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Studies on Kallikreins. IV.[1] Enhancement of Valine Transport Across the Rat Small Intestine

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The effect of a highly purified hog pancreatic kallikrein and synthetic bradykinin on the transport of valine across the rat small intestine was examined by an *in vitro* experiment with the sac of everted intestine and by an *in situ* mesenteric perfusion experiment. The flow of valine from the mucosal to the serosal side of everted intestine was stimulated significantly by the addition of kallikrein to the mucosal fluid in a concentration of 0.1 KU/ml, while bradykinin was effective only by its addition to the serosal fluid.

In a perfusion experiment, valine transport into the vascular system was enhanced significantly by intra-intestinal administration of 0.02—20 KU of kallikrein. The maximum effect was obtained with 2 KU, and the absorption of valine was 1.9 times larger than that of the control. Bradykinin infusion into the mesenteric perfusion system in concentrations of 10^{-2} to 10^{2} nM also increased the transport of valine. A bell-shaped dose-response curve, in which the maximum enhancement was achieved with 1 nM of bradykinin, was obtained similar to the case of kallikrein. This effect of kallikrein appeared soon after its administration into the intestinal lumen and usually lasted about 30 min under the present experimental conditions.

Keywords——kallikrein; bradykinin; valine; intestine; transport; absorption

The pancreas is an organ which abounds in kallikrein. Lewis[3] reported that this enzyme was found in the pancreatic juice and effluxed into the intestinal lumen. Besides this finding, Zeitlin[4] and Erdös, *et al.*[5] showed that there was prekallikrein in the intestine and that this kallikrein would be related to gut pathology. On the physiological rationale of the pancreatic kallikrein, Hilton and Jones[6] suggested that it might cause a functional vasodilatation in the pancreas, as in the submaxillary gland. However, there has been no investigation on the significance of kallikrein in the intestine. Proteolytic activities of the kallikreins are very weak and their substrate specificities on synthetic esters are very limited. Hence it would hardly be possible to deal with kallikreins as one of the digestive enzymes like trypsin or chymotrypsin.

Meanwhile, we found previously that hog pancreatic kallikrein can be absorbed by the rat small intestine,[7] and it was speculated that the absorbed kallikrein would enhance the intestinal absorption of various nutrients as a result of increase permeability of the intestinal epithelial cells or vasodilatation of the mesenteric blood vessels.

To corroborate this assumption, the effect of pancreatic kallikrein and bradykinin on the intestinal absorption of valine was studied in this investigation.

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Materials and Methods

Materials——A highly purified hog pancreatic kallikrein, 1250 KU/mg, was used in this investigation. Synthetic bradykinin triacetate was supplied from the Protein Research Foundation (Osaka). Valine [U-^{14}C] was a product of Daiichi Chemical Co., Tokyo (specific activity, 225 mCi/mmol).

***In Vitro* Experiment with Rat Everted Intestine**——Female Wistar rats, weighing about 150 g, were killed by a blow on the head and exsanguination from the common carotid artery, and the jejunum was immediately removed. A sac of everted intestine of about 5 cm in length was prepared according to the procedure of Wilson and Wiseman.[8] Two sacs, which were filled with 0.6 ml of 0.2—5 mM valine in the Krebs-Ringer bicarbonate buffer (serosal fluid), were placed in a test tube holding 10 ml of the same solution but containing 0.02 μCi of valine[U-^{14}C] (mucosal fluid). Various doses of the samples, hog pancreatic kallikrein or synthetic bradykinin, were added to the mucosal or serosal fluid.

These test tubes were incubated for 60 or 15 min at 37° under constant bubbling with 95% O_2 and 5% CO_2 into the mucosal fluid. After incubation, volume of the serosal fluid was measured and its radioactivity was counted with a liquid scintillation counter (Model LSC-502, Aloka, Tokyo). The amount of valine transported from the mucosal to the serosal side was estimated from the radioactivity in the serosal fluid, and expressed in μmol of valine per g of wet organ per 60 or 15 min.

***In Situ* Mesenteric Perfusion Experiment**——The rat of 150 g body-weight was perfused with Krebs-Ringer bicarbonate buffer saturated with 95% O_2–5% CO_2 gas through the superior mesenteric vascular system (flow rate: 20 ml/hr) under pentobarbital anesthesia.[9] The perfusate was recovered from a polyethylene catheter inserted into the mesenteric vein. After the preliminary perfusion of 10 min, 0.1 μmol of valine, 0.11 μCi of valine[^{14}C], and various doses of kallikrein in 0.6 ml of the same buffer were administered into the ligated jejunum of about 20 cm in length and then the perfusion was continued for 60 min. During this period the recovered perfusate was fractionated every 10 min. The amount of transported valine was estimated from the radioactivity detected in each fraction and expressed in nmol of valine per 10 or 60 min. In the case of bradykinin experiments, 10^{-2} to 10^{2} nM bradykinin in the buffer was infused into the superior mesenteric artery, and 2.6 μmol of valine in 0.5 ml of the buffer was given intra-intestinally together with ^{14}C-valine.

Results

Effect of Hog Pancreatic Kallikrein

The effect of kallikrein on valine transport across everted sac of the jejunum is shown in Fig. 1. In the dose range of 10^{-4} to 1 KU/ml of the mucosal fluid, about 1.5 times greater flow than that of the control was achieved with 0.1 KU/ml concentration, but the amount of

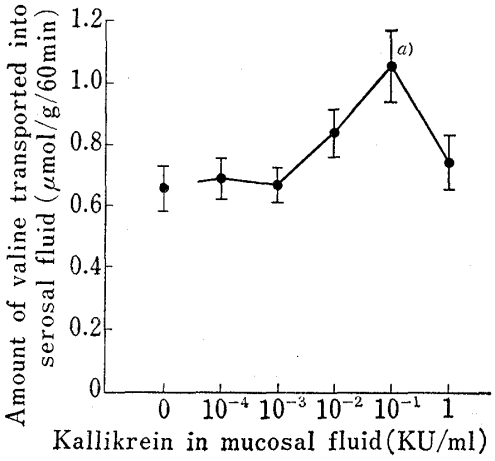

Fig. 1. Effect of Hog Pancreatic Kallikrein on Valine Transport (*in vitro* experiment with sac of everted intestine)

valine concentration: 0.2 mM
mean ± SE of 16 sacs obtained from 4 rats
a) significant difference ($p < 0.05$)

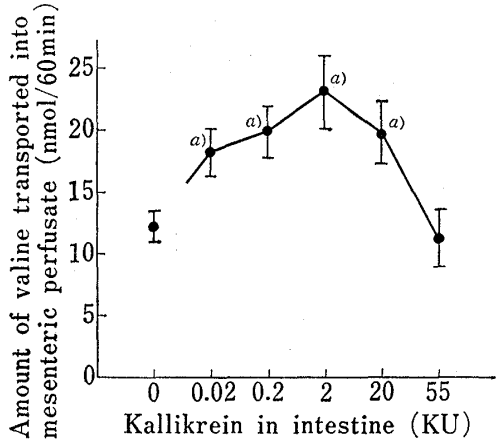

Fig. 2. Effect of Hog Pancreatic Kallikrein Administered into the Intestine on Valine Absorption (*in situ* perfusion experiment)

valine dose: 0.1 μmol
mean ± SE of 5—19 rats
a) significant difference ($p < 0.05$)

8) T.H. Wilson and G. Wiseman, *J. Physiol.* (London), **123**, 116 (1954).
9) C. Moriwaki, K. Yamaguchi, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **22**, 1029 (1974).

transport decreased again with 1 KU/ml of kallikrein. This kallikrein preparation was recognized as pure in disc electrophoresis, hence it was strongly suggested that kallikrein in the mucosal fluid enhanced the valine transport across the everted intestine.

The same result was obtained in the *in situ* mesenteric perfusion experiment. By combined administration of 0.02 to 55 KU of kallikrein and 0.1 μmol of valine into the ligated jejunum, the valine absorption increased by the factor of 1.9 in the presence of 2 KU of kallikrein, and the rate decreased with higher doses (Fig. 2), as in the *in vitro* experiment (Fig. 1).

The perfusate was recovered in the fractions taken every 10 min, so that the time course of valine absorption could be followed (Fig. 3). Valine transport into the perfusate began soon after its intra-intestinal administration, and reached the maximum 10 to 20 min later. Enhancement of valine absorption was observed by the presence of 0.02 to 20 KU of kallikrein in the jejunum, but not with 55 KU. The profiles of 0.02 and 20 KU were similar to that of 0.2 KU, and the effect of kallikrein was most prominent during the first 20 min.

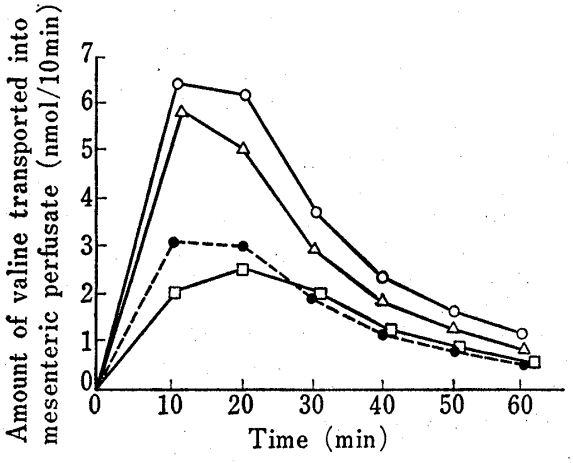

Fig. 3. Time Course of Valine Absorption in the Presence of Hog Pancreatic Kallikrein in the Intestine (*in situ* perfusion experiment)

valine dose: 0.1 μmol
kallikrein dose
---●---: control —△—: 0.2 KU
—○—: 2.0 KU —□—: 55.0 KU

Effect of Bradykinin

The kallikreins are the enzyme which liberates kinins from kininogen, and the effect of bradykinin, one of the most representative kinins, was likewise investigated. No effect, however, could be found on valine transport across the everted intestine by addition of the peptide to the mucosal fluid. Therefore, it was added to the serosal fluid and incubation was carried out for 15 min. In this case, valine transport increased about 1.5 times more than that of the control by the addition of 200 ng of synthetic bradykinin to the serosal side (Fig. 4).

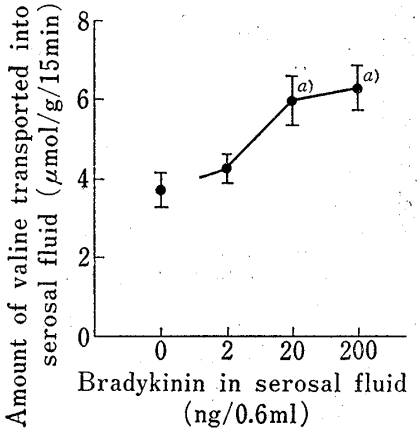

Fig. 4. Effect of Bradykinin on Valine Transport (*in vitro* experiment with sac of everted intestine)

valine concentration: 5 mM
mean ± SE of 16 sacs obtained from 4 rats
a) significant difference ($p < 0.05$)

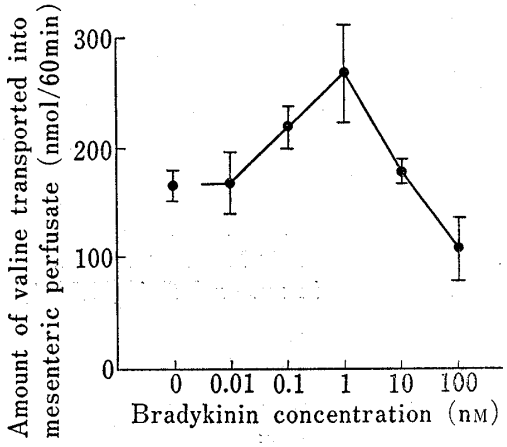

Fig. 5. Effect of Bradykinin Infused into Mesenteric Perfusion System on Valine Absorption (*in situ* perfusion experiment)

valine dose: 2.6 μmol
mean ± SE of 6 rats

Taking this result into acount, an *in situ* experiment of bradykinin effect on valine absorption was carried out with infusion of the peptide into the vascular system. Valine transport into the vascular perfusate increased significantly with 1.0 nM bradykinin perfusion, from 163±11 to 274±52 nmol/60 min (Fig. 5). When the concentration of bradykinin was increased further to 10 or 100 nM, the rate of absorption decreased again. This diminution of the effect in the presence of a higher concentration was also demonstrated in the experiment on intra-intestinal administration of kallikrein.

Discussion

As mentioned above, Hilton and co-workers suggested that the kallikreins caused the functional vasodilatation in various glands,[3,6,10] but Schachter and Beilenson[11] refuted this suggestion basing their argument on several experimental results, mainly obtained from cat submaxillary gland. Besides these investigations, there are many reports on the role of the kallikrein-kinin system, such as the induction of neonatal circulatory changes,[12] its participation in the regulation of electrolyte secretion in the kidney,[13] and its involvement in the inflammatory processes.[14]

The present investigation is an attempt to corroborate the probability that the kallikrein-kinin system has some influence on the intestinal absorption of the nutrients. Although it would be desirable to employ various kinds of nutrients in this investigation, we arbitrarily chose valine, a neutral amino acid transported actively in the intestinal absorptive cells.

As shown in the experimental results, the most effective doses of kallikrein for the stimulation of valine transport were 0.1 KU/ml and 2 KU in the *in vitro* and *in situ* experiments, respectively, and the stimulation of valine transport diminished again with higher kallikrein doses in both experiments. The wet weight of the pancreas of a rat of 150 g body-weight is about 500 mg, and the content of kallikrein in the gland would be no more than 35 KU.[15] Arens and Haberland mentioned that kallikrein content in the intestinal tissue did not exceed 1 KU/g dry weight.[16] In view of these findings regarding the amount of kallikrein, the present results showing that very small quantities of kallikrein stimulated valine transport significantly appears to be reliable as one of the physiological functions of this enzyme. This effect of kallikrein reached its maximum as early as 10 min after administration (Fig. 3), and this fact agrees with our previous finding that the absorption of kallikrein was mostly completed by 30 min after its intra-intestinal administration.[7]

Concerning the mechanism of this effect of kallikrein, it is probable that intra- or intercellular liberation of kinin may take place in the intestinal tissue by the absorbed kallikrein the action of this kinin for vasodilatation or permeability increase results in the appearance of the effect of kallikrein. Valine transport into the mesenteric blood vessel from the intestinal lumen was enhanced by the infusion of bradykinin into the superior mesenteric artery. The profile of this response (Fig. 5) was almost similar to that of the intra-intestinal administration of kallikrein (Fig. 2). These observations will support the above interpretation.

The effective dose of bradykinin on valine transport from the ligated intestine was as small as 1 nM, *i.e.*, about 1 ng/ml. Chou, *et al.*[17] demonstrated that the reduction of vaso-

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resistance and vasodilatation occurred in a dog by the infusion of 0.35 ng/ml of bradykinin solution into the superior mesenteric artery. The kininase activity in the recovered perfusate was almost negligible, and it is assumed that the infused bradykinin could give its effect on the vascular system or others by this small amount.

The question of whether this stimulative effect of kallikrein and bradykinin on valine absorption is the result of an increase in the passive transport through the increase of membrane permeability or an acceleration of the active transport processes, is now being examined in our laboratory, together with studies on the effect of kallikrein on the transport of other amino acids or carbohydrates.

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