Plasma Angiotensin I Converting Enzyme Activity in Rabbits under Various Experimental Conditions*

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Recent findings reported by Ng and Vane\(^1\) show that the conversion of circulating angiotensin I to angiotensin II is carried out rapidly during the passage through the lung and not by an enzyme in the blood. The relation between the so-called “converting enzyme”, which was first found in plasma by Skeggs et al\(^2\) and the enzyme in the lung, which participates in the conversion of angiotensin I to II in vivo, is still discussed. An \textit{in vitro} method, which can measure plasma converting enzyme activity (PCEA) was developed by Boucher et al\(^3\) This method is simple and reproducible and has now applied on rabbits. At the same time, plasma renin activity (PRA) and plasma renin substrate (PRS) were measured in rabbits by Boucher’s \textit{micro-method}\(^4\), which was originally described for rat plasma. In order to clarify whether there is any relation between PCEA and other factors involved in the renin-angiotensin system in rabbits, PCEA, PRA and PRS were measured \textit{in vitro} in normal rabbits, in bilaterally nephrectomized rabbits, and under the conditions of experimental renal hypertension and of sodium restriction, depletion and loading.

Six ml of the blood was taken from the ear vein into a syringe containing heparin for the determination of PCEA, PRA and PRS. PCEA was measured by Boucher’s method with a minor modification to facilitate the use of a small amount of sample. In this modification, plasma was diluted to a half concentration by adding the same amount of Tris-HCl buffer solution 0.1 M, pH 7.4, the amount of isoleucine-5-angiotensin I was increased to 2 µg, and the time of incubation at 37°C was prolonged to 30 minutes. The results were expressed as the amount of ng of angiotensin II formed from 1 µg of angiotensin I per hour by the action of converting enzyme in undiluted plasma. Nephrectomy and clipping of the renal artery were performed under sodium pentobarbital anesthesia (30 mg/kg, intravenously).

Table I shows that there was no significant difference in PCEA in the blood from the carotid artery, the jugular and renal vein, taken within the same collection period after laparotomy under pentobarbital anesthesia in 6 normal rabbits. In 7 rabbits, 24 hours after unilateral nephrectomy a slight decrease of PRA (p<0.02) was observed, but PCEA and PRS remained unchanged. Following bilateral nephrectomy of other 9 rabbits, a significant decrease of PCEA, to 30% of the value before nephrectomy was found after 24 hours (p<0.001), and PRA fell to

<table>
<thead>
<tr>
<th>Site*</th>
<th>PCEA**</th>
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<tbody>
<tr>
<td>Carotid artery</td>
<td>245 ± 25</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>262 ± 15</td>
</tr>
<tr>
<td>Renal vein</td>
<td>262 ± 30</td>
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</table>

Values are means ± SE

*: The blood was taken with the same collection period after laparotomy under pentobarbital anesthesia (30 mg/kg, intravenously).

**: ng of angiotensin II formed per hour from 1 µg of angiotensin I.

** Key Words:** Renin, Angiotensin-I, Converting Enzyme Activity, Nephrectomy, Experimental Renal Hypertension, Sodium Intake

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undetectable levels (p<0.001) and PRS was increased 3 fold (p<0.01). In hypertensive rabbits by constriction of one renal artery, with or without contralateral nephrectomy, PCEA remained unchanged up to 6 to 7 weeks after clipping. A high PRA was observed in rabbits with a clipped renal artery and contralateral kidney removed. There were no significant differences in PCEA between the rabbits on the different amounts of sodium intake.

From these results, the kidney appears to play a part in the maintenance of the level of PCEA, but the mechanism played by the kidney on PCEA levels and a physiological role of the decreased PCEA in nephrectomized animals are remained to be elucidated. It may be difficult to assume that there is some significant role of PCEA in the pathogenesis of experimental renal hypertension in rabbits, and PCEA does not appear to be affected by the same stimuli which are known for the control of PRA.

REFERENCES

DISCUSSION:
Chairman: TATSUO KOKUBU, Osaka Univ.

Dr. Kurihara reported an assay method of plasma angiotensin I converting enzyme activity (PCEA) and the levels of PCEA, plasma renin activity and renin substrate in normal rabbits, in bilaterally nephrectomized rabbits, and under conditions of experimental renal hypertension and of sodium restriction, depletion and loading. He observed a significant decrease of PCEA only following bilateral nephrectomy. He summarized that the kidney appeared to play in the maintenance of the level of PCEA and that PCEA did not appear to be affected by the same stimuli which were known for the control of plasma renin activity. Discussion was done about mainly the procedure of PCEA and its site of the action and its physiological significance. Following paper No.4 which was reported that CE acted not only in pulmonary circulation but also in blood stream, the discussion was focused upon whether angiotensin I was converted to angiotensin II in the other organs such as kidney, adrenals and liver.

Dr. UEMURA (Kagoshima University): Did you have any evidences that angiotensin produced by Boucher method was angiotensin II?
Dr. KURIHARA: Yes, I did. I believe that angiotensin I might be produce by Boucher method from the results of contraction of rat uterus by the produced pressor substance and from the use of EDTA and pH in the incubation system.

Dr. UEDA (Osaka University): How did you treat carboxypeptidase activity?
Dr. KURIHARA: Plasma was preincubated at 5°C for 5 minutes as reported by Boucher et al. in Circulation Res. 26: 1-83, 1970.

Dr. FUKUCHI (Tohoku University): Did you find out any difference between levels of PCEA of renal artery and renal vein?

Dr. KURIHARA: No, I did not. However, I found no difference between PCEA of renal vein and carotid artery.

Dr. KANEKO (Tokyo University): Did you examine about differences between artery blood and vein blood in lung or liver?

Dr. KURIHARA: No, I didn’t.

Dr. FUKUCHI: I suppose that angiotensin I might be converted to angiotensin II in kidney, because we often observe the increase of amount of angiotensin II in the vein blood of kidney with renal artery constriction.

Dr. SARUTA (Keio University): As to the site of action of CE, I got some results indicating adrenal tissue had CEA. So, I suppose that various kinds of organs may have CE.

Dr. SOKABE (Toho University): I think we have to mind biological activity of angiotensin I when we discuss about CEA.

Dr. SATO (Tokyo University): Dr. Sokabe, I would like to ask you about a direct biological activity of angiotensin I.


According to the reports of No.4 and 5, angiotensin I converting enzyme and one of the kininase, kininase II, might be the same enzyme, so-called carboxydipeptidase, and the site of action might be not only lung but also plasma and various kinds of organs. In order to clarify the degree of conversion of angiotensin I to II in the various tissue and its physiological or pathological significance, further studies are necessary.

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