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Studies on Kallikreins. VI.¹⁾ Effect of Pancreatic Kallikrein and Kinin on the Transport of Amino Acids and Glucose across the Rat Small Intestine

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The influence of hog pancreatic kallikrein and kinin on the transport of amino acids or glucose across the rat small intestine was further examined by the *in vitro* everted sac method and by the *in situ* mesenteric perfusion technique. Absorption of methionine and glucose into the vascular system was significantly enhanced, as was that of valine, by intra-luminal administration of kallikrein. Transport of methionine, valine and glucose was also enhanced by addition of bradykinin to the serosal fluid of the everted intestine, but no enhancement of glutamic acid transport was observed under the same conditions.

The stimulation of *in vitro* valine transport by kallikrein was inhibited by aprotinin. Valine transport was not affected by addition of trypsin, plasmin or kallikrein to the serosal side, whereas it was significantly increased by addition of 0.01 KU of kallikrein together with 10 μ g of kininogen to the serosal side.

Valine transport was reduced under anaerobic conditions or by removal of the intestinal epithelial layer. Ouabain or 2,4-dinitrophenol also inhibited the intestinal transport of valine. Under these conditions (except for ouabain treatment), valine transport was not enhanced by addition of bradykinin to the serosal side. In ouabain-treated intestine, however, bradykinin increased valine transport approximately 1.5 times compared with the control, but the transport was not restored to the normal level. Meanwhile, valine absorption was decreased to about 40% of the normal value by *in situ* infusion of 1.0 mM ouabain into the mesenteric vascular system, and the rate was restored to the normal level by intra-luminal administration of kallikrein.

Keywords—transport; absorption; intestine; valine; methionine; glutamic acid; glucose; kallikrein; bradykinin; rat

It has been said that the kallikrein-kinin system causes functional vasodilatation in some secretory glands, such as submaxillary gland²⁾ or pancreas,³⁾ but there is still a conflict of opinion on this point.^{4,5)} Recently it has been shown that glandular kallikreins are involved in a variety of biological functions, such as electrolyte excretion,^{6,7)} cell proliferation,⁸⁾ injury repair,⁹⁾ reproduction,¹⁰⁾ intestinal absorption,^{11,12)} *etc.* We have previously found that intestinal valine absorption was enhanced by intra-intestinal administration of kallikrein or by infusion of kinin into the mesenteric vascular system,¹²⁾ and we suggested that pancreatic kallikrein in pancreatic juice may be related to the absorption of nutrients.

As a continuation of this work, the effects of kallikrein and kinin on the absorption and *in vitro* transport of various amino acids and glucose across the small intestine were studied, and their effects on valine transport were examined under anaerobic conditions and under inhibition of kallikrein or intracellular ATP generation. This paper describes the results of these experiments carried out by the *in vitro* everted sac method and the *in situ* mesenteric perfusion technique.

Experimental

Materials—Highly purified hog pancreatic kallikrein (1250 KU/mg) and aprotinin were donated by Bayer AG (Germany). Bradykinin was purchased from the Protein Research Foundation (Osaka). Trypsin, plasmin and α -chymotrypsin were products of Sigma Chemical Co. (U.S.A.). Ouabain was supplied by Nakarai Chemicals, Co. (Kyoto). L-[U-¹⁴C] valine (225 mCi/mmol), L-[U-¹⁴C] methionine (5.81 mCi/mg), L-[U-¹⁴C] glutamic acid (227 mCi/mmol) and D-[U-¹⁴C] glucose (260 mCi/mmol) were obtained from Daiichi

Chemical Co. (Tokyo). Partially purified bovine kininogen was prepared according to the method of Moriwaki and Schachter.¹³⁾

Methods—*In vitro* experiments with rat everted intestine and *in situ* mesenteric perfusion experiments were carried out as described previously.¹²⁾

Results

The effects of kallikrein on the absorption of methionine, glutamic acid and glucose were studied by *in situ* mesenteric perfusion experiments (Fig. 1). The contents of ¹⁴C-methionine and -glucose in the perfusate recovered from the mesenteric vein were increased significantly by the presence of 10^{-3} KU of kallikrein in the intestinal lumen, but there was no enhancement of the absorption of glutamic acid. The enhancing effects of kallikrein on methionine and glucose absorption appeared as early as 10 min after the administration, and the dose-response profiles were bell-shaped, as in the case of valine.¹²⁾ The transport of valine, methionine and glucose was also stimulated significantly by addition of 10 ng of bradykinin to the serosal fluid of everted jejunum (Fig. 2), whereas *in vitro* glutamic acid transport was not altered by 10 or 100 ng of bradykinin.

Fig. 1. Effect of Kallikrein on the Absorption of Methionine, Glutamic Acid and Glucose (*in situ* perfusion experiment)

Kallikrein and ¹⁴C-labeled sample nutrients were administered in the intestine, and the radioactivity in the perfusate recovered from the mesenteric vein was counted. Methionine (—●—●—) and glutamic acid (—▲—▲—): 0.1 μ mol, glucose (—○—○—): 2.5 μ mol. Mean $\pm$ S.E. of 6 to 8 rats, * significant difference ($p < 0.05$).

Fig. 2. Effect of Bradykinin on the Transport of Valine, Methionine, Glutamic Acid and Glucose (*in vitro* experiment)

A sac of everted intestine to which 0.6 ml of the serosal fluid had been added was placed in the mucosal fluid containing ¹⁴C-labeled sample nutrient. The concentrations of the sample nutrient in the mucosal and serosal fluid were the same. After 30 min incubation, the radioactivity in the serosal fluid was counted. Concentrations of valine, methionine and glutamic acid: 0.2 mM, glucose concentration: 5 mM. Mean $\pm$ S.E. of 6–8 sacs obtained from 3–4 rats, * significant difference ($p < 0.05$).

The enhancement of *in vitro* valine transport by kallikrein was inhibited by aprotinin, a potent kallikrein inhibitor.¹⁴⁾ The transport was increased 1.6 times compared with the control by 0.1 KU/ml of kallikrein in the mucosal fluid, but such elevation was not observed if the kallikrein had first been incubated with aprotinin for 10 min at 37°. Aprotinin (20 KIU/ml) itself did not cause any change in valine transport *in vitro*. Various proteins other than kallikrein, *i.e.* 10^{-3} – 10 μ g/ml of trypsin, α -chymotrypsin or bovine serum albumin, were added to the mucosal fluid, but no enhancement of valine transport was observed.

Addition of 10^{-2} – 10 μ g/sac of trypsin and plasmin or 10^{-3} – 10 KU/sac of kallikrein to the serosal fluid had no effect on the valine transport. Thus, kallikrein stimulated valine transport when it was given on the mucosal side but not on the serosal side. The effect of bradykinin appeared from the serosal side. Therefore, kallikrein was administered in the serosal side with bovine kininogen. As shown in Fig. 3, kallikrein or the kininogen alone did not increase the valine transport, whilst the combined administration of kallikrein (10^{-2} KU/sac) and kininogen (10 μ g/sac) in the serosal fluid caused 1.3 times more valine transport

Fig. 3. Effect of the Combined Administration of Kallikrein and Kininogen to the Serosal Fluid on Valine Transport (*in vitro* experiment)

A: control, B: kallikrein (10^{-2} KU/sac), C: bovine kininogen ($10 \mu\text{g/sac}$), D: kallikrein (10^{-2} KU/sac) and kininogen ($10 \mu\text{g/sac}$). Valine concentration: 0.2 mM . Mean $\pm$ S.E. of 8 sacs obtained from 4 rats, *significant difference ($p < 0.05$).

Fig. 4. Effect of Bradykinin on Valine Transport in the Presence of DNP (*in vitro* experiment)

Valine concentration: 0.2 mM . Mean $\pm$ S.E. of 8 sacs obtained from 4 rats, a: without DNP, b: with DNP (0.1 mM).

than in the control. This stimulation was almost equal to the effect of 10 ng of bradykinin given on the serosal side (Fig. 2).

The presence of 0.1 mM 2,4-dinitrophenol (DNP) in the mucosal fluid caused a remarkable decrease of valine transport to about 50% of the control without DNP. Under these conditions, addition of 10 or 100 ng of bradykinin to the serosal fluid had no effect on the transport (Fig. 4).

An anaerobic state was established by introducing N_2 gas into the mucosal fluid, which did not contain Ca^{2+} . An enhancement of valine transport by bradykinin was observed in the aerobic state, in which Ca^{2+} -free Krebs-Ringer bicarbonate solution was used as the incubation medium, but the transport under anaerobic conditions was only about one-third of that under aerobic conditions. A slight elevation of the transport was seen upon addition of bradykinin

Fig. 5. Effect of Bradykinin on Valine Transport under Anaerobic Conditions (*in vitro* experiment)

Ca^{2+} -free Krebs-Ringer solution was used as the incubation medium. Valine concentration: 0.2 mM . Mean $\pm$ S.E. of 8 sacs obtained from 4 rats, a: aerobic, b: anaerobic.

Fig. 6. Effect of Bradykinin on Valine Transport in the Presence of Various Na^+ Concentrations in the Incubation Medium

Incubation medium: Krebs-Ringer bicarbonate solution in which Na^+ concentration was varied by replacement of Na^+ with Tris. Control: $\bullet$, 10 ng of bradykinin: $\circ$, valine concentration: 0.2 mM . Mean $\pm$ S.E. of 8 sacs obtained from 4 rats, * significant difference ($p < 0.05$).

to the serosal fluid, but it reached only a half of that of the aerobic control even at a dose of 100 ng (Fig. 5).

Valine transport across intestine from which the epithelial layer had been removed by the method of Stern¹⁵⁾ was reduced to less than a half of the normal value, and bradykinin did not have any effect on the transport in this intestine. It is thus necessary to retain the epithelium in order to evoke the stimulatory effect of kinin on the transport. Valine transport was suppressed in parallel with the decrease of Na^+ concentration in the mucosal fluid, and restoration of the transport was seen upon addition of 10 ng of bradykinin to the serosal fluid (Fig. 6).

Fig. 7. Effect of Bradykinin under the Inhibition of Valine Transport by Ouabain (*in vitro* experiment)

Valine concentration: 0.2 mM. Mean $\pm$ S.E. of 8 sacs obtained from 4 rats, a: without ouabain, b: with ouabain (2.5 mM).

Fig. 8. Effect of Kallikrein on Valine Absorption during 1.0 mM Ouabain Perfusion (*in situ* perfusion experiment)

Valine dose: 0.1 μ mol. Mean $\pm$ S.E. of 6 rats, * significant difference ($p < 0.05$), a: without ouabain, b: with ouabain.

It was reported that ouabain, a specific inhibitor of Na, K-ATPase, suppressed the *in vitro* transport of Na^+ and amino acids across the small intestine.¹⁶⁾ Addition of 2.5 mM ouabain to the serosal fluid reduced valine transport to about one-third of the normal level. By a combined addition of ouabain and bradykinin (10 and 100 ng) to the serosal fluid, the transport was increased significantly by a factor of 1.6, but the amount was only about 60% of the normal value (Fig. 7). During infusion of 1.0 mM ouabain into the superior mesenteric artery, 0.1 μ mol of valine was applied to the ligated jejunum. The amount of absorbed valine was reduced to about 40% of the normal value, but it was restored to the normal level by intraluminal administration of 10^{-2} KU of kallikrein. However, valine absorption decreased with higher doses of kallikrein, and a typical bell-shaped dose-response curve was obtained (Fig. 8).

Discussion

We have previously reported that valine transport in rat small intestine was enhanced by addition of pancreatic kallikrein to the mucosal side or of bradykinin to the serosal side.¹²⁾ Kallikrein and kinin stimulated not only the transport of valine, but also that of methionine and glucose, which are transported actively (Figs. 1 and 2). Meng and Haberland¹⁷⁾ reported that the net transport of glucose and 3-O-methyl-glucose was stimulated by addition of 10^{-2} – 10^2 KU/ml of pancreatic kallikrein, and Dennhardt and Haberich¹¹⁾ mentioned that the transport of Na^+ , water and glucose was augmented by intra-intestinal perfusion of pancreatic kallikrein. Thus, pancreatic kallikrein seems to enhance not only valine, methionine and glucose but also Na^+ and water absorption across the small intestine. Meanwhile, the flux of glutamic

acid, which is transported in the intestine by diffusion,¹⁸⁾ was not altered by kallikrein or bradykinin (Figs. 1 and 2). These findings suggest that the kallikrein-kinin system stimulates active transport in the small intestine. A dose of 10^{-3} KU kallikrein was found to be the most effective for enhancement of the absorption of methionine and glucose in the present investigation. In view of the effective dose and the contents of kallikrein in pancreas and intestine, this effect may be one of the physiological functions of kallikrein, as discussed in our previous paper.¹²⁾

Trypsin, α -chymotrypsin and bovine serum albumin had no effect on the intestinal transport of valine, and the effect of kallikrein on the transport was blocked by aprotinin. Taking these results into account, we consider that this stimulation is a specific action of kallikrein.

We have demonstrated that hog pancreatic kallikrein is absorbed from the rat small intestine, though the amount was only about 1% of the administered dose,¹⁹⁾ and we speculated that absorbed kallikrein would affect the transport from the serosal side of the intestine. Addition of kallikrein to the serosal fluid did not enhance valine transport, however. Kallikrein seemed to exert its stimulative effect on the transport only when it was administered in the mucosal fluid. Meanwhile, enhancement of the transport was observed by combined addition of kallikrein and kininogen to the serosal fluid (Fig. 3). These results suggest that kinin generation takes place under the epithelial layer of the intestine and that kinin stimulates active transport in the absorptive cells.

It is widely accepted that the mucosa-to-serosa flux of active solute transport is diminished by addition of DNP²⁰⁾ or under anaerobic conditions.²¹⁾ Under these conditions, the suppressed valine transport was not restored to the normal level by addition of bradykinin to the serosal side of the intestine (Figs. 4 and 5). The intracellular supply of ATP seems to be indispensable for the enhancing effect of bradykinin on valine transport.

Rixon and Whitfield⁸⁾ found that kallikrein or kinin stimulated mitosis in the bone marrow and the thymus, and Ca^{2+} seemed to be necessary for this action. They speculated that kinin facilitated the entry of extracellular Ca^{2+} through some sites in the membrane. However, Ca^{2+} in the incubation medium did not seem to be important for the effect of bradykinin on valine transport, because there was no difference in valine transport in media with and without Ca^{2+} (Fig. 5).

The activity of Na,K-ATPase which is located in the baso-lateral membrane of the intestinal epithelial cell is inhibited by ouabain,²²⁾ and ouabain also suppressed the active transport of amino acids or sugar by blocking Na^+ efflux from the intra- to the extracellular space. The inhibition of valine transport by ouabain was reversed by kallikrein (Fig. 8) or by bradykinin (Fig. 7). These results suggest that the kallikrein-kinin system affects Na,K-ATPase in the baso-lateral membrane of the absorptive cells, and that this action is related to the mechanism of stimulation of the absorption.

As mentioned above, it seems probable that the effect of kallikrein or kinin on the intestinal absorption might be caused by the acceleration of active transport, but not by the increase of passive transport. Some amino acids or sugars are co-transported with Na^+ through the membrane.^{23,24)} Thus, we also suggest that the kallikrein-kinin system might affect the transport of electrolytes across the small intestine, as in the kidney. Further investigations on the role of intestinal kallikrein, which we have already purified,¹⁾ are in progress.

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