Metabolism of Vasoactive Substances in the Lung: 
Change of Metabolism and its Significance 
in Rabbits with Experimental Pneumonitis

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A lot of information about the function of the lung other than gas exchange has been reported within recent past. Among them, the metabolism of vasoactive substances is interested in many investigators because of its influence upon the pathophysiology of the systemic circulation. In this respect we have found that all of the depressor substances tested were inactivated through the pulmonary circulation whereas all the pressor substances were passed through the pulmonary circulation without any change of their pressor activity. We investigated, in this paper, the change of metabolism of vasoactive substances in pathological condition of the lung, and intended to find out the effect of these metabolic change of the lung on systemic circulation.

Materials and Methods

Asp^1^-Ile^8^-angiotensin I (AT I), Asp^1^-Ile^5^-angiotensin II (AT II), bradykinin (BK) (obtained from the Institute for Protein Res., Osaka Univ.), prostaglandine E_1 (PG E_1) (Ono Pharmac. Co., Japan) and adenosine (Takeda Pharmac. Co., Japan) were used as vasoactive substances which have been known to be inactivated in the pulmonary circulation.

In vivo experiments: Experiments were performed as reported before. Rabbits weighing 2.5 Kg were used for the experiment. AT I and BK were injected through the canula in the aorta or in the auricular vein, and the changes of the systemic blood pressure were recorded on the polygraph through the canula which was fixed in the carotid artery. 0.5 µg of AT I and 0.5 µg of BK were injected via aortic canula and the doses which produce the same blood pressure response (equipotential dose) when injected into auricular vein were found out after several different amount of injection. This ratio of equipotential dose (I.v.inj.)/I.a.inj.) assumed to be the capacity of inactivation of vasoactive substances.

Isolated perfused lung preparation: The perfusion was performed in an apparatus according to Levey et al with small modification as reported before. The lungs were ventilated with the air by creating the alternating negative pressure of 10~20 mmHg within the perfusion chamber 20 times/min using cylindrical pump. The perfusion was performed at constant flow rate of 45~60 ml/min of Tyrode’s solution. AT I and BK of several concentration were injected through the rubber tube, and the perfusate was collected consecutively into successive tube about 4 ml for each tube after single passage through the lungs.

Assay of carboxydipeptidase activity: Assay was performed spectrophotometrically according to Cushman and Cheung. Briefly, 0.25 ml of 5 mM Hip-His-Leu in 0.1 M phosphate buffer,
Fig.1. Shows sequential change of equipotential doses of several vasoactive substances in rabbits with experimental pneumonitis. Columns indicate the equipotential doses of the intravenous injection which produce the blood pressure response as high as the intra-aortic injection of fixed amount of vasoactive substances.

TABLE I CARBOPHYDROPEPTIDASE ACTIVITY IN LUNG HOMOGENATE AND IN PLASMA WITH EXPERIMENTAL PNEUMONITIS

<table>
<thead>
<tr>
<th></th>
<th>lung homogenate</th>
<th>plasma</th>
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<tbody>
<tr>
<td></td>
<td>uM/min/g (± SE)</td>
<td>uM/min/ml (± SE)</td>
</tr>
<tr>
<td>Before (normal)</td>
<td>3070 ± 546</td>
<td>267 ± 17</td>
</tr>
<tr>
<td>3rd day</td>
<td>523 ± 102</td>
<td>123 ± 12</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>7th day</td>
<td>1302 ± 178</td>
<td>144 ± 24</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>14th day</td>
<td>1445 ± 142</td>
<td>176 ± 13</td>
</tr>
<tr>
<td>21st day</td>
<td>2493 ± 459</td>
<td>150 ± 45</td>
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</table>

pH 8.3, containing 0.3 M NaCl, and 0.1 ml of enzyme (lung homogenate or plasma) were incubated at 37°C (10 minutes for lung homogenate and 30 minutes for plasma). The amount hippuric acid formed was measured by absorbancy at 228 mμ. The activity was expressed as μM/ml/min for plasma and μM/g/min for the lung.

Assay of bradykinin: Bioassay was performed according to Roblero. Rat weighing 200–250 g was anesthetized by pentobarbital-Na, then small polyethylene tube was inserted from right carotid artery about 3 cm, so the top of the tube always placed at ascending aorta. Bradykinin was injected through this tube and the change of the blood pressure was measured in the left carotid artery and recorded on kymograph. The concentration of BK was calculated by blacketting with standard BK.

Assay of angiotensin II: The amount of formed angiotensin II was assayed using rat uterus in purpose for recognize angiotensin II from angiotensin I by the method as reported before.\textsuperscript{10}

Production of experimental pneumonitis: Rabbits were received two times intravenous injection of Complete Freund’s Adjuvant at the dose of 0.5 mg with seven days interval, and the rabbits after 3rd, 7th, 14th and 21st days of

second treatment were used for in vitro experiments, and the lungs of 3rd days were used for isolated perfusion preparation.

Experiments using lung homogenate: Rabbits were bled from carotid artery and the lungs and heart were removed in a mass. The lungs were washed with \( \frac{1}{200} \) M phosphate buffer through the pulmonary artery. After removal of bronchi and large arteries, lungs were homogenized with Potter homogenizer in a 10-times volume of \( \frac{1}{200} \) M phosphate buffer, pH7.2. The homogenate was sonicated with sonicator (Sonic, U.S.A.) followed by centrifugation. Carboxydipeptidase activity for each normal and pathological lung was measured using the supernatant as mentioned above.

RESULTS

In Vivo experiments: The results were summerized in Fig. 1. The change of inactivating activity in the pathological lung for vasoactive substances were expressed as the change of equipotential doses of intravenous injection to the intro-aortic injection. The equipotential dose for BK became lower in the rabbits with pneumonitis than in the normal rabbits and lowest on 3rd day of second injection of Freund’s adjuvant, namely 0.85 \( \mu \)g of intravenous injection gave same effect to 0.5 \( \mu \)g of intro-aortic injection whereas in normal rabbits 2.2 \( \mu \)g of intravenous injection was necessary to produce the same blood pressure change as high as 0.5 \( \mu \)g of intro-aortic injection. This equipotential dose increased gradually and recovered to normal control levels in 21st day. This change were pararell with the change of microscopic findings of the lung. This decrease of equipotential dose was also observed in PG E\(_1\). This fact suggested that the inactivating capacity for some vasoactive substances were decreased in the pathological lung.

The blood pressure of the experimental group and normal group were 110.3 \( \pm \) 2.35 and 90.4 \( \pm \) 2.63 mmHg respectively means significant decrease of the blood pressure in experimental group. There was no difference of the body weight between these two groups.

Carboxydipeptidase activity in lung homogenate: Carboxydipeptidase activity in the lung homogenate measured by using Hip-His-Leu as the substrate are shown in Table I. The activity in the lung and in plasma were significantly decreased on 3rd day and 7th day of the experiment. The carboxydipeptidase activity in normal lung homogenate was 3070 \( \mu \)g/min/gm of wet weight of the lung and that on the 3rd day was 523 \( \mu \)g/min/gm, meaning 83% decrease of
the activity. The activity became increased gradually onto 21st day. The activity of the enzyme in plasma was also changed in the same way as in lung homogenate. The activity was 267 μM/min/ml in normal rabbits and 123 μM/min/ml on 3rd day of the experiment, meaning 54% decrease of the activity. The change of the enzyme activity in lung homogenate and in plasma were parallel with the histological changes of the lung.

Removal of BK and activation of AT I in single perfusion in isolated lung preparation: 15 μg of BK and 1.5 μg of AT I were used for perfusion experiments because this dose were found to be adequate to produce the difference after preliminary experiments. 82% of BK were removed in normal lungs and 80% of BK were removed in pathological lungs, there was no difference of inactivating capacity between them. The activation of AT I in normal and pathological lungs were 54% and 50% respectively, no difference was observed also in activating capacity between normal and pathological lungs. This results were different from the results of in vivo experiment and in the experiment using lung homogenate. The results of in vivo experiments, experiments of isolated lung perfusion and in vitro experiments using lung homogenate were summarized in Fig. 2. The left part of the figure shows the in vivo experiment of normal and in rabbits on 3rd day of the pneumonitis, expressed by the ratio of equipotential dose, and the middle part shows carboxydiipeptidase activity in the lung homogenate. As far BK, the results of these experiment shows same tendency. However, the results of perfusion experiment (right paty of the figure) is different from the former.

Presence of carboxydiipeptidase inhibitor in pathological lungs: In purpose to find out the reason of this significatn decrease of the carboxydiipeptidase activity in pathological lung and in plasma, the homogenate of pathological lungs was added to the normal control system for the carboxydiipeptidase activity. The carboxydiipeptidase activity in normal lung homogenate were suppressed by adding pathological lung homogenate, and this suppression were parallel with the amount of pathological lung homogenate added.

DISCUSSION

It was very interesting that the inactivation of BK and PG E₁ was decreased in pathological lungs. The systemic blood pressure of the rabbits with experimental pneumonitis was significantly lower than that of normal control group. This fall of blood pressure might not be all due to the metabolic changes of the lung, but might have some relation to them. This fact gives a dimly light to the hypothesis that the metabolism of the lung might have some relation to the systemic blood pressure.

As to the relationship between angiotensin I converting enzyme, kininase and carboxydiipeptidase, the results were very confusing. The activity of converting enzyme was not changed in in vivo experiment and perfusion experiment. The activity of kininase was decreased in in vivo experiment and in vitro experiment using lung homogenate, but was not changed in perfusion experiment. This complete different results in each method might derived from the complete difference of the methodology. Possible explanations for the discrepancy of these enzyme activity were that the affinity of this enzyme for the substrate were different in each substrate especially in the unusual condition like inflammation, and that the existence of the inhibitor of the enzyme modified the apparent activity of the enzyme.

It is very interesting that the carboxydiipeptidase activity in the lung homogenate and in plasma was significantly decreased in rabbits with pathological lung. A lot of enzyme are circulating in plasma, but only few enzymes are decreased their activity in some pathological state. It is the rule that the activity of the enzyme in plasma usually respond to some pathological condition by increasing their activity. This unusual decrease of carboxydiipeptidase activity in plasma and in lung homogenate might be explained by the existence of the inhibitor in the pathological state. The presence of the inhibitor of the carboxydiipeptidase is interested in the relation to the pathogenesis of inflammation. The purification and the properties of this inhibitor are under investigation.

REFERENCES
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UEDA, E., et al.


Discussion:

Chairman: Dr. K. YAMAMOTO, Osaka City Univ.

Dr. TAKEDA (Tokyo Univ.): 1) You reported that the metabolism of vasoactive substances (bradykinin and angiotensin I) was different between in vivo experiment and perfusion experiment. What do you think this difference?
2) Did you determine the enzyme activity using angiotensin I or bradykinin as a substrate with the diseased lung homogenate?
Dr. UEDA: 1) We did not use the whole blood as a perfusate in the perfusion experiment.

Even in the control experiment, the inactivation capacity to bradykinin was higher in the perfusion experiment than in the in vivo experiment.
The difference between the data obtained from both experiments may be explained by the difference of experimental method and condition.
2) We did not use bradykinin and angiotensin I as a substrate, because there are some methodological difficulties to determine the enzyme activity of lung homogenate using these substrates.

Dr. SOKABE (Jichi Med. School): You pointed out that unknow inhibitor obtained from lung tissue could inhibit both angiotensin I activation and bradykinin inactivation. What do you think the decrease of the activity of bradykinin inactivation alone in the lung with pneumonitis?
Dr. KOKUBU (Ehime Univ.): In the future problem, angiotensin I converting enzyme, kininase and the unknown inhibitor must be purified, and then the response of the inhibitor on both enzyme activities should be determined.
Dr. UEDA: In this experimental system, it is difficult to show an activation of angiotensin I, so that I suppose even in the diseased lung the activation of angiotensin I will be hardly recognized.

If the order of the affinity of this enzyme to the substrate were bradykinin > inhibitor > angiotensin I, our data were not inconsistent.

Dr. KURIHARA (Tokyo Univ.): My question is methodological one. I wonder whether can we determine the metabolism of angiotensin I in the pulmonary circulation by the response on blood pressure? In other words, the response on blood pressure may be influenced by not only alternate metabolism of angiotensin I but also the changes on responsivenes of blood vessels.
Dr. UEDA: I think the measuring of inactivation of vasoactive substances in the lung by the method of “equi-pressor dose” is not satisfy, so that in the present study, the perfusion experiment of the lung and the enzymatic experiment in vitro were additionary carried out.
Dr. YAMAMOTO: I agree with Dr. Kurihara’s opinion. When a natural occurring substance in the body, it might better to determine chemically and biologically.

I would like to introduce our recent data briefly. According to Dr. Abe following acute reduction of renal artery perfusion pressure, renin release increased markedly with an increase of angiotensin I, but not angiotensin II concentration in the stenosed renal vein. A similar result was obtained after intrarenal arterial infusion of homologous renin. In these experiments, renal vasoconstriction was parallel with angiotensin II concentration in artery but not in renal vein. Moreover, intrarenal arterial infusion of angiotensin II inhibitor (1sar, 8levo-angio II) could not abolish autoregulation of the renal circulation.

These data suggest that released renin does not show biological action through local formation of angiotensin II, rather angiotensin I is converted to angiotensin II mainly in the extrarenal circulation, probably in the lung circulation, and then appear their biological action.