration 1 mg/ml) or diisopropyfluorophosphate (5 μl of 5 g/100 ml solution in isopropyl alcohol in 1 ml of plasma) was added to the plasma.

**Acute haemorrhagic shock in the rat**

A 12 months old female rat spontaneously hypertensive Okamoto rat, weighing 260 g, was used. A fine polyethylene tube was inserted into the aorta under anaesthesia 2 days before the experiment (Matsunaga, Komuro, Yamamoto, Hara, Ogino, Yamori & Okamoto, 1974). On the day of experiment, eight samples (1.5 ml each) were taken from the catheter at 7 min intervals, without anaesthesia. Each sample was divided into two parts. One part was frozen immediately. The other part was cryoactivated.

Renin concentration was measured by the method of Matsunaga, Yamamoto, Hara, Ogino & Okamoto (1975), and the renin substrate was measured by converting all of the substrate in 50 μl of plasma into angiotensin I according to the method previously reported (Matsunaga, Kira, Yamamoto & Ogino, 1974).

**Results**

**Effects of trypsin and enzyme inhibitors**

As shown in Table 1, trypsin activated PRA of both NR and HR plasma to almost the same values as those found after the cold incubation. However, trypsin induced only slight activation of renin activity in plasma which had already been kept at −5°C for 4 days, suggesting a common mechanism in trypsin-induced activation and in cryoactivation. The increase in renin activity by cryoactivation was closely correlated to the increase obtained by incubation with trypsin (r = 0.88, P < 0.001). N-Ethylmaleimide which has been used to inhibit a conversion of big renin into small active renin (Inagami, Hirose, Murakami & Matoba, 1977), did not inhibit cryoactivation. On the other hand, renin activities obtained after cold incubation with soyabean trypsin inhibitor or DFP were 75–89% lower than those obtained after cold incubation without any inhibitor. N-Ethylmaleimide and soyabean trypsin inhibitor did not affect the determination of renin activity.

**Acute haemorrhagic shock in the rat**

Blood samples taken at 7 min intervals, showed a gradual rise in renin activities at first and a relatively rapid rise in two samples at the end of this experiment in both non-cryoactivated (PRA) and cryoactivated (TRA) plasma. The ratios of TRA to PRA were almost constantly (1.7–1.9) throughout the experiment. There were no significant differences in plasma renin concentrations and in renin substrate concentrations between non-cryoactivated plasma and cryoactivated plasma.

**Discussion**

The results suggest a similar mechanism in the activation by trypsin and low temperature. A trypsin-like enzyme is likely to be involved in both cases. On the other hand, the activation of plasma renin is likely to be different from the activation of so-called big renin in kidney extracts, since N-ethylmaleimide, which protects the activation of big renin, had no effect on cryoactivation of plasma renin.

In the rat, exposure of plasma to low temperature changed neither plasma renin concentration nor the amount of renin substrate, whereas renin activity was elevated. This indicates that cryoactivation is not due to a conversion of renin precursor, prorenin, into an active form. An alternative explanation for this mechanism is a destruction of an inhibitor of the renin–renin sub-

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**Table 1. Effects of trypsin and enzyme inhibitors on cryoactivation**

<table>
<thead>
<tr>
<th></th>
<th>NR</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity</td>
<td>1.54 ± 0.18</td>
<td>6.05 ± 0.10</td>
</tr>
<tr>
<td>Total renin activity</td>
<td>7.45 ± 0.35</td>
<td>12.14 ± 0.50</td>
</tr>
<tr>
<td>Trypsinized</td>
<td>6.41 ± 0.05</td>
<td>11.95 ± 0.55</td>
</tr>
<tr>
<td>Trypsinized after CRA</td>
<td>8.22 ± 0.10</td>
<td>12.28 ± 0.10</td>
</tr>
<tr>
<td>CRA with NEM</td>
<td>8.36 ± 0.55</td>
<td>13.11 ± 0.10</td>
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<tr>
<td>CRA with SBTI</td>
<td>2.85 ± 0.06</td>
<td>6.73 ± 0.11</td>
</tr>
<tr>
<td>CRA with DFP</td>
<td>3.10 ± 0.01</td>
<td>7.57 ± 0.12</td>
</tr>
</tbody>
</table>

Mean values ± SEM are shown (n = 4)
substrate reaction, or, less likely, a production of an activator, by the action of a trypsin-like enzyme. The constant ratio of TRA to PRA during haemorrhagic shock in the rat would also support this view.

References


