Preference of Peyer's Patches to Jejunal Epithelium for Intestinal Absorption of Oligopeptides, Tyrosylglycylglycine and D-Kyotorphin

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The intestinal absorption of oligopeptides, peptidase-degradable tyrosylglycylglycine (TGG) and peptidase-resistant L-tyrosyl-D-arginine (D-kyotorphin, D-KTP) across Peyer's patches (PP) in rabbit intestine were studied using an Ussing-type chamber and *in situ* closed perfusion methods. The clearance for the serosal appearance of intact TGG across PP by the Ussing-type chamber method was a little higher than that across the jejunal epithelium (JE). Meanwhile, the *in situ* closed perfusion experiment showed that the clearance for the plasma appearance of intact TGG across PP was about 10 times that of JE. Furthermore, it was shown that the clearance for the plasma appearance of D-KTP in PP was about twice that in JE by the *in situ* closed perfusion method, indicating that the membrane permeability of PP was higher than that of JE. Therefore, these results indicate that PP had less metabolic peptidase activity than JE, and that the PP was a suitable site for the intestinal absorption of oligopeptides, especially peptidase-degradable peptides.

Key words Peyer's patches; D-kyotorphin; tyrosylglycylglycine; intestinal absorption; intestinal metabolism

It is well known that the intestinal absorption of both peptides and proteins is poor. Our previous study reported that rabbit Peyer's patches (PP) transported high molecular peptides more effectively, with less hydrolysis than the jejunal epithelium (JE).²⁾ In this report, the transport of oligopeptides, tyrosylglycylglycine (TGG, a part of leucine enkephalin) and L-tyrosyl-D-arginine (D-kyotorphin, D-KTP) across PP was studied using two experimental methods which measured metabolic activity and membrane permeability. In rat intestine, aminopeptidase-degradable TGG³⁾ was too unstable to be transported,⁴⁾ whereas peptidase-resistant D-KTP, which was reported to be stable in the brain,⁵⁾ was stable enough to be transported in the small intestine.⁶⁾

MATERIALS AND METHODS

TGG and D-KTP were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Other reagents were of analytical grade.

Transport Experiment Using Ussing-Type Chamber JE and proximal PP were removed from male New Zealand white rabbits (2–3 kg) which had been fasted for about 24 h. The tissues were isolated from rabbit intestine under anesthesia induced by the intraperitoneal administration of urethane (dose of 3.3 g/rabbit). After the muscular layer was stripped from intestinal tissue, the intestinal tissue were mounted on the Ussing-type chambers using the same method described in our previous report.²⁾ Eleven milliliters of Ringer solution containing TGG (10 mM) was added to the mucosal side, and 11 ml of Ringer solution was added to the serosal side. Transport experiments were performed at 37 °C. One milliliter of Ringer solution on the serosal side was sampled every 20 min, then an additional 1 ml of Ringer solution was supplemented to maintain a constant serosal volume.

Transport Experiment by *in Situ* Closed Loop Perfusion The *in situ* closed loop perfusion experiment followed the method of Ho *et al.*⁷⁾ A glass perfusion cell

was inserted into the small intestine to face the PP or JE (0.4 cm²). After perfusion of Ringer's buffer for 10 min, 10 ml of peptide solution (10 mM) dissolved in Ringer's buffer was perfused at a flow rate of 5 ml/min. Blood from the intestinal vein was sampled every 10 min by a peristaltic pump at a flow rate of 0.08 ml/min.

Assay of TGG and D-KTP TGG, D-KTP and tyrosine were determined by HPLC, as reported previously.²⁾ Briefly, a column (Finepak SIL C18-5, Jasco, Tokyo) and fluorescence detector (821-FP, Jasco, Tokyo, Ex: 278 nm, Em: 305 nm) were used. The flow rate of the mobile phase composed of 4% acetonitrile in 10 mM ammonium formate (pH 4.5) was 1.0 ml/min.

Data Analysis Clearance for the serosal or plasma appearance (CL_{apr}) of TGG and D-KTP and its 95% confidence interval (95% CI) were calculated from the amounts transported during the period from 20 to 60 min.

RESULTS AND DISCUSSION

Figure 1 shows the time course of the serosal appearance of intact TGG in the Ussing chamber experiment. This indicates that the transported amount of intact TGG across PP was a little larger than that of JE (Fig. 1). CL_{apr} in PP and JE were 1.61×10^{-3} and 0.939×10^{-3} $\mu\text{l}/\text{min}/\text{cm}^2$, respectively (Table 1).

Figure 2 shows the time course of the appearance of intact TGG in plasma in the *in situ* closed perfusion experiment. CL_{apr} of TGG in PP and JE were 15.9×10^{-3} and 1.26×10^{-3} $\mu\text{l}/\text{min}/\text{cm}^2$, respectively (Table 1). This indicates that the absorption of TGG across PP was definitely higher than that across JE. CL_{apr} of TGG in JE was similar to that in the Ussing chamber experiment. Since TGG was reported to be metabolized by an aminopeptidase, bestatin-inhibitable peptidase,³⁾ the preference of CL_{apr} in PP to JE was considered to be derived from the lower activity of aminopeptidase in PP than JE. This is supported by the report of Hayakawa and Lee.⁸⁾ These results indicate that PP is a suitable site

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Fig. 1. Appearance of TGG on the Serosal Side across PP (a) and JE (b) by the Ussing-Type Chamber Experiment

TGG on the mucosal side: 10 mM. Data represent means $\pm$ S.E. ($n=3$).

Table 1. Comparison of Clearances (CL_{apr}) for the Serosal or Plasma Appearance of TGG and D-KTP in PP and JE

Peptides	Exp. method	PP ($\times 10^{-3}$ $\mu\text{L}/\text{min}/\text{cm}^2$)		JE ($\times 10^{-3}$ $\mu\text{L}/\text{min}/\text{cm}^2$)	
		CL_{apr}	95% CI	CL_{apr}	95% CI
TGG	Ussing chamber	1.61	-0.93—4.15	0.939	-3.61—5.48
TGG	<i>In Situ</i>	15.9	13.7—18.1	1.26	0.87—1.66
D-KTP	<i>In Situ</i>	338.0	292.8—383.1	94.4	68.5—120.2

Values were calculated from the data shown in Figs. 1—3. CI, confidence interval.

for intestinal absorption of not only high molecular peptides but also peptidase-degradable oligopeptides.²⁾

Figure 3 shows the time course of appearance of D-KTP in plasma across PP and JE in the *in situ* closed perfusion experiment. CL_{apr} in PP and JE were 0.338 and 0.0944 $\mu\text{L}/\text{min}/\text{cm}^2$, respectively (Table 1). This indicates that D-KTP was transported across PP to a greater extent than across JE. Meanwhile, in rat everted small intestine (jejunum), the absorption clearance of D-KTP was 0.465 $\mu\text{L}/\text{min}/\text{cm}^2$ (calculated from 0.516 $\mu\text{L}/\text{min}$ per cm of intestine⁶⁾ by assuming that the luminal radius is 0.178 cm⁹⁾, which was about one-tenth that of D-glucose (20 mM).¹⁰⁾ Since D-KTP was stable against peptidase,⁶⁾ the transportability of D-KTP, which does not contain a metabolic factor, represents the permeability preference of PP to JE.

The preference of TGG and D-KTP transport across PP in comparison to JE was observed in the *in situ* closed perfusion experiment. However, this preference was not observed in the Ussing chamber experiment as shown for TGG in this study, and as reported for D-KTP previously.²⁾ The Ussing-type chamber experiment in this study is devoid of blood flow or the muscular layer barrier, and thereby, the transport process to the serosal side was simple. Meanwhile, the *in situ* closed perfusion experiment preserved the blood flow, which is a primary factor for maintaining physiological conditions; in addition, the same transport pathway of drug from the luminal side into the intestinal vein as *in vivo* was observed. Furthermore, the CL_{apr} of TGG in JE in the Ussing chamber experiment was similar to that of the *in situ* closed perfusion experiment, indicating that the two experiments worked well and the results of PP absorption were not artificial. For the PP absorption study, therefore, the *in situ* closed perfusion experiment was considered to be more suitable than the Ussing-type chamber experiment. It also

Fig. 2. Plasma Appearance of TGG across PP (a) and JE (b) by the *in Situ* Closed Perfusion Experiment

TGG in perfusate: 10 mM. Data represent means $\pm$ S.E. ($n=3$).

Fig. 3. Plasma Appearance of D-KTP across PP (a) and JE (b) by the *in Situ* Closed Perfusion Experiment

D-KTP in perfusate: 10 mM. Data represent means $\pm$ S.E. ($n=3$).

seems that the more complicated transport process in the *in situ* closed perfusion experiment than in the Ussing chamber experiment resulted in variance of transported amounts in PP.

In conclusion, the absorption preference of PP to JE was shown in the *in situ* experiment for both TGG (about 10 times) and D-KTP (about twice), but in practice the area of PP is much smaller than that of JE in the intestine. Therefore, an efficient delivery to PP is necessary to actually improve the intestinal absorption of peptides through PP.

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