Angiotensinase Activity Using Human Crude Angiotensin

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Angiotensinase is believed to consist of several enzymes which destroy angiotensin. Although angiotensinase has not been identified, angiotensinase activity has been demonstrated in number of tissues including plasma, red blood cell, kidney, liver, pancreas, adrenal gland and intestine. There are many different opinions concerning angiotensinase activities in several diseases. For instance, Hickler et al. reported that elevated plasma angiotensinase activities in patients with renovascular hypertension, advanced essential hypertension, hypertension related to advanced chronic glomerulonephritis and pheochromocytoma. In contrast, Itskovitz et al. reported that no significant differences were found between the normotensives and hypertensive groups at any level of blood pressure. One of the important causes of these conflicting results will be attributed to the techniques commonly used at present, which are based on the in vitro degradation of the pressor activity of synthetic valine-5 angiotensin II amide (Hypertensin, Ciba) by plasma, using bioassay by rats. In addition, homogeneity of angiotensin-angiotensinase reaction and angiotensin concentration to be used come into question. Now that human pure angiotensin has been made, it is necessary to measure angiotensinase activity by the method using this human pure angiotensin to elucidate the angiotensin-angiotensinase reaction more precisely.

But, as it is difficult to obtain a large amount of human pure angiotensin, we describe herein the results which were measured by the method using human crude angiotensin and discuss the difference from results based on the degradation of synthetic valine-5 angiotensin II amide.

Methods and Materials

Human crude angiotensin was made by the method of Brown et al. with minor modification. Namely, renin substrate which was eluted from human serum, and human renin which was extracted from human kidneys were incubated at 37.0°C for 24 hours. During this term renin substrate was completely converted to angiotensin. Thus this yielded human crude angiotensin was adjusted to the pressor effect of 0.1 μg/cc of synthetic angiotensin.

We measured angiotensinase activity by the method of Hickler et al. using this human crude angiotensin and synthetic angiotensin. On this occasion, incubation time with serum was 15 minutes for synthetic angiotensin and 120 minutes for human crude angiotensin respectively, on considering biological half-life of two kinds of angiotensin.

Serum specimens for simultaneous analyses of angiotensinase activities were obtained from following subjects: normal 3, primary aldosteronism 2, renovascular hypertension 2, glomerulonephritis 3 essential hypertension in adults 5, hypertension with unknown cause in younger adults 10, liver cirrhosis with ascites 3 and fulminant hepatitis 1.

In the following, tissue angiotensinase activity was measured by the same method.

Tissue specimens were obtained from a kidney and a liver from 33 year-old healthy man who died shortly after sustaining head trauma. Sections of renal cortex, papilla, medulla and liver were homogenized in physiological saline solution (0.4 ml per

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Degradation of Synthetic Angiotensin

![Graph showing degradation of synthetic angiotensin over time.]

Fig. 1. Progressive degradation of pressor activity of angiotensin II by serum.

mg of renal and liver tissue) respectively. The homogenates were centrifuged at 3000 rpm for 15 minutes, after which the supernatant containing angiotensinase was separated and kept in ice until analysis.

RESULTS

Fig. 1 shows progressive degradation of synthetic angiotensin and human crude angiotensin by serum. The reduction in pressor activity after incubating synthetic angiotensin with serum for 15 minutes and 30 minutes was approximately 40 per cent and 70 per cent respectively.

Unlike synthetic angiotensin, the reduction in pressor activity of human crude angiotensin was markedly delayed. After 120 minutes it was about 40 per cent and after 6 hours barely it reached to 80 per cent.

Fig. 2 and 3 show simultaneous analyses of serum angiotensinase activities using synthetic angiotensin and human crude angiotensin in normal subjects and various groups of patients.

In every group, significant correlations were demonstrated between results using synthetic angiotensin and results using human crude angiotensin. For instance, serum angiotensinase activities in primary aldosteronism were suppressed on both occasions. On the other hand, liver cirrhosis with ascites and fulminant hepatitis showed increased activities.

Fig. 4 shows angiotensinase activities in the human renal cortex, medulla, papilla and liver by the methods using degradation of synthetic angiotensin and human crude angiotensin respectively.

Both methods showed approximately similar results, that is, angiotensinase activity was four to five times greater in the renal cortex than in the medulla and papilla.

Fig. 2. Serum angiotensinase activities in healthy subjects and various groups of patients (by the method using synthetic angiotensin, 'Hypertensin Ciba')

<table>
<thead>
<tr>
<th>Destruction Rate of Angiotensin</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Normal Subjects</td>
<td>50</td>
</tr>
<tr>
<td>Primary Aldosteronism</td>
<td>40.2</td>
</tr>
<tr>
<td>Renovascular Hypertension</td>
<td>28.5</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>46.1</td>
</tr>
<tr>
<td>Essential Hypertension in Adults</td>
<td>38.2</td>
</tr>
<tr>
<td>Hypertension with Unknown Cause in Younger Adults</td>
<td>47.8</td>
</tr>
<tr>
<td>Liver Cirrhosis and Fulminant Hepatitis(*)</td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td>61.2</td>
</tr>
</tbody>
</table>

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### Discussion

It is unknown how this yielded human crude angiotensin is in conformity with human pure angiotensin yielded by Arakawa.

Comparing with synthetic angiotensin, both are heat-stable and soluble in the 95 per cent ethanol. But there is a significant difference between the velocity of the destruction of synthetic angiotensin and human crude angiotensin.

Ueda et al. also reported delayed destruction of human crude angiotensin by a method resembling the one we used.

It was reported that human pure angiotensin yielded by Arakawa was more easily destroyed by human plasma than our human crude angiotensin. Therefore, one of the causes of this delayed destruction of our human crude angiotensin may be attributed to some substances mixed in it, which may govern angiotensin-angiotensinase reaction. And then, purification of this human crude angiotensin has a possibility to accelerate its destruction. As serum and tissue angiotensinase activities using human crude angiotensin showed same tendency with the results using synthetic angiotensin, it is inferred that even though we use more purified human angiotensin the results show same tendency. Thus, when we measure angiotensinase activity, any of them is recommended.

Above mentioned, as human crude angiotensin is destroyed more slowly than synthetic angiotensin, it is pertinent to find definite difference of angiotensinase activity and to avoid unspecific destruction. Apart from measurement of serum angiotensinase activity, it is unknown whether the difference of the velocity of destruction of synthetic angiotensin and human angiotensin plays a role in the generation of renal hypertension or not. To make clear this problem, it will be necessary to observe the in vivo destruction of human angiotensin.

### Conclusion

1. We measured serum and tissue angio-
tensinase activities by the method of Hickler et al. using human crude angiotensin and synthetic angiotensin.

2. Human crude angiotensin was destroyed by serum more slowly than synthetic angiotensin.

3. Angiotensinase activities measured by the method using human crude angiotensin showed same tendency with the results using synthetic angiotensin.

REFERENCES


