PepT1 mRNA Expression Is Induced by Starvation and Its Level Correlates with Absorptive Transport of Cefadroxil Longitudinally in the Rat Intestine

Kazumasa Naruhashi,[1] Yoshimichi Sai,[1,2] Ikumi Tamai,[1,2] Nagao Suzuki,[1] and Akira Tsuji[1,2,3]

Received February 6, 2002; accepted 24 June 2002

Purpose. To establish how closely intestinal transport activity for beta-lactam antibiotics is correlated with PepT1 expression, absolute expression level of PepT1 mRNA and transport activity were determined longitudinally in the small intestine of fed and starved rats.
Methods. For evaluation of absolute expression levels of PepT1 mRNA, quantitative RT-PCR by LightCycler® was used. The transport function was determined by quantifying the absorptive transport of cefadroxil across intestinal tissue sheets in a Ussing chamber.
Results. PepT1 mRNA expression was highest at the lower region and lowest at the upper region in the fed rats. The value of PepT1 was about 1/5~1/6 of that of GAPDH. The expression level in the starved rats was increased in all segments, but more profoundly in the upper region. Cefadroxil transport across intestinal tissue was higher in the lower region and lower in the upper region in fed rats, and increased in the upper region in starved rats. An excellent correlation was observed between expression levels and the permeability coefficients ($r^2 = 0.859$, $p < 0.05$).
Conclusions. The intestinal transport of cefadroxil is directly proportional to PepT1 expression, suggesting that the PepT1 expression level in the rat small intestine is the major determinant of the absorption of peptide-like compounds.

KEY WORDS: PepT1; cefadroxil; intestine; mRNA; transport.

INTRODUCTION

The existence of a peptide transport system in the small intestine has been well established, and this system plays a major role in the absorption of nitrogen (1). It is also well known that certain hydrophilic beta-lactam antibiotics are transported via this peptide transport system (1). PepT1, an oligopeptide transporter, has been isolated from the rabbit (2), human (3), rat (4,5), and mouse (6) small intestines. Injection of PepT1 cRNA into *Xenopus laevis* oocytes induces electrogenic transport activity, which results as a consequence of peptide/H^+ cotransport. The substrate specificity of PepT1 is relatively broad: the transporter recognizes not only di/tripeptides and peptide mimetics (7,8) but also some drugs that do not have peptide structure, such as valacyclovir (8,9). The kinetic properties of this transporter have been studied thoroughly, and the transport activity of PepT1 for di/tripeptides and beta-lactam antibiotics was found to be closely related to the intestinal transport and *in vivo* absorption (10). In addition, uptake of glycylsarcosine is negligible in oocytes injected with rat intestinal mRNA with antisense PepT1 cRNA (10). Thus, it is accepted that PepT1 contributes predominantly to intestinal absorption of oligopeptides and beta-lactam antibiotics in the small intestine. Recently, Chu *et al.* (11) documented a correlation between the expression level of PepT1 protein and absorptive function in human PepT1-transduced Caco-2 cells and rats.

It is well known that the functions of the intestine vary longitudinally. In relation to this, the expression patterns of some enzymes and transporters have been reported to be heterogeneous. In addition, the absorptive function of the intestine is altered under various conditions, such as dietary regulation (12,13), starvation (14), anticancer drug-induced intestinal injury (15,16), sigma-ligand (17), insulin (18), *etc.* Intestinal expression patterns of PepT1 have also been reported in terms of mRNA and protein (19,20). However, because of methodological reasons, these reports only provide information concerning relative levels of PepT1. Recently, a versatile approach to quantifying absolute level of mRNA expression by means of PCR (LightCycler®) was developed by Roche Diagnostics GmbH (Manheim, Germany), which has surmounted some of the methodological considerations that limit the usefulness of quantifying mRNA.

The purpose of this study was to examine the pattern of distribution of absolute expression level of PepT1 mRNA longitudinally in the small intestine of freely fed rats and starved rats. We also investigated whether the absorptive functional activity correlates with PepT1 expression level. Intestinal regional dependence of absorption is usually studied by the closed intestinal loop method or by using isolated intestinal tissue in the unit of length or apparent surface area. Therefore, it was thought preferable in the present study to quantify the expression level of transporters in a unit of length, but not tissue weight or protein amount, to evaluate the relationship between the absorptive functional ability and expression of transporters. Accordingly, to quantify the expression level of PepT1, the small intestine was divided into eight segments, and the absolute level of PepT1 mRNA expression in each segment was measured by a real-time PCR method using LightCycler®, which makes it possible to quantify sensitively, precisely, and reproducibly the target mRNA (21). Then, to confirm that peptide transport in rats can be largely ascribed to the intestinal PepT1, the functional activity in corresponding segments of the small intestine was estimated by measuring cefadroxil transport across rat small intestinal tissue mounted in a Ussing chamber, in addition to measuring the disappearance of cefadroxil from intestinal closed loops.

MATERIALS AND METHODS

Materials

Cefadroxil was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were obtained commercially and were of reagent grade.

[1] Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920-0934, Japan
[2] CREST, Japan Science and Technology Corporation, 4-1-8 Motomachi, Kawaguchi 332-0012, Japan
[3] To whom correspondence should be addressed. (e-mail: tsuji@kenroku.kanazawa-u.ac.jp)

ABBREVIATIONS: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPLC, high-performance liquid chromatography; MES, 2-(*N*-morpholino)ethanesulfonic acid; PCR, polymerase chain reaction; PepT1, proton-coupled oligopeptide transporter; RT, reverse transcription.

Animals

Six- to 7-week-old male Sprague-Dawley rats were purchased from Japan SLC (Hamamatsu, Japan). Control (fed) rats were given free access to normal chow and water until used for experiments. Starved rats received no food but had free access to water for 48 h before experiments. To avoid the effect of circadian rhythm, all animals were killed at 10 a.m. of the day of experiments. Animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

RNA Preparation

Rats were anesthetized with intraperitoneal administration of pentobarbital (50 mg/kg), and then the whole small intestine was immediately excised and divided into eight segments of the same length, numbered as 1 (upper) to 8 (lower). The length of each segment was 14.7 ± 0.3 and 13.2 ± 0.4 cm in fed and starved rats, respectively. Each segment was opened, and the content was washed out with ice-cold saline solution. Excess liquid was removed with filter paper. Each segment was frozen in nitrogen liquid and crushed. The crushed tissue was mixed with buffer RLT/beta-mercaptoethanol (100:1) and homogenized by using a syringe equipped with a 20-gauge needle. Total RNA was extracted with RNeasy Mini Kits (Qiagen, Venlo, The Netherlands) according to the manufacturer's protocol.

RNA purity was evaluated spectrophotometrically by using the O.D.260/O.D.280 ratio, and samples having a value of >1.7 were used. The concentration of total RNA was calculated from the O.D. 260 value.

Total RNA was subjected to RT reaction by using M-MLV Reverse Transcriptase (Life Technologies Inc., Gaithersburg, MD, USA).

Standard of PepT1 and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) for PCR

mRNA extracted from rat intestine was reverse transcribed to cDNA. This cDNA was amplified by heat-block PCR to the saturation point with specific primers under the same conditions described below. The amplified product was purified with a QIAquick purification kit (Qiagen). After agarose gel electrophoresis, the amount was determined from the absorbance (A260) measured with a Bio Photometer (Eppendorf), and this material was used as the standard cDNA. It was serially diluted from 10^8 to 10^2 to copies/μL with salmon sperm DNA so that a standard curve could be obtained for quantification.

Quantitative RT-PCR Method

Quantification of the mRNA message coding for GAPDH and PepT1 was performed by using LightCycler® technology (Roche Diagnostics). Primer sets were as follows: GAPDH forward primer (5′-GTTACCAGGGCTGCCTTCTC-3′) and GAPDH reverse primer (5′-GGGTTTCCCGTTGATGACC-3′), which produce a 168-bp amplicon (21); and PepT1 forward primer (5′-CACAGCGCCAGCAACTATCA-3′) and PepT1 reverse primer (5′-GATATTACCGATGGCCACGG-3′), which produce a 360-bp amplicon. The cDNA from 10 ng of RNA was used for GAPDH- or PepT1-specific PCR. The PCR cycles were as follows: GAPDH: initial denaturation at 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 0 s and combined annealing extension at 60°C for 10 s and 72°C for 0 s. PepT1: initial denaturation at 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 0 s and combined annealing-extension at 60°C for 0 s and 72°C for 10 s, followed by fluorescence emission reading at 78°C for 10 s. Each sample of cDNA was diluted with salmon sperm DNA to three different concentrations and used for PCR. The quantification value was calculated from the average values of three determinations of the three different dilutions.

Intestinal Absorption Study by the Loop Method

The procedure has been described in detail elsewhere (22). Closed loops (14 cm) of segments 1, 4, and 8 were prepared by ligation at both ends. Seven hundred microliters of isotonic 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (5 mM KCl, 100 mM NaCl, 10 mM MES, 85 mM mannitol, polyethylene glycol 0.01%; pH 6.4; the osmolarity was 290 mOsm/kg) containing cefadroxil (1 mM) was administered into each loop as a bolus. After 15 min, the solution in the loop was collected. Then, the loop was rinsed and the sample was collected, giving a total volume of 5 mL with isotonic MES buffer. The pH of the sample was measured with a pH meter. The fraction of absorbed cefadroxil was estimated from the difference between the dose administered and the remaining amount in the loops.

Transport Experiments by the Ussing-Type Chamber Method

Rat intestinal tissue sheets were prepared as described previously (22). The exposed area was 0.5 cm^2. The volume of bathing solution on each side was 5 mL, and the temperature was maintained at 37°C. The test solution was composed of 128 mM NaCl, 5.1 mM KCl, 1.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 21 mM $NaHCO_3$, 1.3 mM KH_2PO4, 10 mM NaH_2PO_4, and 5 mM glucose and was gassed with 95% O_2/5% CO_2 before and during the transport experiments.

Analytic Method

The amount of cefadroxil was measured by high-performance liquid chromatography (HPLC). A TSK gel ODS-80Ts (150 × 4.6 mm I.D., Tosoh, Tokyo, Japan) was used as the analytic column heated at 35°C. The ultraviolet detector (UV-875; Japan Spectroscopic Co., Kyoto, Japan) was set at 240 nm. The mobile phase consisted of 0.1 M CH_3COOH, including 0.01 M sodium 1-pentanesulfonate (adjusted to pH 3.0 with NaOH)-acetonitrile (93:7, v/v), and the flow rate was 0.9 mL/min. The assay was linear over the concentration range of 0.5–50 μM.

The amount of inulin was measured by the method previously reported (23).

Data Analysis

Expression levels of mRNA are given as the copy numbers in each segment (copy/segment) or per unit mRNA amount (copy/μg RNA). Transport was estimated in terms of

permeation ($\mu L/cm^2$) obtained by dividing the amount transported ($\mu mol/cm^2$ or $\mu g/cm^2$) by the initial concentration of test compound on the donor side ($\mu mol/\mu L$ or $\mu g/\mu L$). The permeability coefficient (cm/s) was obtained from the slope of the linear portion of the permeation-time curve. All data are expressed as means ± SEM, and statistical analysis was performed by using Student's *t* test. A difference between means was considered to be significant when $p < 0.05$.

RESULTS

Quantitative Analysis of RNA Amount along the Small Intestine

In fed rats, no significant difference in the total RNA level was observed among the eight segments. Compared with fed rats, a tendency to decrease was observed in the lower region of the small intestine in starved rats. By contrast, the total RNA level showed a tendency to increase in the upper to middle segments of the small intestine from starved rats.

Expression of GAPDH mRNA along the Small Intestine

First, we quantified the expression level of mRNA of GAPDH, which is a housekeeping gene and is conventionally used as an internal standard. The content of GAPDH was expressed per total RNA and per segment (Fig. 1).

In fed rats, the expression level was 2-fold higher in the upper region than in the lower region. In starved rats, the expression level was decreased in the upper region compared with that in fed rats.

Expression of PepT1 mRNA along the Small Intestine

This study aimed to compare the expression level of PepT1 mRNA with absorptive function in each segment. Because there were regional differences of GAPDH mRNA expression in this study, we evaluated the expression level of PepT1 per segment. This evaluation reveals the *in vivo* regional differences in absorptive function. Figure 2 shows the expression level of PepT1 mRNA in each segment of small intestine from fed and starved rats. In fed rats, the expression level in segment 7 was highest, being ~3.7 times that in segment 1, which showed the lowest expression. In starved rats, the expression level was significantly increased in segments 1–5 compared with that in fed rats. The expression level of PepT1 relative to GAPDH was 1/2.8–1/21.6 and 1/1.6–1/8.4 in fed and starved rats, respectively.

Absorption of Cefadroxil from Rat Small Intestinal Loops

The absorption of cefadroxil from the intestine was examined by the *in situ* closed loop method. Cefadroxil is hydrophilic and is a representative substrate for PepT1 (9). Because PepT1 expression was low in the upper region and high in the lower region, and the induction of PepT1 expression by starvation was prominent in the middle region, we chose segments 1, 4, and 8 for this evaluation. Table I shows cefadroxil absorption and pH changes. In fed rats, the extent of absorption in 15 min was variable among the segments, being fastest in segment 4 and slowest in segment 8. The apparent absorptive function did not correlate with the expression level of PepT1. Although the experimental solution (MES buffer) was adjusted to pH 6.0, the changes of pH were considerable. Indeed, the pH of the administered solution at the end of the experiment was 6.51, 6.29, and 7.90 in segments 1, 4, and 8, respectively. Because protons are the driving force for PepT1

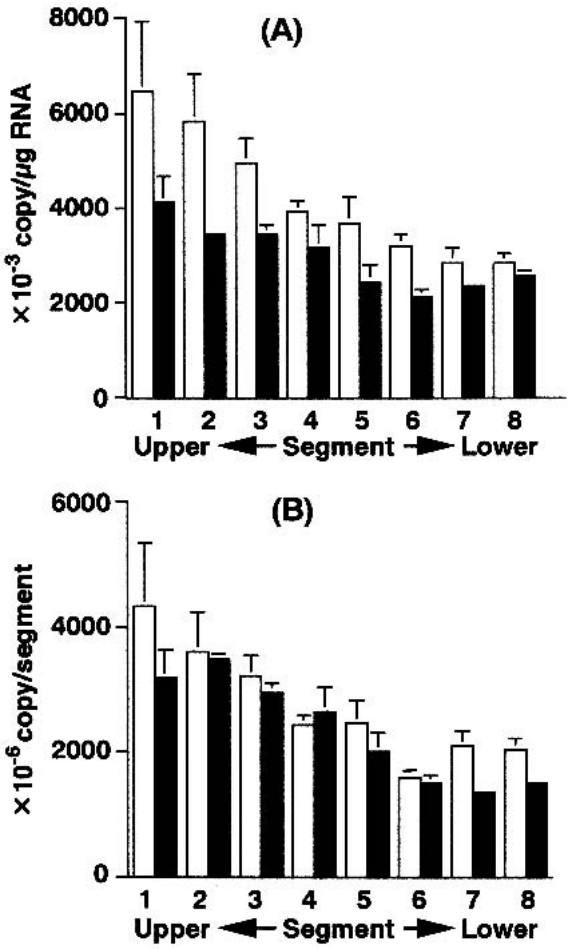

Fig. 1. Expression level of GAPDH mRNA in each segment from small intestine of fed and starved rats. The quantified data are expressed per total RNA (A) or per segment (B). Open and closed columns represent fed and starved rats, respectively. Each column represents means ± SEM of three rats.

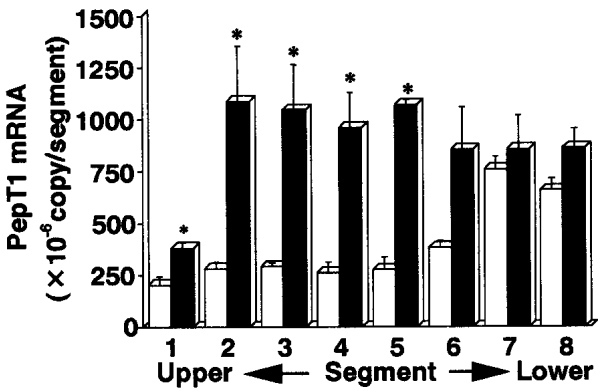

Fig. 2. Expression level of PepT1 mRNA in each segment from small intestine of fed and starved rats. Open and closed columns represent fed and starved rats, respectively. Each column represents means ± SEM of three rats. *$p < 0.05$, significant difference between fed and starved rats.

Table I. Cefadroxil Absorption and pH Changes Using the *in Situ* Loop Method in Various Regions of Intestine from Fed Rats

Region	Absorption (%)	pH after experiment
Upper (segment 1)	71.17 ± 1.49	6.51
Middle (segment 4)	85.65 ± 1.33	6.29
Lower (segment 8)	39.91 ± 2.92	7.90

Note: In the loop absorption experiment, 0.7 mL of isotonic MES buffer containing 1 mM cefadroxil (pH 6.0) was administered into a loop of the upper (segment 1), middle (segment 4), or lower (segment 8) region. Luminal fluid in each loop was collected 15 min after administration, and the pH of the solution was measured. The fraction absorbed was estimated as the difference between dose administered and remaining amount in the loops. Each value represents means ± SEM of three experiments.

and it is difficult to maintain a constant pH in the *in situ* closed loop method, we decided to use the Ussing chamber method to evaluate the absorptive function at constant pH.

Absorptive Transport of Rat Small Intestinal Tissue in a Ussing Chamber

Table II shows the absorptive transport of cefadroxil across rat intestinal tissue of segments 1, 4, and 8, as evaluated in a Ussing chamber. The pH at the end in this method was not significantly altered from the initial pH. In fed rats, in the presence of pH gradient (mucosal/serosal = 6.0/7.4), permeability coefficients of cefadroxil (1 mM) showed a regional dependence with an increase in the order of segment 1 < segment 4 < segment 8. When glycylsarcosine (5 mM) was added in the donor solution, the permeability of cefadroxil was significantly decreased in all three segments. These results indicate that the absorptive transport in this experimental method can be ascribed to active transport via PepT1.

In starved rats, the permeability coefficient showed a tendency to increase in segment 1 and was significantly increased in segment 4, where the PepT1 induction by starvation was most prominent.

In fed rats, segments 1, 4, and 8, transport of inulin across intestinal tissues was significantly smaller than transport of cefadroxil.

Correlation between Absorptive Transport of Cephadroxil across Rat Intestinal Tissue Mounted in the Ussing Chamber and PEPT1 Expression in Rat Intestine

When the absorptive transport of cefadroxil was evaluated with the Ussing chamber method and compared to intestinal PepT1 mRNA expression levels in fed and starved rats, a significant correlation was observed with $r^2 = 0.859$ ($p < 0.05$) (Fig. 3). Inulin transport, which indicates the paracellular transport by passive diffusion, across rat intestinal tissue was approximately equal to the y-axis value of the extrapolated regression line.

DISCUSSION

The aims of the study were to evaluate the absolute value of PepT1 mRNA expression along the intestinal tract and to demonstrate whether the expression level is well correlated with the transport activity of PepT1. We quantified the absolute expression level by real-time PCR method by LightCycler®, and the transport activity was determined by cefadroxil transport across rat intestinal tissue mounted in a Ussing chamber. Thus, we determined the distribution pattern of PepT1 mRNA expression along the small intestine in absolute, not relative, terms in a unit of length and examined its correlation with absorptive function for the PepT1 substrate cefadroxil. Thus, we determined the distribution pattern of PepT1 mRNA (absolute values, not relative) along the length of the intestine and made a correlation comparison with the absorptive function for the PepT1 substrate cefadrxil.

Although expression levels of various genes and proteins, including transporters such as PepT1 (19,20), were previously studied, absolute values were obtained because of methodologic considerations. Ogihara *et al.* (19) documented PepT1 expression in the intestine by Western blotting and reported staining for PepT1 in the jejunum and ileum were stronger than that in the duodenum. Although denser bands indicate higher expression, this is a qualitative analysis. Regarding mRNA, the conventional RT-PCR method relies on endpoint analysis, which is, at best, semiquantitative because of variations in amplification efficiency in the later cycles of the PCR. Moreover, most of the studies using this approach evaluate the expression level after a correction based on expression of an internal standard, such as beta-actin or GAPDH.

Table II. Cefadroxil and Inulin Transport across Rat Intestinal Tissue from Various Regions as Shown with the Ussing Chamber Method

	Permeability coefficient ($\times 10^6$ cm/s)		
	Upper (segment 1)	Middle (segment 4)	Lower (segment 8)
Control	3.26 ± 0.29	4.99 ± 0.50	8.20 ± 1.01
+ glycylsarcosine (5 mM)	0.456 ± 0.244*	2.10 ± 0.64*	1.74 ± 0.17*
Starvation	5.88 ± 0.77	10.9 ± 1.5*	8.81 ± 0.92
Inulin	1.75 ± 0.31*	2.58 ± 0.42*	1.89 ± 0.17*

Note: The time course of cefadroxil (1 mM) transport was evaluated with the Ussing chamber method. The experimental solution was adjusted to pH 6.0 and 7.4 on the mucosal and serosal sides, respectively. The time course of inulin (10 mg/mL) transport was also evaluated with the Ussing chamber. The pH of the experimental solution was adjusted to 7.4 on both the mucosal and serosal sides. Each value represents means ± SEM of 4–9 experiments. *$p < 0.05$, significantly different from the corresponding control.

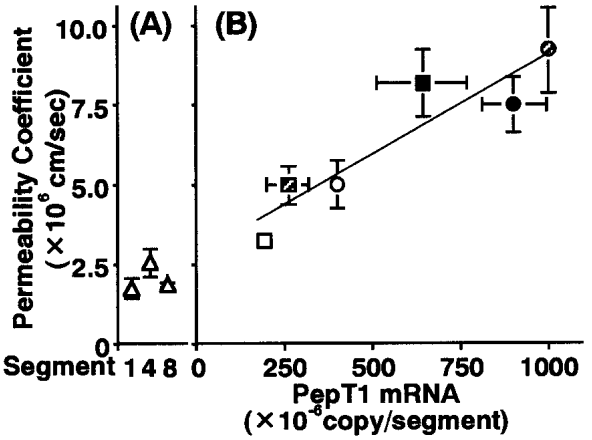

Fig. 3. Correlation between PepT1 mRNA expression level and permeability coefficient of cefadroxil in intestinal tissues of fed and starved rats. (A) Inulin permeability in the rat intestinal tissue mounted in the Ussing chamber. Absorptive permeability in the intestinal tissues from control rats. (B) Absorptive permeability of cefadroxil and PepT1 expression level. Segment 1 (□), segment 4 (▨) and segment 8 (■) from control rats: Segment 1 (○), segment 4 (◍) and segment 8 (●) from starved rats. A significant correlation was observed ($r^2 = 0.859$, $p < 0.05$). Each point represents means ± SEM of 4– 9 experiments.

We report here for the first time the distribution of PepT1 mRNA along the small intestine according to its absolute expression level, as determined by real-time detection of PCR amplification using LightCycler®. We quantified GAPDH and PepT1 expression levels in the small intestine longitudinally. Regional differences in GAPDH expression were found in both fed and starved rats (Fig. 1). Therefore, correction based on the GAPDH expression level may not be adequate, and so we evaluated the expression level per segment along the intestine. PepT1 mRNA expression was high in the lower region and low in the upper region (Fig. 2). It is assumed that PepT1 functions more efficiently in the upper region than in the lower region because the luminal pH is lower in the upper region. The reason for the higher expression level of PepT1 mRNA in the lower region may be to allow the animal to take up peptides throughout the whole small intestine. The expression levels of PepT1 mRNA were about 1/5–1/6 of those of GAPDH mRNA. Among transporters, PepT1 mRNA expression was 5–8 times higher than MRP2 or MDR1a mRNA expressions (unpublished data). Therefore, PepT1 seems to be the predominant transporter in terms of mRNA expression. We also quantified the expression level in starved rats, because it has been reported that starvation induces PepT1 expression in the rat small intestine (14). To avoid unidentified circadian effects, starvation was initiated at 10 a.m., and all experiments were performed 48 h later. The induction of PepT1 mRNA expression was observed in all segments by starvation and pronounced in the middle to upper segments (Fig. 2). Shiraga *et al.* (13) reported that amino acids and dipeptides directly stimulate the transactivation of the PepT1 gene promoter and induce PepT1 expression. We consider that the induction of PepT1 mRNA by starvation and that by amino acids and dipeptides are attributable to different regulatory mechanisms, although the precise mechanism(s) is still unknown. It has been reported that intestinal peptide transport is relatively resistant to starvation or malnutrition, compared with amino acid transport (14). Therefore, the increase in PepT1 expression by starvation may be associated with resistance to starvation: a starved animal is short of nitrogen for survival, and by inducing PepT1 expression, the animal can take up nitrogen more efficiently.

It is believed that peptide mimetics are absorbed via PepT1 in the small intestine. This is because the kinetic parameters estimated from *in situ* and *in vivo* studies are consistent with those from characterization studies (e.g., using oocytes injected with PepT1 cRNA) (9). However, it remains necessary to confirm a direct correlation between PepT1 expression level and *in vivo* absorptive function. Therefore, we evaluated the absorption capacity via PepT1 by measuring the absorption of cefadroxil, a good substrate for PepT1.

Despite the lower expression level of PepT1 mRNA in segment 1, absorption from segment 1 was unexpectedly higher than that from segment 4 or 8 in the closed loop absorption experiment (Table I). Sawamoto *et al.* (24) also found in a conventional *in situ* loop study, the absorption rate constant of cephalexin, which is also a good substrate for PepT1, was highest in the duodenum and lowest in the lower ileum. However, in the present study, the pH at the end of the experiments (15 min) was much higher in segment 8 than in segment 4 or 1. Because protons are an important driving force for transport via PepT1, it is likely that the pH had a profound effect on absorption, and the values obtained in the closed loop system may not reflect the PepT1 expression level. To avoid the effect of pH change, we quantified absorptive function with the Ussing chamber method, because the volumes of the reservoirs in this apparatus are relatively large and the pH can be well maintained.

As determined with the Ussing chamber, the regional variation of the cefadroxil transport was very similar to PepT1 mRNA expression levels in the intestinal tissues from fed rats (Table II). Moreover, in the upregulated tissue (starved rat), the transport of cefadroxil was increased. There was a significant correlation ($r^2 = 0.859$, $p < 0.05$) between absorptive transport of cefadroxil and PepT1 expression (Fig. 3), indicating that cefadroxil transport depends on the PepT1 mRNA expression. In the present study, the concentration of cefadroxil in the donor solution was 1.0 mM, which is much smaller than the reported Michaelis constant (Km) (5.9 mM) (25) and, therefore, is suitable to detect a saturable component. Moreover, the absorptive permeability was decreased approximately to the level of the inulin permeability by addition of glycylsarcosine (Table II). Therefore, the variations of cefadoxil transport detected by this method can be accounted for by the differences in the expression level of PepT1.

Because we evaluated the function of PepT1 by the transport of cefadroxil across rat intestinal tissue, the drug should permeate not only the brush-border membrane but also the basolateral membrane. It was reported that a peptide transport system, which recognizes both peptides and peptide-like drugs, is important for permeation across basolateral membrane in Caco-2 cells (26,27). However, the Km values of the basolateral peptide transporter for the peptides and peptide-like drugs are higher than those of PepT1, and this basolateral peptide transporter is a facilitated transporter. Therefore, it is assumed that permeation across the apical membrane via PepT1 is time limiting, and it is reasonable that

PepT1 mRNA expression and cefadroxil transport across the intestinal tissue correlated significantly in this study. Tomita *et al.* (28) reported that the transport activity of rabbit intestinal epithelial cells from duodenum was higher than that from ileum, whereas the same group reported that the jejunum and ileum gave stronger staining for PepT1 by Western blotting in rat. Accordingly, the discrepancy may be due to species difference.

In this study, we quantified mRNA level with quantitative real-time PCR, because the detection sensitivity is much higher than that of other methods. However, protein levels remain undetermined. However, it was suggested that the increased population of PepT1 in the brush-border membrane of intestinal mucosal cells after 1-day of fasting in rats was pretranslational (12,29) (i.e., the abundance of PepT1 mRNA accompanies the increase in PepT1 protein mass). Therefore, it is reasonable to assume that the correlation between mRNA level and cefadroxil transport measured in the Ussing chamber reflects a correlation between functional PepT1 protein mass and cefadroxil transport.

Various factors should be taken it into consideration when establishing the amount of intestinal absorption *in vivo*. Cephalexin is well known to be strongly absorbed in the upper small intestine (30). On the other hand, Sawamoto *et al.* (24) reported that the major absorption site of cephalexin *in vivo*, in fasted state, should be below the lower jejunum because of the much higher rate of transit in the upper small intestine. We showed in the present study a good correlation between PepT1 expression and cefadroxil transport. If this information is taken into consideration in addition to the luminal pH, transit time, *etc.*, simulation of drug absorption *in vivo* should become more accurate.

Very recently, it was reported that the expression level of PepT1 quantified by Western blotting measurement in human PepT1 overexpressing Caco-2 cells or rat jejunum was significantly correlated with cephalexin uptake or intestinal loop single perfusion of cephalexin, respectively (11). Thus, these findings provide further support for the above assumption.

In conclusion, the longitudinal expression pattern of PepT1 mRNA in the small intestine was determined by measuring absolute amounts with use of a real-time PCR method. Expression of PepT1 mRNA was low in the upper region and gradually increased toward the lower region in fed rats. Expression of PepT1 mRNA was induced by starvation, and the induction was most prominent in the upper region. The absorption capacity for a PepT1 substrate, cefadroxil, closely paralleled the mRNA expression level of PepT1 in both fed and starved rat intestines, suggesting that PepT1 expression is the main determinant of the absorptive function for cefadroxil.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mr. Masahide Fukudo.

REFERENCES

1. A. Tsuji and I. Tamai. Carrier-mediated intestinal transport of drugs. *Pharm. Res.* **13**:963–977 (1996).
2. Y. J. Fei, Y. Kanai, S. Nussberger, V. Ganapathy, F. H. Leibach, M. F. Romero, S. K. Singh, W. F. Boron, and M. A. Hediger. Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368**:563–566 (1994).
3. R. Liang, Y. J. Fei, P. D. Prasad, S. Ramamoorthy, H. Han, T. L. Yang-Feng, M. A. Hediger, V. Ganapathy, and F. H. Leibach. Human intestinal H^+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. *J. Biol. Chem.* **270**: 6456–6463 (1995).
4. H. Saito, M. Okuda, T. Terada, S. Sasaki, and K. Inui. Cloning and characterization of a rat H^+/peptide cotransporter mediating absorption of beta-lactam antibiotics in the intestine and kidney. *J. Pharmacol. Exp. Ther.* **275**:1631–1637 (1995).
5. K. Miyamoto, T. Shiraga, K. Morita, H. Yamamoto, H. Haga, Y. Taketani, I. Tamai, Y. Sai, A. Tsuji, and E. Takeda. Sequence, tissue distribution and developmental changes in rat intestinal oligopeptide transporter. *Biochim. Biophys. Acta* **1305**:34–38 (1996).
6. Y. J. Fei, M. Sugawara, J. C. Liu, H. W. Li, V. Ganapathy, M. E. Ganapathy, and F. H. Leibach. cDNA structure, genomic organization, and promoter analysis of the mouse intestinal peptide transporter PEPT1. *Biochim. Biophys. Acta* **1492**:145–154 (2000).
7. I. Tamai, N. Tomizawa, A. Kadowaki, T. Terasaki, K. Nakayama, H. Higashida, and A. Tsuji. Functional expression of intestinal dipeptide/beta-lactam antibiotic transporter in *Xenopus laevis* oocytes. *Biochem. Pharmacol.* **48**:881–888 (1994).
8. T. Terada, H. Saito, M. Mukai, and K. Inui. Characterization of stably transfected kidney epithelial cell line expressing rat H^+/peptide cotransporter PEPT1: localization of PEPT1 and transport of beta-lactam antibiotics. *J. Pharmacol. Exp. Ther.* **281**: 1415–1421 (1997).
9. I. Tamai, N. Tomizawa, T. Takeuchi, K. Nakayama, H. Higashida, and A. Tsuji. Functional expression of transporter for beta-lactam antibiotics and dipeptides in *Xenopus laevis* oocytes injected with messenger RNA from human, rat and rabbit small intestines. *J. Pharmacol. Exp. Ther.* **273**:26–31 (1995).
10. I. Tamai, T. Nakanishi, K. Hayashi, T. Terao, Y. Sai, T. Shiraga, K. Miyamoto, E. Takeda, H. Higashida, and A. Tsuji. The predominant contribution of oligopeptide transporter PepT1 to intestinal absorption of beta-lactam antibiotics in the rat small intestine. *J. Pharm. Pharmacol.* **49**:796–801 (1997).
11. X. Y. Chu, G. P. Sanchez-Castano, K. Higaki, D. M. Oh, C. P. Hsu, and G. L. Amidon. Correlation between epithelial cell permeability of cephalexin and expression of intestinal oligopeptide transporter. *J. Pharmacol. Exp. Ther.* **299**:575–582 (2001).
12. R. H. Erickson and J. R. Gum, Jr., M. M. Lindstrom, D. McKean, and Y. S. Kim. Regional expression and dietary regulation of rat small intestinal peptide and amino acid transporter mRNAs. *Biochem. Biophys. Res. Commun.* **216**:249–257 (1995).
13. T. Shiraga, K. Miyamoto, H. Tanaka, H. Yamamoto, Y. Taketani, K. Morita, I. Tamai, A. Tsuji, and E. Takeda. Cellular and molecular mechanisms of dietary regulation on rat intestinal H^+/peptide transporter PepT1. *Gastroenterology* **116**:354–362 (1999).
14. H. Ogihara, T. Suzuki, Y. Nagamachi, K. Inui, and K. Takata. Peptide transporter in the rat small intestine: ultrastructural localization and the effect of starvation and administration of amino acids. *Histochem. J.* **31**:169–174 (1999).
15. H. Tanaka, K. I. Miyamoto, K. Morita, H. Haga, H. Segawa, T. Shiraga, A. Fujioka, T. Kouda, Y. Taketani, S. Hisano, Y. Fukui, K. Kitagawa, and E. Takeda. Regulation of the PepT1 peptide transporter in the rat small intestine in response to 5-fluorouracil-induced injury. *Gastroenterology* **114**:714–723 (1998).
16. K. Naruhashi, M. Nadai, M. Nakao, N. Suzuki, T. Nabeshima, and T. Hasegawa. Changes in absorptive function of rat intestine injured by methotrexate. *Clin. Exp. Pharmacol. Physiol.* **27**:980–986 (2000).
17. T. Fujita, Y. Majikawa, S. Umehisa, N. Okada, A. Yamamoto, V. Ganapathy, and F. H. Leibach. sigma Receptor ligand-induced up-regulation of the H^+/peptide transporter PEPT1 in the human intestinal cell line Caco-2. *Biochem. Biophys. Res. Commun.* **261**: 242–246 (1999).
18. M. Thamotharan, S. Z. Bawani, X. Zhou, and S. A. Adibi. Hormonal regulation of oligopeptide transporter pept-1 in a human intestinal cell line. *Am. J. Physiol.* **276**:C821–826 (1999).
19. H. Ogihara, H. Saito, B. C. Shin, T. Terado, S. Takenoshita, Y. Nagamachi, K. Inui, and K. Takata. Immuno-localization of H^+/

peptide cotransporter in rat digestive tract. *Biochem. Biophys. Res. Commun.* **220**:848–852 (1996).
20. Y. Sai, I. Tamai, H. Sumikawa, K. Hayashi, T. Nakanishi, O. Amano, M. Numata, S. Iseki, and A. Tsuji. Immunolocalization and pharmacological relevance of oligopeptide transporter PepT1 in intestinal absorption of beta-lactam antibiotics. *FEBS Lett.* **392**:25–29 (1996).
21. C. T. Wittwer, K. M. Ririe, R. V. Andrew, D. A. David, R. A. Gundry, and U. J. Balis. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. *Biotechniques* **22**:176–181 (1997).
22. K. Naruhashi, I. Tamai, N. Inoue, H. Muraoka, Y. Sai, N. Suzuki, and A. Tsuji. Involvement of multidrug resistance-associated protein 2 in intestinal secretion of grepafloxacin in rats. *Antimicrob. Agents Chemother.* **46**:344–349 (2002).
23. Z. Dische and E. Borenfreund. A new spectrophotometric method for the detection and determination of keto sugars and trioses. *J. Biol. Chem.* **192**:583–587 (1951).
24. T. Sawamoto, S. Haruta, Y. Kurosaki, K. Higaki, and T. Kimura. Prediction of the plasma concentration profiles of orally administered drugs in rats on the basis of gastrointestinal transit kinetics and absorbability. *J. Pharm. Pharmacol.* **49**:450–457 (1997).
25. C. Y. Yang, A. H. Dantzig, and C. Pidgeon. Intestinal peptide transport systems and oral drug availability. *Pharm. Res.* **16**:1331–1343 (1999).
26. T. Terada, K. Sawada, H. Saito, Y. Hashimoto, and K. Inui. Functional characteristics of basolateral peptide transporter in the human intestinal cell line Caco-2. *Am. J. Physiol.* **276**:G1435–G1441 (1999).
27. M. Irie, T. Terada, K. Sawada, H. Saito, and K. Inui. Recognition and transport characteristics of nonpeptidic compounds by basolateral peptide transporter in Caco-2 cells. *J. Pharmacol. Exp. Ther.* **298**:711–717 (2001).
28. Y. Tomita, M. Takano, M. Yasuhara, R. Hori, and K. Inui. Transport of oral cephalosporins by the H+/dipeptide cotransporter and distribution of the transport activity in isolated rabbit intestinal epithelial cells. *J. Pharmacol. Exp. Ther.* **272**:63–69 (1995).
29. M. Thamotharan, S. Z. Bawani, X. Zhou, and S. A. Adibi. Functional and molecular expression of intestinal oligopeptide transporter (Pept-1) after a brief fast. *Metabolism* **48**:681–684 (1999).
30. H. Maekawa, Y. Takagishi, K. Iwamoto, Y. Doi, and T. Ogura. Cephalexin preparation with prolonged activity. *Jpn. J. Antibiot.* **30**:631–638 (1977).