Transport of Low and High Molecular Peptides Across Rabbit Peyer's Patches

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The permeability of peptides across rabbit jejunal epithelium (JE) and Peyer's patches (PP) was compared. Kyotorphin (L-tyrosyl-L-arginine) was almost completely hydrolyzed during its membrane transport in both PP and JE, but [D-Arg^2]Kyotorphin (L-tyrosyl-D-arginine) was less hydrolyzed in PP than in JE. Since the permeability of intact [D-Arg^2]Kyotorphin was almost equal in PP and JE, no superiority of PP to JE was found for dipeptide transport. More intact fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (FITC-BSA) and concanavalin A (FITC-Con A) were transported in PP than in JE. At both absorption sites, the transport of the intact FITC-Con A was superior to that of the intact FITC-BSA. Colchicine significantly reduced the total transport of the intact and degradation forms of both peptides and the reduction ratio was greater in PP than in JE. Accordingly, it was suggested that PP can be used as prominent absorption sites for polypeptides since they have lower peptidase activity and higher endocytosis activity than JE.

KEY WORDS: Peyer's patches; absorption; degradation; polypeptide; dipeptide; endocytosis.

INTRODUCTION

High molecular peptide drugs are rapidly hydrolyzed by enzymes in the gastrointestinal (GI) tract and in the epithelial membrane, resulting in loss of activity. In addition, the large molecular mass and low lipophilicity of the peptides induce a low membrane permeability, and thus the most feasible oral dosing is not practical (1,2).

Intestinal Peyer's patches (PP) exist in the jejunum, the ileum, and the appendix of various animals, including humans, as small, white, and sparsely populated spots (3). PP include morphologically unique microfold or membranous cells (M cells). The short microvilli of M cells make the macromolecules readily accessible to the membrane surface, and a paucity of lysosomal hydrolyzing enzymes in M cells enables macromolecules to permeate virtually intact (4–6). Also, endocytosis of extracellular macromolecules through binding to specific receptors or by adsorption to membrane surface is a characteristic of M cells (7). Thus, PP are expected as possible absorption sites for oral administration of peptides. Macromocular absorption in PP has been observed morphologically by optical and electrical microscopes but few quantitative data are available. The Ussing-type chamber technique has shown that horseradish peroxidase (8) and glycoprotein (RU41740) (9) are more effectively absorbed in the intact form in piglet PP than jejunal epithelium (JE) and in rabbit PP than in duodenum, respectively.

In this study, to use PP as effective absorption sites for peptides, we examined the basic transport characteristics in rabbit PP, i.e., the permeability and degradation of dipeptide and polypeptide in the rabbit PP, and compared them with those of rabbit JE. Kyotorphin (L-tyrosyl-L-arginine) and [D-Arg^2]Kyotorphin (L-tyrosyl-D-arginine) were used since these two stereoisomers can be easily determined by HPLC and their enzymatic degradation can be compared (10). Bovine serum albumin (BSA) and the lectin concanavalin A (Con A), which are absorbed by fluid-phase endocytosis and adsorptive endocytosis respectively (7), were selected as representative polypeptides.

MATERIALS AND METHODS

Chemicals

Kyotorphin, [D-Arg^2]Kyotorphin, colchicine, fluorescein isothiocyanate (FITC)-labeled BSA (FITC-BSA; apparent molecular weight 67,000), and FITC-Con A (apparent molecular weight 102,000) were purchased from Sigma Chemical Co., St. Louis, MO. FITC-BSA was used after removing the fraction with a molecular weight of less than 50,000 by filtration (Centricut Mini, Krabou, Osaka, Japan). For FITC-Con A, the void fraction obtained from Sephadex G-25 was used. Both peptides were used after their fractions were lyophilized. Sephadex G-25 and G-75 were purchased from Pharmacia LKB, Biotechnology AB, Uppsala, Sweden. Other reagents were of analytical grade or better.

Membrane Permeation Experiments

JE and proximal PP were removed from male New Zealand white rabbits (2–3 kg) which were fasted for about 24 hr. The tissues were isolated under urethane anesthesia intraperitoneally administered at a dose of 3.3 g/rabbit. After the muscular layer was stripped from both tissues, they were mounted on the Ussing-type chambers (exposed area, 0.75 cm^2), in the same manner as discussed in our previous report (11,12). Eleven milliliters of Ringer solution containing various permeant compounds was added to the mucosal side, and 11 mL of Ringer solution was added to the serosal side. Permeation experiments were performed at 37°C. One milliliter of sample was removed from the serosal side at 20-min intervals, then 1 mL of Ringer solution was supplied to maintain a constant serosal volume. The concentrations of the permeant compound solutions were as follows: Kyotorphin and [D-Arg^2]Kyotorphin, 10 m*M;* FITC-BSA, 0.1%; and FITC-Con A, 0.01%. To inhibit the permeation, 0.1 m*M* colchicine was added to the mucosal side.

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Degradation of FITC-BSA and FITC-Con A

After completion of the permeation experiment (100 min) 3 mL each of the mucosal and serosal samples was applied to a Sephadex G-75 column (3-cm inner diameter × 36-cm length). Phosphate buffer (20 m*M*, pH 8.0) was eluted through the column at a rate of 1.0 mL/min, and 5 mL each of the fractions was collected to determine FITC-BSA and FITC-Con A fluorometrically. Serosal samples of FITC-Con A were lyophilized, then redissolved in a small amount of Ringer solution before application to the Sephadex G-75 column.

Assay

For Kyotorphin and [D-Arg^2]Kyotorphin, the sample solution was treated by final concentration of 5% $HClO_4$ and the supernatant obtained after removing the precipitate was analyzed by HPLC. The intact and degraded forms (tyrosine) were separately determined by HPLC. The HPLC conditions were as follows: column, JASCO Finepak SIL C18-5 (Japan Spectroscopic Co. Ltd., Tokyo); mobile phase, 4.0% (v/v) acetonitrile in 10 m*M* ammonium formate (pH 4.5); flow rate, 1.0 mL/min; fluorescence detector, 278 nm for exitation and 305 nm for emission; internal standard, L-phenylalanine; injection volume, 20 μL; retention time, 6.0 min for Kyotorphin, 9.4 min for [D-Arg^2]Kyotorphin, 5.4 min for tyrosine, and 8.2 min for phenylalanine. To determine the total amount of intact and degraded FITC-BSA and FITC-Con A, the serosal samples were diluted twice with Ringer solution and the concentrations were determined fluorometrically at 490 and 520 nm for excitation and emission, respectively. The concentrations in the Sephadex G-75 fractions were determined fluorometrically without dilution as described above.

RESULTS

No permeation of intact Kyotorphin was found in either PP or JE but the amount appearing in the serosal side as the degradation form, i.e., tyrosine (nmol/cm^2), over 100 min was greater in JE than in PP (338 ± 14 vs 183 ± 49). Contrary to Kyotorphin, however, permeation of intact [D-Arg^2] Kyotorphin into the serosal side was also found in both absorption sites. The permeability of total [D-Arg^2]Kyotorphin (intact form and tyrosine) was three times greater in JE than in PP (Table I). In JE, the permeability of intact [D-Arg^2]Kyotorphin was one-third of the total permeation, but in PP the permeabilities were similar, indicating that the degradation of [D-Arg^2]Kyotorphin in PP was minor.

Table I. Serosal Amount of Total (Intact and Degradation Forms) and Intact [D-Arg^2]Kyotorphin Across Jejunal Epithelium (JE) and Peyer's Patches (PP) over 100 Min[a]

	JE	PP
Total	241 ± 81	63.0 ± 24.6
Intact	68.0 ± 27.5*	42.6 ± 22.9[b]

[a] Each value represents the mean ± SE of four rabbits.
[b] Not significantly different from total ($P > 0.05$).
* $P < 0.01$ vs total.

Table II. Serosal Amount of Total (Intact and Degradation Forms) FITC-Bovine Serum Albumin (FITC-BSA) and FITC-Concanavalin A (FITC-Con A) Across Jejunal Epithelium (JE) and Peyer's Patches (PP) over 100 Min, and Percentage of the Intact Form to the Total[a]

	JE	PP
FITC-BSA		
Control		
Amount (μg/cm^2)	0.450 ± 0.054	0.506 ± 0.068
Intact percentage	8.37 ± 1.35	17.2 ± 1.8*
+ Colchicine		
Amount (μg/cm^2)	0.195 ± 0.024**	0.145 ± 0.005**
Intact percentage	6.20 ± 1.30	5.47 ± 1.21**
FITC-Con A		
Control		
Amount (μg/cm^2)	0.792 ± 0.209	1.432 ± 0.202*
Intact percentage	20.7 ± 2.7	67.3 ± 10.1*
+ Colchicine		
Amount (μg/cm^2)	0.452 ± 0.160	0.332 ± 0.144**
Intact percentage	16.9 ± 2.6	23.3 ± 5.3***

[a] Each value represents the mean ± SE of four to seven rabbits.
* $0.01 < P < 0.05$ vs JE.
** $P < 0.01$ vs the control.
*** $0.01 < P < 0.05$ vs the control.

The amount of total FITC-BSA and FITC-Con A (the intact and degradation forms) appearing in the serosal side over 100 min is shown in Table II. In both PP and JE, their amount was less than 0.1% of the initial mucosal amount. The amounts of FITC-BSA were almost equal in both sites, but the amount of FITC-Con A was significantly greater in PP than in JE.

The results of gel filtration for FITC-BSA and FITC-Con A collected from the serosal side of JE and PP for 100 min are shown in Figs. 1 and 2, respectively. The intact forms of both compounds were eluted at about 100 mL, and the degradation forms appeared at about 300 to 400 mL for FITC-BSA and at 200 to 400 mL for FITC-Con A. Since Sephadex G-75 can fractionate compounds less than about 80,000 in molecular weight, the single peak for FITC-Con A might include degraded forms of lower molecular weight than that of intact FITC-Con A. Thus, the fraction was further eluted through Sephacryl S-200, which can fractionate molecular weights between 5000 and 250,000. A single peak was eluted, showing no degradation of Con A (data not shown). Both compounds in the mucosal side were regarded as not being degraded during the permeation experiment, since a single peak of the intact form was obtained from the mucosal sample, in the same manner as shown in Figs. 1 and 2.

The percentage of the peak area of the intact form to the total in Figs. 1 and 2 is also listed as the control in Table II. The percentage for both compounds was significantly greater in PP than in JE. Comparing the percentages between FITC-BSA and FITC-Con A in both absorption sites, those for FITC-Con A were significantly greater than those for FITC-BSA (Table II).

Colchicine, which is known as an endocytosis inhibitor (13), decreased the serosal amount of total FITC-BSA and FITC-Con A and the decreasing ratio was greater in PP than

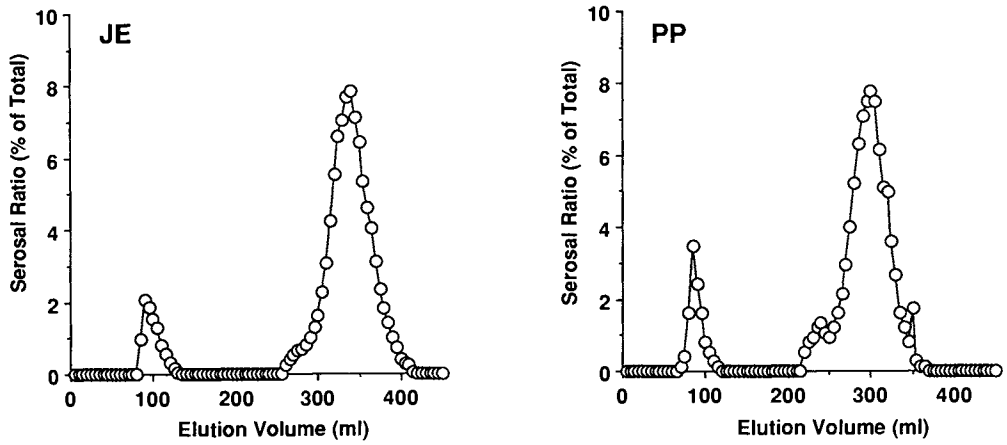

Fig. 1. Typical chromatographic profile (Sephadex G-75) of FITC-BSA transported across jejunal epithelium (JE) and Peyer's patches (PP) over 100 min. The fractions around 100 and 300–400 mL represent intact FITC-BSA and the degradation forms, respectively.

in JE. In PP, the colchicine effect was greater for FITC-Con A than for FITC-BSA.

DISCUSSION

Neither Kyotorphin nor [D-Arg^2]Kyotorphin was degraded in the mucosal side and no appearance of the degradation form, i.e., tyrosine, in the mucosal side was found for either dipeptide during the permeation experiment (data not shown). Therefore, the serosal amount of the sum of the intact form and tyrosine was used as a measure of the permeability of the peptides.

The permeability of Kyotorphin and [D-Arg^2]Kyotorphin was less in PP than in JE. Kyotorphin was almost completely degraded in both sites, but degradation of [D-Arg^2] Kyotorphin was minor in PP. These results suggest that PP is not superior to JE for dipeptide transport but low peptidase activity in PP is effective for peptide absorption as the intact form (Table I).

Since no degradation of FITC-BSA and FITC-Con A was observed in the mucosal side (data not shown), the serosal amount of the total (intact + degradation forms) was taken as a measure of permeability (Table II). The permeability of FITC-BSA was similar in PP and in JE. That of FITC-Con A, on the other hand, was greater in PP than in JE. The appearance ratios (%) of the intact forms to the total for both compounds were greater in PP than in JE (Table II, Figs. 1 and 2), suggesting that both peptides can be transported with less hydrolysis in PP than in JE.

Since the inhibitory effect of colchicine on the serosal amount of total FITC-BSA or FITC-Con A was significantly greater in PP than in JE (Table II), both compounds were shown to be more effectively transported by endocytosis in PP than in JE. Ducroc *et al.* used the metabolic inhibitors, 2-deoxyglucose and sodium azide, to examine the endocytic transport of horseradish peroxidase (8) and indicated that PP have more effective endocytosis activity, in agreement with our results. Also, our results indicated that the inhibitory

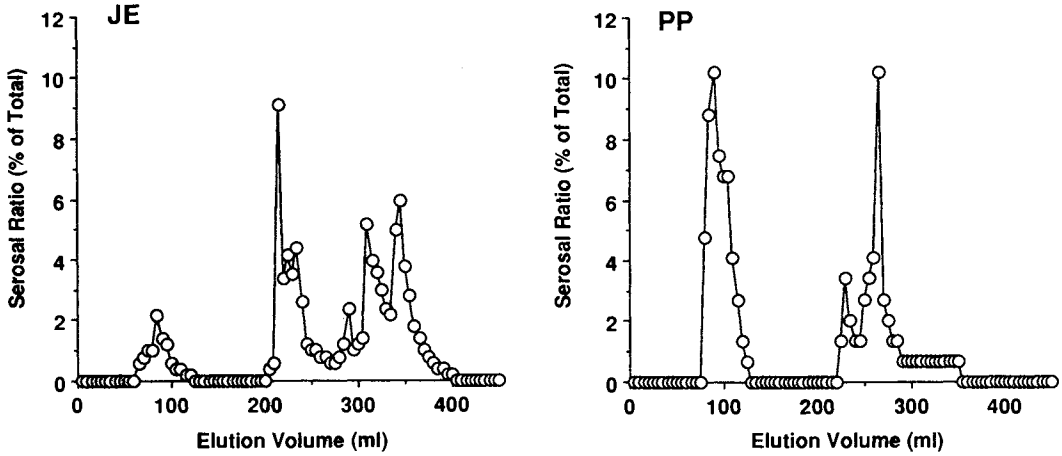

Fig. 2. Typical chromatographic profile (Sephadex G-75) of FITC-Con A transported across jejunal epithelium (JE) and Peyer's patches (PP) over 100 min. The fractions around 100 and 200–400 mL represent a higher molecular fraction including intact FITC-Con A and a lower molecular fraction including the degradation forms, respectively.

effect of colchicine in PP was greater for FITC-Con A than for FITC-BSA. Accordingly, Con A is more specifically transported by endocytosis than BSA. Although it is reported that BSA is transported by fluid-phase endocytosis through PP, the degradation during transport was analyzed simply by phosphotungstic acid precipitation of intact ^{125}I-BSA (14). Lectins such as Con A are reportedly taken up into PP by adsorptive endocytosis (7,15). However, this was demonstrated visually by electron micrography and no chemical analysis was given for the transported compound qualitatively or quantitatively. In this study, we quantitatively showed that although PP have less absorption ability for low molecular peptides, they can transport high molecular peptides more effectively with less hydrolysis than JE, and that this advantage is prominent for Con A. These results suggest that PP can be used as prominent absorptive sites of polypeptides if targeting to PP is successful. Because of the absorbability of FITC-Con A, conjugation with Con A appears promising.

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