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Studies on Kallikreins. VII.[1] Effects of Kallikrein and Some Autacoids on *in Vitro* Transport of Valine and Sodium Ion

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The effect of kallikrein and some autacoids on the transport and uptake of valine in the rat small intestine was examined, *in vitro*, by the method of sac of everted intestine and by measurement of uptake of valine in the tissue. Kallidin and rat intestinal kallikrein (RIK) stimulated the intestinal transport of valine, like bradykinin and pancreatic kallikrein. Kallidin was more effective than bradykinin or Met-Lys-bradykinin. RIK stimulated the transport significantly when it was added to either the mucosal or serosal side. Moreover, the accumulation of valine into the small intestine was enhanced by bradykinin, and a similar result was obtained by the combined addition of rat pancreatic kallikrein and rat kininogen to the medium.

The net mucosa-to-serosa Na^+ transport was enhanced by the addition of bradykinin to the serosal side of the everted intestine, and Na^+ transport was further increased by adding bradykinin to the serosal fluid and glucose to the mucosal. The net Na^+ transport was suppressed by the addition of ouabain in the serosal fluid, but was restored to the normal level by bradykinin. Measurement of Na^+ in the intestine showed that addition of bradykinin to the medium reduced the uptake of Na^+ into the tissue and enhanced the efflux of Na^+ from the intestine to the medium. Glucose- or valine-evoked transmural potential difference in jejunum segment was elevated by the addition of kallidin to the serosal side, but the transepithelial resistance was not altered.

Thus, we suggest that Na^+ transport in the small intestine is stimulated by the action of kinins on the serosal side of the intestine, and this acceleration of Na^+-flux seems to be accompanied with the enhancement of amino acid and glucose transport.

Keywords——transport; uptake; intestine; sodium ion; valine; kinins; kallikrein; rat

It has been shown that various autacoids, such as prostaglandin, serotonin, *etc.*, affect the gastrointestinal motility or the intestinal blood flow,[2,3] but little is known about their effects on nutrients transport. We have investigated the effect of the kallikrein–kinin system on the intestinal nutrients transport and found that bradykinin, one of the autacoids, enhanced the mucosa-to-serosa transport of valine, methionine or glucose when it was present on the serosal side of the intestine.[1] Therefore, it is of interest to investigate whether other autacoids as well as kinins are relevant to solute transport in the intestine or not.

It is well-known that the influx of some sugars and amino acids into epithelial cells of the small intestine depends on the extracellular Na^+ concentration.[4,5] Many investigators[6–9] have reported that the transmural potential difference (PDt) across the small intestine was increased by the addition of a solute, which was transported actively, to the mucosal side of the intestine. Thus, if the action of kinins on the intestinal epithelial cells were associated with the active transport, kinins would stimulate not only Na^+ transport but also solute-evoked PDt change simultaneously.

The present paper deals with the effects of some autacoids on the intestinal transport of valine and with the influence of kallikrein extracted from rat pancreas or intestine on the transport. Furthermore, the effects of kinin on Na^+ transport and solute-evoked PDt change in rat small intestine were investigated in order to elucidate the mechanism of the enhanced intestinal solute transport.

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Experimental

Materials——Synthetic bradykinin, kallidin and Met-Lys-bradykinin were purchased from the Protein Research Foundation (Osaka). Serotonin and histamine dihydrochloride were from E. Merck (Germany). L-[U-^{14}C] valine (225 mCi/mmol), ^{22}Na–NaCl (carrier–free, 135 mCi/mmol) and acetylcholine chloride was from Daiichi Chemical Co. (Tokyo). Prostaglandin E_1 (PGE_1) and E_2 (PGE_2) were gifts from Ono Yakuhin Ltd. (Osaka). Rat pancreatic kallikrein (RPK; 143 KU/A_{280}) and rat intestinal kallikrein (RIK; 64.2 KU/A_{280}) were purified according to the methods of Hojima *et al.*[10] and Moriwaki *et al.*,[11] respectively. Rat kininogen from the pseudoglobulin fraction of heated plasma which was prepared by the method of Moriwaki and Schachter[12] was further purified by diethylaminoethyl (DEAE)-cellulose chromatography. Ouabain and *N*-2-hydroxylethylpiperazine-*N′*-2-ethansulfonic acid (HEPES) were the products of Boehringer Mannheim Co. (Germany) and Nakarai Chemicals Co. (Kyoto), respectively.

Methods——*In Vitro* Experiment with Rat Everted Intestine: The method was described in our previous paper.[13] The amount of transported valine was estimated from the radioactivity of ^{14}C-valine in the serosal fluid. Sodium concentration was determined with a Shimadzu AA-620 atomic absorption spectrometer (Kyoto). The net amount of Na^+ transported was calculated from the difference of Na^+ amount in the serosal fluid before and after the experiment.

Measurement of *in Vitro* Uptake of Valine and Na^+ into the Intestinal Tissue: An isolated intestinal segment of about 5 cm length from a Wistar rat was cut open along the mesenteric border. Two segments were preliminarily incubated at 37°C for 5 min in Krebs–Ringer bicarbonate solution (pH 7.4) and then transferred to a plastic cup containing 10 ml of the same solution with or without 4 μCi of ^{22}Na. The segments were incubated at 37°C for 1 to 20 min under constant gassing with 95% O_2 and 5% CO_2. After incubation, the wet weight of each segment was measured and the radioactivity of ^{22}Na in the segment was counted with an Aloka PSM-1206 well-type scintillation counter (Tokyo) with a sodium iodide crystal. The experiment on valine uptake into the intestinal segment was carried out as described above in Krebs–Ringer bicarbonate solution containing 2 μmol of valine and 0.3 μCi of ^{14}C-valine. The radioactivity in the segment was counted after combustion in an Aloka ASC-111 auto-oxidizer (Tokyo). The amount of valine or Na^+ taken up in the intestinal segment was expressed in μmol or μg per g wet intestine per a definite time, respectively.

Measurement of Transmural Potential Difference (PDt) and Transepithelial Resistance (Rt): The jejunum was removed from a Wistar rat fasted for 12 h, and cut along the mesenteric border. A small segment (about 1×1 cm) was mounted between the windows (0.28 cm^2 each) of two small chambers ($1\times1\times2$ cm and $0.75\times1\times2$ cm), and these chambers were clamped tightly. The mucosal and serosal chambers were filled with 1.0 and 0.75 ml of HEPES-buffered sulfate solution (Na_2SO_4 19.25, NaOH 1.95, $KHCO_3$ 2.5, $MgSO_4$ 1.0, $CaSO_4$ 2.0 and mannitol 243.0 mM, pH 7.4, 37°C aerated with 95% O_2 and 5% CO_2), respectively. PDt was measured with a preamplifier (Nihon Kohden, MEZ-8101) connected to Ag–AgCl electrodes in the mucosal and the serosal fluids *via* siliconated salt bridges filled with 1.5% agar in saturated KCl by setting the potential of the mucosal side to be 0 mV. Another set of Ag–AgCl electrodes was placed close to both sides of the jejunum segment to pass current and Rt was measured by applying pulses of 1 to 5 μA (duration about 1 s).

Results

Effect of Various Autacoids on Valine Transport

When 10 ng/sac of bradykinin, kallidin or Met–Lys-bradykinin was added to the serosal side of the everted intestine, bradykinin and kallidin enhanced valine transport, but Met–Lys-bradykinin did not affect the transport significantly (Fig. 1). Addition of kallidin caused about 1.6 times greater transport than that of the control at 30 min of incubation, and kallidin was the most effective among the three peptides in enhancing the transport. However, the addition of these peptides (100 ng/ml) to the mucosal fluid failed to stimulate the transport (data not shown). The effects of PGE_1, PGE_2, acetylcholine, histamine and serotonin on valine transport were also examined *in vitro*. Within the dose ranges tested from 10^{-4} to 10 ng/sac, the addition of 10^{-1} ng of PGE_1 and 10^{-3} ng of PGE_2 to the serosal fluid produced significant stimulation of valine transport at 30 min of incubation (Fig. 2). Both serotonin and histamine (10^{-4}—10^{-2} μg/sac) were ineffective, while about 2-fold increased transport compared to the control was observed with 1 μg/sac of acetylcholine (Fig. 2).

Effect of RPK and RIK on Valine Transport

When RPK or RIK (10^{-5}—10^{-2} KU/ml) was added to the mucosal fluid of the everted intestine, a stimulative effect on valine transport was obtained with 10^{-3} KU/ml of both kal-

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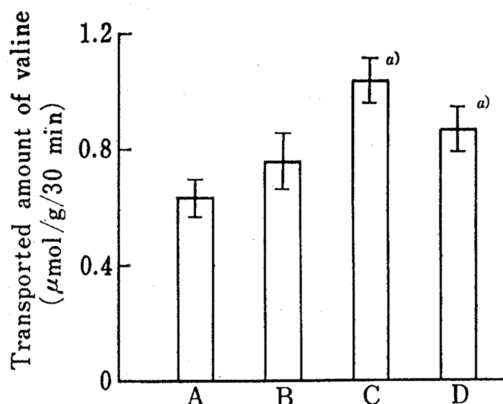

Fig. 1. Effects of Bradykinin, Kallidin and Met-Lys-Bradykinin on Valine Transport

A: control, B: Met-Lys-bradykinin (10 ng/sac), C: kallidin (10 ng/sac), D: bradykinin (10 ng/sac).
Valine concentration: 0.2 mM.
Mean ± S.E. of 8 sacs obtained from 4 rats.
a) Significant difference ($p<0.05$).

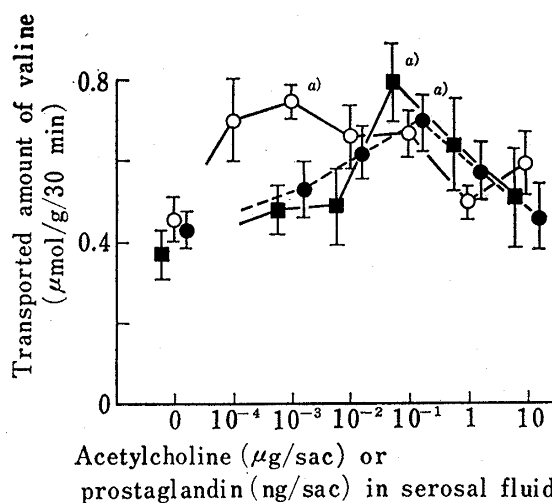

Fig. 2. Effects of Acetylcholine, Prostaglandin E_1 and E_2 on Valine Transport

—■—: acetylcholine, --●--: PGE_1, —○—: PGE_2.
Valine concentration: 0.2 mM.
Mean ± S.E. of 8 sacs obtained from 4 rats.
a) Significant difference ($p<0.05$).

likreins at 60 min of incubation. The transport profiles of valine in the presence of RPK and RIK were similar to that in the case of hog pancreatic kallikrein reported previously.[13] When 10^{-5} to 10^{-2} KU of RPK or RIK was added to 0.6 ml of the serosal fluid, the most effective dosages to increase the transport at 30 min incubation were 10^{-2} and 10^{-4} KU/sac of RPK and RIK, respectively. Figure 3 shows only the effect of RIK, since both kallikreins are similarly effective. Although RIK was found to be effective when added to the mucosal fluid as well as the serosal, its stimulating action on valine transport was stronger and faster when it was added to the serosal side.

Effect of Kallikrein and Kinin on Valine Uptake in the Intestinal Segment

The effect of bradykinin on ^{14}C-valine uptake in the isolated intestinal segment is shown in Fig. 4. Valine uptake increased about 1.5-fold in the presence of 0.1 or 1 ng/ml bradykinin at 15 min of incubation, but a higher concentration (10 ng/ml) was less effective. The synergism of RPK and kininogen was further examined. Though neither 10^{-4} KU of RPK nor 10 μg of the kininogen showed any effect on valine uptake, the combined addition caused about 1.5-fold greater uptake than that of the control (Fig. 5). The dose-response relationship between dosages of RPK or rat kininogen and the amount of valine accumulation in the intestine was not examined in detail. The results shown in Fig. 4 and Fig. 5 suggest that a suitable amount of kinin liberated from kininogen by kallikrein may be necessary to stimulate the accumulation of valine in the intestine.

Effect of Bradykinin on Na^+ Transport and Uptake

The effect of bradykinin on the net mucosa-to-serosa (M→S) Na^+ transport across the sac of everted intestine was examined without an Na^+ gradient (10 mM Na^+ in both mucosal and serosal fluids). The sodium concentration of the serosal fluid increased within 5 min, and reached a steady-steate after 5 min incubation. Addition of 10 ng of bradykinin to the serosal fluid caused a stimulative effect on Na^+ transport (M→S); the net Na^+ transport was enhanced about 1.4 times compared to the control after 5 min incubation (Fig. 6). In this experiment, a medium which contained 10 mM Na^+ was employed because this concentration was suitable to detect a slight change of Na^+ amount. Almost the same effect of bradykinin was found in another experiment in which ^{22}Na transport (M→S) was determined in Krebs–Ringer solu-

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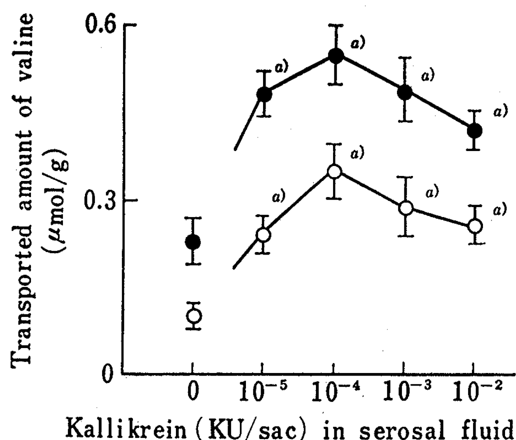

Fig. 3. Effect of Rat Intestinal Kallikrein on Valine Transport across the Everted Intestine

—○—: 15 min incubation, —●—: 30 min incubation.
Valine concentration: 0.2 mM.
Mean ± S.E. of 6 sacs obtained from 3 rats.
a) Significant difference ($p<0.05$).

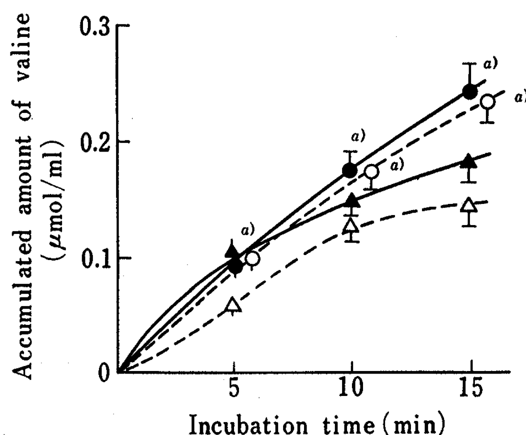

Fig. 4. Effect of Bradykinin on the Accumulation of Valine in Rat Jejunum

--△--: control, --○--: 0.1 ng/ml of bradykinin, —●—: 1 ng/ml of bradykinin, —▲—: 10 ng/ml of bradykinin.
Valine concentration: 0.2 mM.
Mean ± S.E. of intestinal segments.
a) Significant difference ($p<0.05$).

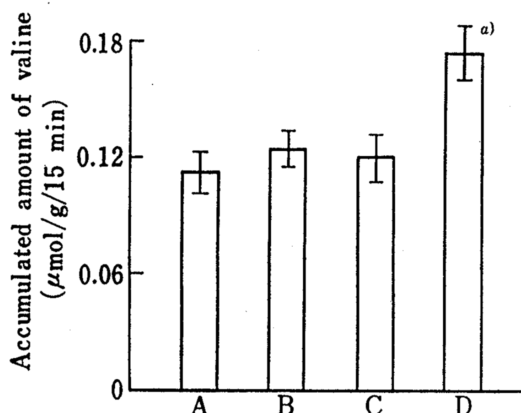

Fig. 5. Effect of the Combined Addition of Rat Pancreatic Kallikrein and Rat Kininogen on Valine Uptake in the Isolated Jejunum

A: control, B: 10 μg of kininogen, C: 10^{-4} KU of kallikrein, D: 10 μg of kininogen and 10^{-4} KU of kallikrein.
Valine concentration: 0.2 mM.
Mean ± S.E. of 6 intestinal segments.
a) Significant difference ($p<0.05$).

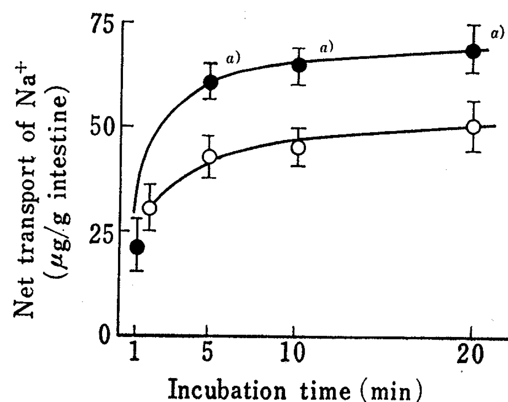

Fig. 6. Effect of Bradykinin on Net Na^+ Transport

The initial Na^+ concentration in serosal and mucosal fluids was 10 mM.
—○—: control, —●—: 10 ng of bradykinin.
Mean ± S.E. of 8 sacs obtained from 4 rats.
a) Significant difference ($p<0.05$).

tion which contained 143 mM Na^+ (Table I). This stimulative effect of bradykinin on the net Na^+ transport was not observed after a preliminary treatment of the serosal side of the intestine with 1 mM ouabain for 20 min (data not shown).

Addition of 5 mM glucose to the mucosal fluid augmented the net Na^+ transport, and about 1.25 times greater transport than that of the control was observed after 20 min. This glucose-evoked increase of the net Na^+ transport was further enhanced by adding 10 ng of bradykinin. Giving ouabain in the serosal fluid reduced the glucose-evoked Na^+ transport to the control level, but this inhibition was restored by the combined addition of ouabain and bradykinin (Fig. 7).

Effect of bradykinin on Na^+ uptake in the jejunum segment was investigated by a ^{22}Na tracer experiment. As shown in Fig. 8, the uptake of ^{22}Na in the intestine increased as a

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Table I. Influence of Bradykinin on ^{22}Na Transport in a Sac of Everted Intestine

Incubation (min)	% of ^{22}Na transport[a)] Control	(n)[b)]	BK 10 ng	(n)[b)]
1	0.31±0.02	(6)	0.38±0.06	(5)
5	2.46±0.13	(6)	2.79±0.09[c)]	(5)
10	5.45±0.12	(6)	5.88±0.38	(5)
20	12.1 ±1.2	(6)	12.3 ±1.5	(5)

a) (Total cpm in serosal fluid/initial cpm in mucosal fluid/g wet weight)×100.
b) Experimental number.
c) Significant difference ($p<0.05$).

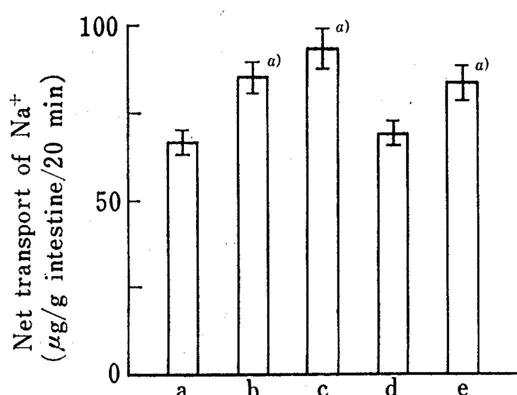

Fig. 7. Effects of Ouabain and Bradykinin on Net Na+ Transport in the Presence of Glucose

The initial Na+ concentration in serosal and mucosal fluids was 10 mM.
Glucose (G): 5 mM, bradykinin (BK): 10 ng, ouabain (Oua): 1 mM.
Mean±S.E. of 8 sacs obtained from 4 rats.
a) Significant difference ($p<0.05$).
a: control, b: G, c: G+BK, d: G+Oua, e: G+BK+Oua.

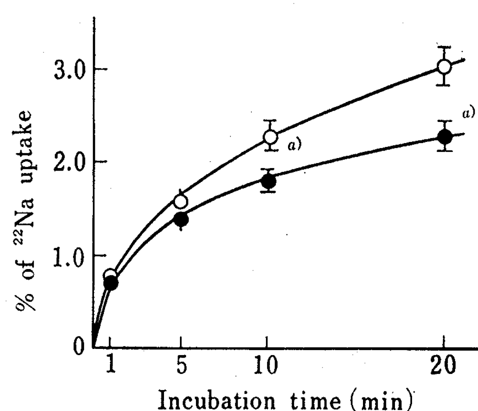

Fig. 8. Effect of Bradykinin on Accumulation of ^{22}Na in Rat Jejunum

—○—: control, —●—: 10 ng of bradykinin.
Mean±S.E. of 6 intestinal segments.
a) Significant difference ($p<0.05$).

function of time, but it was suppressed by 10 ng of bradykinin in the incubation medium. A significant reduction of ^{22}Na uptake was observed at 10 and 20 min.

The efflux of Na^+ from the intestine to the incubation medium was, however, accelerated by bradykinin. The intestinal segment was preliminarily incubated in Krebs–Ringer solution containing 10 μCi of ^{22}Na for 20 min in order to make the jejunum take up ^{22}Na sufficiently. Then the segment was transferred to ^{22}Na-free Krebs–Ringer solution or the solution containing 1 ng/ml bradykinin and incubated for various periods. Figure 9 shows the efflux profiles of ^{22}Na from the intestine to the incubation medium. After 1 min incubation, 26.5% of the initial radioactivity in the jejunum was lost from the tissue segment in the bradykinin-containing medium whereas only 14% was lost in the control. Therefore, it was confirmed that Na^+ efflux was significantly enhanced by bradykinin.

Effect of Kallidin on Glucose- or Valine-Evoked PDt and Rt

Glucose was added in a stepwise manner to the mucosal chamber and glucose-evoked PDt changes were recorded. The magnitude of PDt increase paralleled the elevation of glucose concentration (1.0—20 mM). The glucose-evoked PDt changes were more significant with kallidin-treated jejunum whose serosal side had been preliminarily contacted with kallidin for 4 min (Fig. 10). The maximum effect of kallidin was observed at 1 ng/0.75 ml of serosal fluid with 2—20 mM glucose, but the responses became smaller with higher doses of kallidin.

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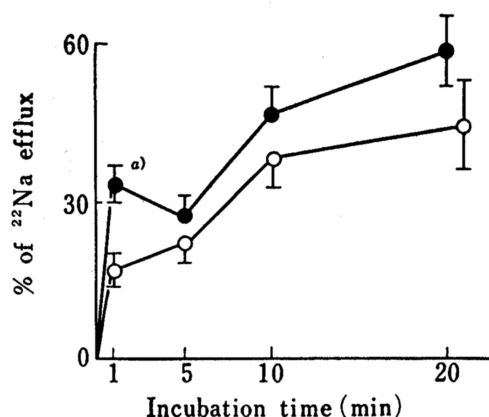

Fig. 9. Stimulatory Effect of Bradykinin on Na^+ Efflux from the Intestinal Segment

—○—: control, —●—: 10 ng of bradykinin.
Mean ± S.E. of 6 sacs obtained from 3 rats.
a) Significant difference ($p < 0.05$).

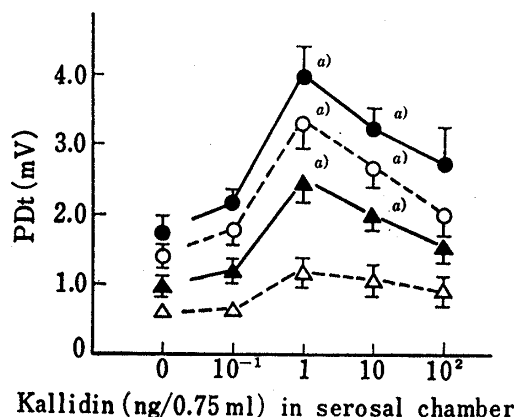

Fig. 10. Effect of Kallidin on Transmural Potential Difference (PDt) evoked by Various Amounts of Glucose

--△--: 2 mM glucose, —▲—: 5 mM glucose,
--○--: 10 mM glucose, —●—: 20 mM glucose.
Mean ± S.E. of 6 experiments.
a) Significant difference ($p < 0.05$).

Rt was measured during the course of these experiments. When glucose was not added to the mucosal chamber, the Rt values of the control and 1 ng kallidin-treated jejunum segments were 134.2 ± 9.22 and 127.3 ± 7.37 $\Omega \cdot cm^2$, respectively. Meanwhile, in the presence of 20 mM glucose, the Rt values of these jejunum segments were 122.1 ± 7.9 and 118.8 ± 6.2 $\Omega \cdot cm^2$, respectively. Thus, Rt changes were negligible under these conditions.

The effect of kallidin on valine-evoked PDt was also examined. Treatment of the serosal surface with 10 ng/0.75 ml of kallidin changed the magnitude of the valine (5 mM)-evoked PDt from 0.4 ± 0.02 mV (control) to 0.94 ± 0.06 mV.

Discussion

On the basis of the enhancing effects of kallikrein and kinin on nutrients transport, we have assumed that their action is attributable to stimulation of the active transport processes in the small intestine, and have called attention to the Na^+ transport in the intestine.[1] Recent studies suggest that there are at least three pathways of Na^+ influx into the epithelial cells of the small intestine; (1) a co-transport system of Na^+ and Cl^- which is inhibited by theophylline,[14] (2) a glucose- or amino acid-dependent Na^+ transport system,[4,5,15] and (3) a system independent of the above two systems.[16] In the present investigation, addition of bradykinin to the serosal fluid stimulated Na^+ transport in the absence of glucose (Fig. 5 and Table I), but bradykinin did not restore the ouabain-suppressed transport of Na^+ to the normal level (data not shown). Crocker and Willavoys[17] reported that in the presence of glucose, the transport of Na^+ and water across the everted sac of rat jejunum was stimulated only when bradykinin was added to the serosal fluid. In our investigation, we confirmed that glucose-mediated Na^+ transport was further accelerated by adding bradykinin to the serosal fluid (Fig. 7), and we found that the Na^+ efflux from the intestine was stimulated by bradykinin (Fig. 9). These results suggest that bradykinin may directly or indirectly affect Na^+, K^+-adenosine triphosphatase (ATPase) in the baso-lateral membrane of the epithelial cells, and thus accelerate the process of Na^+ efflux from the cells.

Okada *et al.*[18,19] reported that the presence of actively transported amino acids or sugars evoked a PDt change across the intestine without any change of Rt. Moreover, it is known that the magnitude of solute-evoked PDt change depends on Na^+ concentration in the mucosal side of the intestine, and PDt change is not evoked by solute in the absence of Na^+ in the

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mucosal fluid.[6-9] Considering these observation, we attempted to study the relationship between Na^+ movement across the membrane and kinin by an electrophysiological technique, and we confirmed that the glucose- or valine-evoked PDt change was significantly enhanced by adding kallidin to the serosal chamber without causing an Rt shift (Fig. 8), but an addition of kallidin to the mucosal chamber suppressed the glucose-evoked PDt changes slightly (data not shown). In order to obtain a larger PDt change,[9,20] SO_4^{2-} was used instead of Cl^- as a major anion of the incubation medium. As a result of using SO_4^{2-}, the co-transport of Na^+ and Cl^- became negligible and the conductance of the extracellular shunt pathway was also repressed. Therefore, these electrophysiological data suggest that kinin stimulates the electromotive force located along the serosal basement of the epithelial layer, and enhances the net mucosa-to-serosa flux of solute-coupled cation.

Several reports have described the participation of various autacoids on Na^+ transport in the intestinal tract,[21-24] but little is known about the effect of the autacoids on amino acid transport. Addition of kinins, PGE_1, PGE_2 and acetylcholine to the serosal fluid enhanced the mucosa-to-serosa transport of valine (Fig. 1 and Fig. 2), but histamine and serotonin did not stimulate the transport (data not shown). These results indicate that not every autacoid with similar pharmacological actions affects the nutrients absorption in the small intestine. It was reported that addition of pharmacological doses of PGE's to serosal fluid inhibited Na^+ transport in the intestine,[21,25,26] whilst much smaller doses of PGE's caused a stimulation of the unidirectional flux of Na^+.[26,27] Taking the relation between Na^+ flux and the uphill transport of amino acid into account, the present finding that relatively low doses of PGE's stimulate valine transport is of interest.

Finch and Hird[28] reported that the accumulation of various amino acids in rat small intestine reached a peak in about 15 min, and saturation phenomena were observed thereafter. In the present investigation, the same profile of valine uptake was obtained, and a combined addition of RPK and rat kininogen or bradykinin alone enhanced the accumulation of valine in the isolated jejunum segment (Fig. 3 and Fig. 4). These data suggest that the kallikrein–kinin system affects the concentrative process which is indispensable to the active transport.

In a series of our papers,[1,13] we demonstrated that kallikreins and kinins enhanced the intestinal absorption of various nutrients which were transported actively. This phenomenon seems to be due to the action of kinins on the serosal side of the intestine. The kallikrein–kinin system also stimulated Na^+ transport in the intestine, as described in this paper, and the effect of kinin on Na^+,K^+-ATPase might be the mechanism of this action.[1] Thus, it is most likely that kinins have a stimulating effect on Na^+ transport in the intestine, as in the kidney,[29] and the enhanced solute transport by kinins may be a result of the accelerated Na^+ efflux.

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