Kininase Activity in Human Blood in Normal and Pathological Conditions

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Kininase activity in blood was estimated in 17 healthy subjects and 162 patients with various diseases. The assay method consists of the incubation of synthetic bradykinin with venous blood at 37°C for 10 minutes and the quantitation of remaining bradykinin with the guinea-pig ileum.

In healthy subjects, kininase activities were 53–72%, with the mean of 64.4±4.93%. Markedly high activities were observed in patients with hyperthyroidism and in those with liver diseases. In patients with bronchial asthma, relatively low activities were found. No significant changes in the activity were found in other diseases.

In 1949, Rocha e Silva and his co-workers discovered an active polypeptide which is released by incubation of pseudoglobulin fraction of plasma protein with trypsin or venom of the snake Bothrops jararaca, and this polypeptide was named bradykinin. Recently, it has been assumed that kinin plays an important role as a mediator in the pathogenesis of various diseases such as hereditary angioneurotic edema, acute pancreatitis, bronchial asthma, carcinoid syndrome and allergic diseases.

On the other hand, kinin is destroyed very rapidly in plasma. This inactivation is due to a kinin-destroying enzyme, kininase, which is contained in human plasma fraction IV-1. Recently, a kininase which destroys bradykinin and kallidin by cleaving their C-terminal arginine was found and named carboxypeptidase N by Erdös and Sloane.

In order to study the participation of kinin in the pathogenesis of various diseases, it is necessary to call attention on the activity of kininase in blood. The assay method and estimated values of kininase activity in human venous blood under healthy and pathological conditions will be described in the present paper.

Materials and Methods

A) Materials

Seventeen healthy persons and 162 patients with various diseases were tested. The patients consisted of 34 with liver diseases, 34 with hyperthyroidism, 21 with endocrine diseases, 19 with hematological diseases, 17 with cardiovascular diseases,
16 with neurological diseases, 9 with renal diseases, 4 with bronchial asthma, 4 with pancreatic diseases and 4 with collagen diseases.

B) Methods

Collection of blood samples: A siliconized syringe was moistened with heparin solution (1,000 units/ml). Venous blood was drawn from the antecubital vein into the syringe under fasting condition, and transferred to a siliconized tube.

Incubation: Synthetic bradykinin was diluted to a concentration of 0.25 μg/ml with Tyrode solution. One milliliter of this bradykinin solution was transferred to another siliconized tube and warmed to 37°C in a water bath. Then, 0.1 ml of venous blood was added by a siliconized pipette and incubated for 10 minutes at 37°C. After the incubation, the tube was immediately cooled to 0°C in an iced water bath to stop further inactivation of bradykinin. The bradykinin remaining in the incubated mixture was assayed on the isolated guinea-pig ileum.

Bioassay: A guinea-pig was killed by a blow on the head, weighed and bled from the carotid arteries. The terminal ileum was freed from the mesentery and washed with warmed Tyrode solution through its lumen. A 2–3 cm segment of the anal portion of the ileum was cut out and suspended in a 10 ml bath filled with aerated Tyrode solution containing atropine sulphate (10⁻⁵) and promethazine hydrochloride (10⁻⁷) at 34–36°C.

Contractions of the ileum strip were recorded on a smoked kymograph with a lever. Samples were assayed by comparing their contracting activities with those of synthetic bradykinin by the four point method. The kininase activity was expressed by the rate of destruction of bradykinin under the condition mentioned above.

C) Comment on the methods

Preliminary experiments were done to determine a suitable condition for the assay method.

Siliconization of glass apparatuses: In 1957, Keele and his co-workers reported that contact of blood with glass surfaces activates the enzymes responsible for plasma kinin release. Hence, if blood samples were handled with unsiliconized glass wares kinin forming enzymes would be activated and liberate bradykinin during the incubation, and the quantities of kinin would increase at the end of the incubation, resulting in an apparent decrease in kininase activity. Actually, the activity of kininase was found to be lower when blood samples were collected by glass wares without siliconization than when the assay was carried out in the standard fashion. From this result, the glass apparatus to be used should be completely siliconized.

Effect of temperature on kininase activity: Since it is generally recognized that the activity of the enzyme is greatly influenced by temperature, the kininase activity was studied at different incubation temperatures. Fig. 1 shows the effect of temperature on the destruction rate of bradykinin by kininase in two blood specimens. The maximum activity was found at 50°C and the activity
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Fig. 1. Effect of temperature on kininase activity. 0.75 µg of synthetic bradykinin in 1 ml of Tyrode solution was incubated with 1 ml of venous blood for 3 min.

decreased progressively as temperature fell. Since a temperature of 50°C was not physiological, the incubation was carried out at 37°C. At 0°C the kininase was inactive over several hours.

Influence of pH on kininase activity: The activity of kininase in human venous blood was examined at various pHs (Fig. 2).

It was found that the optimum pH of kininase was around 7.4. Below this pH, the activity was rapidly reduced and disappeared completely at 5.6 or lower, but

Fig. 2. Influence of pH on kininase activity. 0.25 µg of synthetic bradykinin in 1 ml of Tyrode solution was incubated with 0.5 ml of venous blood at 37°C for 3 min.
its activity was still recognizable at such a strongly alkaline pH as 10.4.

**Time course of bradykinin inactivation:** Since kininase in blood is so active that the added bradykinin is too rapidly inactivated to be recovered for the estimation of kininase activity in a few minutes, suitable dilution of blood should be considered for the evaluation of kininase activity. The time course of bradykinin inactivation was studied at various blood dilutions and at different bradykinin concentrations.

As illustrated in Fig. 3, in A system in which blood was diluted twofold, 80 per cent of bradykinin was destroyed in 3 minutes and the half-life was approximately 1 minute. In B (sixfold dilution) and C (11 fold) systems, the half-life was 4 minutes and 10 minutes, respectively. Elevenfold dilution of blood was adopted for the standard procedure.

The assay method used for the present study proved to be very accurate and could be carried out with ease.

**Results**

Blood kininase activities in 17 healthy subjects and in 162 patients with various diseases are shown in Fig. 4 and Table 1.

1) **Healthy subjects**

Seventeen healthy persons of both sexes were subjected to study as normal controls. The values of kininase activities were 53–72% (mean 64.4±4.93%).
TABLE 1. Kininase activity in healthy subjects and patients with various diseases

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Number of cases</th>
<th>Kininase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>17</td>
<td>59–72</td>
</tr>
<tr>
<td>Liver diseases</td>
<td>34</td>
<td>69–100</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>34</td>
<td>67–100</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>4</td>
<td>50–67</td>
</tr>
<tr>
<td>Pancreatic diseases</td>
<td>4</td>
<td>50–71</td>
</tr>
<tr>
<td>Collagen diseases</td>
<td>4</td>
<td>60–71</td>
</tr>
<tr>
<td>Endocrine diseases</td>
<td>21</td>
<td>60–86</td>
</tr>
<tr>
<td>Hematological diseases</td>
<td>19</td>
<td>50–87</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td>17</td>
<td>44–87</td>
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<td>16</td>
<td>47–78</td>
</tr>
<tr>
<td>Renal diseases</td>
<td>9</td>
<td>50–75</td>
</tr>
</tbody>
</table>

Fig. 4. Bradykininase activity in various diseases. Dotted lines show the mean values. ▲ and □, see text (p. 70).
Fig. 5. Bradykininase activity of the blood of patients with various liver diseases. Dotted lines show the mean values.

Fig. 6. Relationship between blood bradykininase activities and serum transaminase activities in patients with acute infectious hepatitis. Dotted lines show the mean values.
2) *Liver diseases*

Thirty-four patients with various liver diseases were tested. Almost all of them showed significantly high enzyme levels. The kininase activities of 29 cases in which the diagnosis had been established clinically or histologically are shown in Fig. 5. Patients with acute hepatitis had the highest enzyme activity. The mean value found in 17 cases of acute hepatitis was $85.9 \pm 7.00\%$, 5 of chronic hepatitis $83.2 \pm 7.86\%$, 5 of cirrhosis $84.3 \pm 10.65\%$ and 2 of cholangiolitic hepatitis $69.5 \pm 9.50\%$.

In the patients with acute infectious hepatitis, kininase activity had some relations with serum transaminase activities (Fig. 6). On the other hand, cirrhotic patients who had high kininase activities showed normal serum transaminase activities. No significant relationships were found between kininase activity and other laboratory findings on liver function such as icterus index, bromsulfalein, cephalin-cholesterol flocculation, thymol turbidity, zinc sulfate, and alkaline phosphatase tests.

3) *Hyperthyroidism*

In 32 out of 34 patients with hyperthyroidism, kininase activities were markedly elevated. The mean value was $86.5 \pm 7.69\%$.

It is well known that hyperthyroidism is often accompanied by liver dysfunction. In one of the tested cases with hyperthyroidism, however, any abnormal liver function was not detected by routine laboratory tests.

As shown in Fig. 7, a significant relationship was found between basal metabolic

![Figure 7](image-url)  
**Fig. 7.** Relationship between blood bradykininase activities and basal metabolic rates in patients with hyperthyroidism.
rate and kininase activity. High enzyme levels fell down to the normal range after the patients had been made euthyroid by treatment.

4) *Bronchial asthma*

Four asthmatic patients were tested during the attacks. Three of them showed lower activities than the normal level. The mean value was $54.3 \pm 7.36\%$.

5) *Pancreatic diseases*

Enzyme activities in 4 cases of pancreatic diseases were examined. One of them was that of adenoma of the pancreas and others were cases of mild acute pancreatitis. The estimated values were within the normal range.

6) *Collagen diseases*

Two patients with Weber-Christian disease, each one patient with systemic lupus erythematosus, polymyositis and dermatomyositis were examined. They had the normal enzyme activities except in one case. This patient with Weber-Christian disease showed an increased kininase activity as high as 100%. In this case, however, remarkable fatty degeneration of the liver was revealed at the postmortem examination. Because of this exceptional finding, this patient was excluded from Table I, but shown in Fig. 4 with $\square$). In another patient with Weber-Christian disease, the enzyme activity was increased after the development of serum hepatitis (shown as $\blacktriangleright \rightarrow \blacktriangle$ in Fig. 4).

In spite of an extremely high blood kinin content, normal kininase activity was demonstrated in a case of dermatomyositis.

7) *Other diseases*

Eighty-two patients with endocrine (hyperthyroidism was excluded), blood, cardiovascular, nervous and renal diseases were studied. A significant deviation from the normal level was found in none of the patients. In addition, all 4 cases of myxedema had normal enzyme levels.

**DISCUSSION**

It is well known that kininases exist in plasma,7 blood corpuscles,10 various tissues11, saliva12 and urine.13 Kininase activity in blood is so active that the half-life of bradykinin is less than 1 minute in circulating blood.14

An adequate and accurate method is necessary for the study of kininase activity in blood. A simple procedure for the estimation of blood kininase activity has been developed in this laboratory. The method was based on biological assay after incubation of synthetic bradykinin and venous blood. Using this method, the whole kininase activity of blood was measured in health and various diseases. Consequently, it was found that blood kininase activity was significantly elevated in liver diseases and hyperthyroidism. At present, the pathological significance of increased kininase activity in such diseases is quite obscure.

According to Erdős and his co-workers' report,8 human plasma fraction IV-1 contains a carboxypeptidase-type enzyme which inactivates kinin by cleaving its
carboxy-terminal arginine. This enzyme is distinguished from pancreatic carboxypeptidase B, and was named carboxypeptidase N. They also reported that, in patients with liver cirrhosis, the activity of carboxypeptidase N which was measured spectrophotometrically with hippuryl-L-lysine as the substrate was significantly lower than normal. However, opposite data were obtained in the present experiments. The result suggests that increased kininase in liver cirrhosis might be different from carboxypeptidase N. It may be assumed that several peptidases exist in circulating blood and kinin is a common substrate of more than one peptidase.

It has already been reported that patients with bronchial asthma have a plasma kininase activity lower than normal. The same result was obtained in the present experiment. On the other hand, it has been already ascertained in this laboratory that kinin content in blood from asthmatic patients is increased. The details will be reported elsewhere. From these findings, it seems probable that kinin plays some role in the pathogenesis of bronchial asthma.

In cases of acute pancreatitis, no significant difference was found from the healthy control. But it must be kept in mind that in all these cases the patients were not severely diseased and in the stage of convalescence. Therefore, it is not concluded that kininase activity is normal in acute pancreatitis.

Acknowledgment

The author is grateful to Prof. Tatsuo Torikai for his leadership and to Drs. Keishi Abe, Kaoru Yoshinaga and Tatsuo Sato for their advice and co-operation. Bradykinin was supplied by the courtesy of Sandoz Ltd., Basle.

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