PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

Peptide Transporter 1 Is Responsible for Intestinal Uptake of the Dipeptide Glycylsarcosine: Studies in Everted Jejunal Rings from Wild-type and Pept1 Null Mice

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ABSTRACT: The purpose of this study was to determine the relative importance of peptide transporter 1 (PEPT1) in the uptake of peptides/mimetics from mouse small intestine, using glycylsarcosine (GlySar). After isolating jejunal tissue from wild-type and *Pept1* null mice, 2 cm intestinal segments were everted and mounted on glass rods for tissue uptake studies. [^{14}C]GlySar (4 μM) was studied as a function of time, temperature, sodium and pH, concentration, and potential inhibitors. Compared with wild-type animals, *Pept1* null mice exhibited a 78% reduction in GlySar uptake at pH 6.0 at 37°C. GlySar uptake showed pH dependence, with peak values between pH 6.0 and 6.5 in wild-type animals, whereas no such tendency was observed in *Pept1* null mice. GlySar exhibited Michaelis–Menten uptake kinetics and a minor nonsaturable component in wild-type animals. In contrast, GlySar uptake occurred only by a nonsaturable process in *Pept1* null mice. GlySar uptake was significantly inhibited by dipeptides, aminocephalosporins, angiotensin-converting enzyme inhibitors, and the antiviral prodrug valacyclovir; these inhibitors had little, if any, effect on the uptake of GlySar in *Pept1* null mice. The findings demonstrate that PEPT1 plays a critical role in the uptake of GlySar in jejunum and suggest that PEPT1 is the major transporter responsible for the intestinal absorption of small peptides. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:767–774, 2011

Keywords: *in vitro* models; intestinal absorption; membrane transport/transporters; peptide transporters; pharmacokinetics; transgenics

INTRODUCTION

Mammalian proton-coupled oligopeptide transporters (POTs) consist of four members, including the high-capacity, low-affinity peptide transporter 1 (PEPT1; SLC15A1); the low-capacity, high-affinity transporter PEPT2 (SLC15A2); and the peptide/histidine transporters PHT1 (SLC15A4) and PHT2 (SLC15A3).[1–4] PEPT1, the first member cloned from the POT family, is abundantly expressed in the apical epithelium of small intestine and is responsible for the intestinal absorption of small peptide fragments (dipeptides and tripeptides) from the diet. The transport process is electrogenic via the symport of a peptide and a proton (1:1 stoichiometry) across the membrane and into the enterocyte.[5] However, PEPT1 is not the only POT in the intestine because PEPT2 is found in glial cells and tissue-resident macrophages of the enteric nervous system.[6] Although it is unlikely that PEPT2 is involved in the absorption of di/tripeptides from these deep neuromuscular layers of the gastrointestinal tract, this outcome is not a certainty. Moreover, transcripts of PHT1 and PHT2 have been found in intestinal tissue segments,[7] and immunohistochemical analyses have indicated that PHT1 is expressed in the villous epithelium of small intestine.[8]

PEPT1 can recognize a wide spectrum of substrates that vary in molecular size, net charge, and solubility, with the potential to transport 400 different dipeptides and 8000 different tripeptides, along with select angiotensin-converting enzyme (ACE) inhibitors such as captopril and enalapril.[9,10] PEPT1 has also been used as a target to improve the poor bioavailability of antiviral drugs such as acyclovir via a prodrug

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approach with valacyclovir. However, it is unclear at present how important PEPT1 actually is relative to other potential transporters and processes (e.g., passive uptake) in the absorption of peptides and peptide-like drugs. As suggested previously with glycylsarcosine (GlySar)[11] and cephalexin,[12] a passive absorption component in the absence of PEPT1 may be greater than originally anticipated.

A novel way to address the relative importance of intestinal PEPT1 versus other transport processes would be to generate an animal model with a defect in intestinal peptide transport. In an effort to develop a PEPT1 knockout animal, Fei et al.[13] first cloned the mouse *Pept1* gene and reported on the cDNA structure, genomic organization, and promoter analysis of mouse PEPT1. Almost a decade later, Hu et al.[11] described for the first time the development of *Pept1* null mice and their preliminary validation and phenotypic analysis in the intestine. In that report, only a cursory examination of the absorption mechanism of GlySar was investigated where *in vitro* intestinal uptake and *in situ* single-pass intestinal perfusion studies were performed at only one concentration and pH value.

With this in mind, the objective of the current study was to define the relative importance of PEPT1 in the absorption of GlySar from the jejunal tissue of wild-type and *Pept1* null mice. Thus, 2 cm intestinal segments were everted and mounted on glass rods for subsequent tissue uptake studies. Radiolabeled GlySar was then studied as a function of time, temperature, sodium and pH, concentration dependence, and potential inhibitors.

MATERIALS AND METHODS

Chemicals

[^{14}C]GlySar (106 mCi/mmol) was purchased from Amersham Biosciences (Chicago, Illinois) and [^{3}H]mannitol (20 Ci/mmol) from American Radiolabeled Chemicals (St. Louis, Missouri). Unlabeled histidine, sarcosine, glycine, carnosine, glycylglycine (GlyGly), GlySar, cephapirin, cephalothin, cephradine, cefadroxil, lisinopril, captopril, enalapril, tetraethylammonium (TEA), and 4-acetamido-4′-isothiocyano-2,2′-disulfonic acid (SITS) were obtained from Sigma-Aldrich (St. Louis, Missouri). Acyclovir and valacyclovir were kind gifts of GlaxoSmithKline (Durham, **North Carolina**). Hyamine hydroxide was purchased from ICN Pharmaceuticals (Costa Mesa, **California**). All other chemicals were obtained from standard sources and were of the highest quality available.

Animals

Weight- and gender-matched *Pept1*$^{+/+}$ (wild-type) and *Pept1*$^{-/-}$ (null) mice, 8 to 10 weeks of age, were used for the proposed studies. The *Pept1* null mice were generated on a C57BL/6 mouse background as described previously.[11] The animals were kept in a temperature-controlled environment, with 12 h light and dark cycles, and access to a standard diet and water *ad libitum*. All mouse studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Preparation of Everted Jejunal Rings

Following anesthesia with sodium pentobarbital (60 mg/kg i.p.), the mouse abdomen was opened, the proximal jejunum was isolated (i.e., ~2 cm distal to the ligament of Trietz), and two 2 cm segments were transferred to an ice-cold incubation medium. Composition of the incubation medium was as follows (in mM): 129 NaCl, 5.1 KCl, 1.4 $CaCl_2$, 1.3 NaH_2PO_4, and 1.3 Na_2HPO_4 (pH 6.0).[14] For pH-dependent analyses, different combinations of 10 mM of Tris and/or MES were added to the incubation buffer to achieve pH values of 5.5 to 8.0, with osmolarity and concentration of sodium being held constant. For sodium-dependent analyses, choline chloride was used to replace the sodium chloride for a low-sodium buffer (pH 6.0). After a rapid wash, the jejunal segments were everted and then fixed over glass rods (3 mm diameter) by surgical threads. Everted jejunal segments were equilibrated in incubation medium gassed with 5% CO_2–95% O_2 at 37°C (water bath) for 5 min. Following the equilibration period, each jejunal segment was placed in 1 mL of incubation medium maintained at 37°C and containing 4 μM of [^{14}C]GlySar (plus 0–40 mM of unlabeled GlySar) and 2 μM of [^{3}H]mannitol (an extracellular marker). Dipeptide uptake was terminated by transferring each segment to an ice-cold wash buffer (same as the incubation medium). The jejunal segments were then washed for 20 s, blotted on filter paper, weighed, and soaked overnight in 0.33 mL of 1 M hyamine hydroxide (tissue solubilizer). A 6 mL aliquot of CytoScint™ scintillation cocktail was added to the tissue and radioactivity was determined by a dual-channel liquid scintillation counter.

The jejunal uptake of radiolabeled GlySar (pmol/mg of tissue weight) was calculated as follows[15]: Uptake = GlySar – Mannitol × R, where R is the ratio of [^{14}C]GlySar to [^{3}H]mannitol in the media, and Mannitol × R provides an estimate of the extracellular content of GlySar.

Data Analysis

The concentration-dependent jejunal uptake of GlySar in wild-type and *Pept1* null mice was fitted simultaneously to an equation containing a Michaelis—Menten term and a nonsaturable component such

that:

$$v = \frac{V_{max} \cdot C}{K_m + C} + K_d \cdot C$$

where V_{max} is the maximal rate of saturable uptake, K_m is the Michaelis constant, K_d is the rate constant for nonsaturable processes, and C is the substrate concentration (i.e., GlySar). Uptake results of the two jejunal segments were combined for each mouse.

Statistics

Data are reported as mean ± standard error (SE) ($n \geq 3$ per genotype). Statistical differences between wild-type and *Pept1* null mice were determined using a two-sample t test. A probability of $p \leq 0.05$ was considered significant. Statistical differences between multiple treatment groups were determined by analysis of variance, and pair-wise comparisons were carried out using Dunnett's test ($\alpha = 0.05$). For analyses using linear and nonlinear least-squares regression, the quality of fit was determined by evaluation of the coefficient of determination (r^2) and the SE of parameter estimates and by visual inspection of residuals. All statistical analyses were performed using Prism version 5.01 (GraphPad Software, Inc., San Diego, **California**).

RESULTS

Time-dependent Uptake of Glysar

The uptake of 4 μM of GlySar was initially studied in whole everted jejunal tissue isolated from wild-type mice. As shown in Figure 1, GlySar displayed a linear uptake for approximately 30 s ($r^2 = 0.999$). The value of y-intercept was not significantly different from zero ($p \geq 0.05$), suggesting that nonspecific binding was negligible. On the basis of these results and to maximize radiotracer uptake in the linear region, an uptake time of 20 s was used for the subsequent experiments in wild-type and *Pept1* null mice. GlySar reached a plateau value of 1.3 pmol/mg at about 7.5 min into the incubation period.

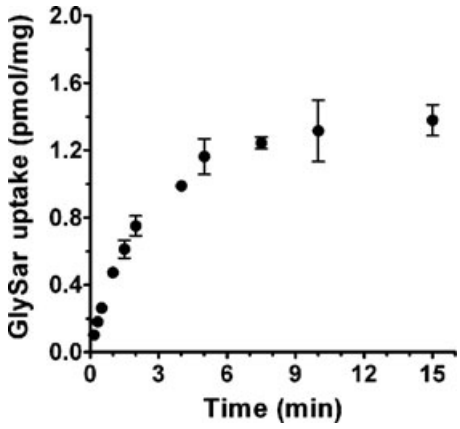

Figure 1. Time-dependent uptake of [^{14}C]GlySar in the jejunum of wild-type mice (4 μM of GlySar in external medium). Studies were performed at 37°C in the incubation buffer, pH 6.0. Data are expressed as mean ± SE ($n = 3$–4).

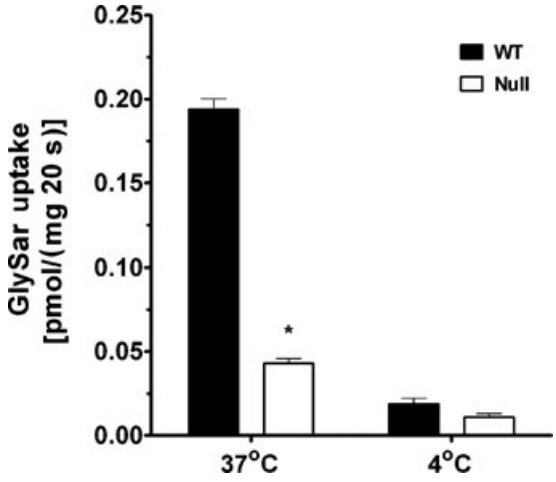

Figure 2. Temperature-dependent uptake of [^{14}C]GlySar in the jejunum of wild-type (WT) and *Pept1* null (Null) mice (4 μM of GlySar in external medium). Studies were performed at 37°C and 4°C in the incubation buffer, pH 6.0. Data are expressed as mean ± SE ($n = 3$–4). $*p \leq 0.001$ for null versus wild-type animals at 37°C.

Temperature-dependent Uptake of Glysar

Pept1 null mice demonstrated a 78% reduction in the uptake of 4 μM of GlySar, as compared with wild-type animals, in everted jejunal rings at 37°C (Fig. 2). In contrast, no differences in GlySar uptake were observed between the two genotypes at 4°C. At the lower temperature, GlySar uptake was only 6% to 10% of control values in wild-type mice (37°C). These results suggest that PEPT1 is responsible for the majority of GlySar uptake in the jejunum of wild-type mice (~80%), with only a small percentage of intestinal uptake being governed by a temperature-dependent process other than PEPT1 (~10%–14%).

Sodium-independent and Ph-dependent Uptake of Glysar

As shown in Figure 3, the jejunal uptake of GlySar was comparable between high-sodium and low-sodium incubation buffers for each genotype. This result in our *in vitro* system is consistent, with PEPT1 being an Na^+-independent transporter. Because PEPT1 is a POT, GlySar uptake was examined as a function of buffer pH (i.e., from 8.0 to 5.5). As shown in Figure 4, the uptake profile of GlySar

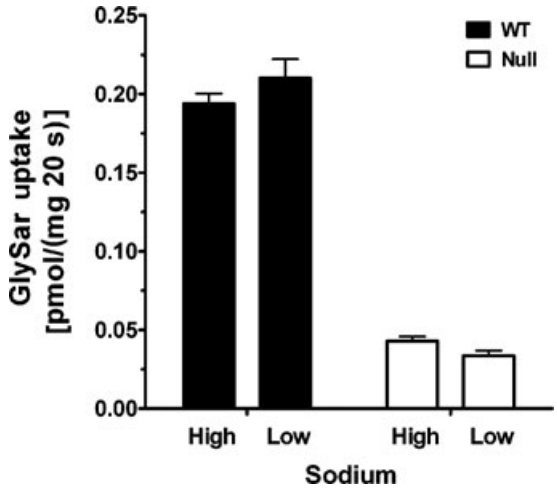

Figure 3. Sodium-dependent uptake of [^{14}C]GlySar in the jejunum of wild-type (WT) and *Pept1* null (Null) mice (4 μM of GlySar in external medium). Studies were performed at 37°C with different concentrations of sodium (see the text) in the incubation buffer, pH 6.0. Data are expressed as mean ± SE (n = 3–4).

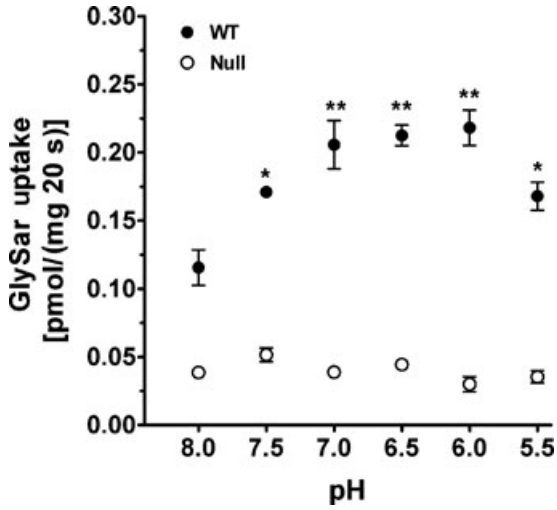

Figure 4. pH-dependent uptake of [^{14}C]GlySar in the jejunum of wild-type (WT) and *Pept1* null (Null) mice (4 μM of GlySar in external medium). Studies were performed at 37°C in Tris–MES containing incubation buffer. Data are expressed as mean ± SE (n = 3). For each genotype, $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$ as compared with pH 8.0.

was "bell shaped" in wild-type mice, with peak values at pH 6.0 to 6.5. In contrast, GlySar uptake was relatively flat in *Pept1* null mice and substantially lower than the uptake observed in wild-type animals.

Concentration-dependent Uptake of Glysar

A saturable uptake of GlySar was observed in the jejunum of wild-type mice when evaluated over a wide concentration range (i.e., from 0.1 to 40 mM). As shown in Figure 5, wild-type animals demonstrated both Michaelis–Menten and nonsaturable uptake processes in which V_{max} = 233 pmol/mg/20 s, K_m = 9.96 mM, and K_d = 8.06 nL/mg/20 s. In contrast, *Pept1* null mice exhibited only a linear uptake for the nonsaturable process. As a result, the carrier-mediated component accounted for about 75% of the total uptake and the nonspecific component for about 25% of the total uptake in wild-type mice. Kinetic parameters for the jejunal uptake of GlySar are summarized in Table 1.

Table 1. Uptake Kinetics of GlySar in Everted Jejunal Rings of *Pept1*$^{+/+}$ (Wild-Type) and *Pept1*$^{-/-}$ (Null) Mice*

Parameters	Wild-Type Mice	*Pept1* Null Mice
V_{max} (pmol/mg/20 s)	233 (36)	–
K_m (mM)	9.96 (3.59)	–
K_d (nL/mg/20 s)	8.06 (0.33)	8.06 (0.33)
V_{max}/K_m (nL/mg/20 s)	23.4	–
% Carrier-mediated	74.4	–
% Nonsaturable	25.6	–
r^2	0.9382	0.9564

* Parameter estimates are expressed as mean ± SE. % Carrier-mediated was calculated as follows: 100 × V_{max}/K_m divided by ($V_{max}/K_m + K_d$). % Nonsaturable was calculated as follows: 100 × K_d divided by ($V_{max}/K_m + K_d$). r^2 is the coefficient of determination.

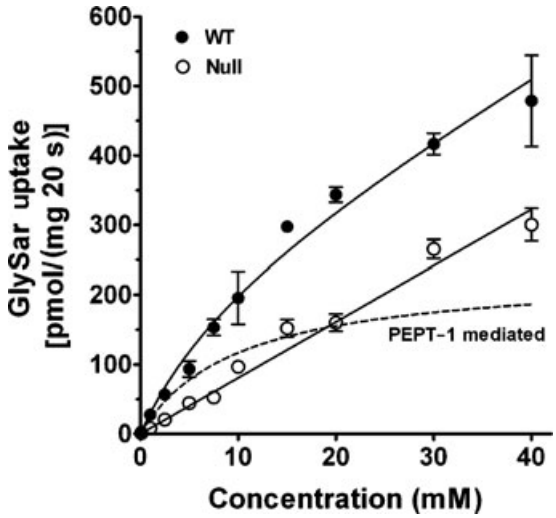

Figure 5. Concentration-dependent uptake of [^{14}C]GlySar in the jejunum of wild-type (WT) and *Pept1* null (Null) mice (0.1–40 mM of total GlySar in external medium). Studies were performed at 37°C in the incubation buffer, pH 6.0. Data are expressed as mean ± SE (n = 3). The dashed line represents the PEPT1-mediated component.

Inhibitor Analysis of Glysar Uptake

Potential inhibitors of GlySar uptake were classified into six groups: (1) amino acids, (2) dipeptides, (3) cephalosporins, (4) ACE inhibitors, (5) organic anion and cation, and (6) antiviral drug and prodrug. As shown in Figure 6a, neither histidine, sarcosine,

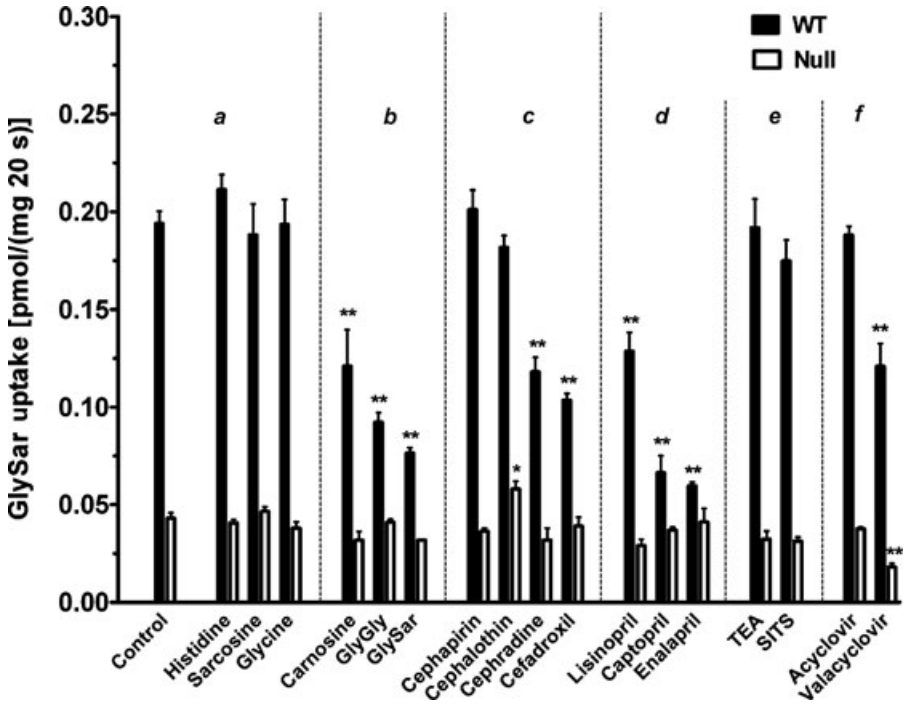

Figure 6. Effect of potential inhibitors (10 mM) on the uptake of [^{14}C]GlySar in the jejunum of wild-type (WT) and *Pept1* null (Null) mice (4 μM of GlySar in external medium). Studies were performed at 37°C in the incubation buffer, pH 6.0. GlyGly, glycylglycine; GlySar, glycylsarcosine; TEA, tetraethylammonium; SITS, 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid. Data are expressed as mean ± SE (n = 3–6). For each genotype, $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$ as compared to control values.

nor glycine inhibited the jejunal uptake of GlySar, relative to control values, in both wild-type and *Pept1* null mice. In contrast, carnosine, GlyGly, and unlabeled GlySar significantly inhibited the uptake of radiolabeled GlySar in wild-type mice (i.e., reduced to 39%–62% of control values); inhibition of GlySar by these dipeptides was not observed in *Pept1* null mice (Fig. 6b). Potential cephalosporin inhibitors were divided into two groups: those containing an α-amino carbon (cephradine and cefadroxil) and those lacking an α-amino carbon (cephapirin and cephalothin). Cephradine and cefadroxil substantially reduced the jejunal uptake of GlySar in wild-type mice (i.e., reduced to 54%–61% of control values), whereas GlySar uptake was unchanged in the presence of cephalothin and cephapirin. In addition, none of the cephalosporin drugs had an inhibitory effect on GlySar uptake in *Pept1* null mice (Fig. 6c). Select ACE inhibitors, such as lisinopril, captopril, and enalapril, significantly inhibited the uptake of GlySar in wild-type mice (i.e., reduced to 31%–66% of control values); no effect of ACE inhibitors on GlySar uptake was observed in *Pept1* null mice (Fig. 6d). Neither TEA nor SITS showed an inhibitory effect on GlySar uptake in wild-type and *Pept1* null mice (Fig. 6e). Finally, the prodrug valacyclovir, but not acyclovir, inhibited the jejunal uptake of GlySar uptake in both genotypes (Fig. 6f).

DISCUSSION

Protein digestion and assimilation in the small intestine begin with its degradation by a spectrum of proteases and peptidases, which are located in the luminal space or bound to brush border membranes of enterocytes.[16] The resultant di/tripeptide substrates and free amino acids are then absorbed by intestinal PEPT1 and the many amino acid transport systems present in this region.[17] Likewise, the oral absorption of aminocephalosporin antibiotics, some ACE inhibitors, and antitumor or antiviral ester prodrugs can be improved by targeting intestinal PEPT1.[9] However, the extent to which nutritional nitrogen and peptide-like drugs are absorbed in small intestine via PEPT1 versus other uptake mechanisms, including passive processes, is uncertain.

Previous studies have characterized the functional characteristics of mouse PEPT1, using a variety of *in vitro* methods. In this regard, mammalian cells and *Xenopus* oocytes expressing mouse PEPT1 have demonstrated that GlySar uptake is stimulated by reduced pH and the dipeptide is transported with low affinity (K_m of 0.6 mM at −50 mV).[13] In a subsequent study,[18] cefixime uptake in mouse PEPT1 cRNA-injected oocytes was stimulated 100-fold as compared with water-injected oocytes. Moreover, the

apical uptake of cefixime in everted sacs prepared from upper small intestine was pH dependent and inhibited by excess concentrations of GlySar. However, these studies were insufficient in that mouse intestinal tissue was not used in the former study[13] and transport kinetics (V_{max}, K_m) and substrate specificity were not examined in the latter study.[18]

Gene knockout is an elegant approach to characterize the physiological, pharmacological, and pathophysiological roles and/or significance of a specific transport protein. With this in mind, Hu et al.[11] first reported the development and phenotypic analysis of *Pept1* null mice and their preliminary validation. These initial studies were important in comparing the *in vitro* uptake and *in situ* permeability of GlySar from jejunum with *in vivo* oral absorption of the dipeptide. However, a full validation was not performed in that driving forces (i.e., the influence of temperature, sodium, and pH) and saturation kinetics were not examined and validation of transport specificity was lacking.

In the present study, several new findings were revealed in comparing the transport characteristics of wild-type and *Pept1* null mice. Specifically, we found that (1) the PEPT1-independent "residual" uptake of GlySar in wild-type mice was 22% at physiological temperature whereas the residual uptake in both genotypes at 4°C was 6% to 10%; (2) GlySar uptake was sodium independent in both genotypes and that uptake was pH dependent in wild-type mice (i.e., maximum uptake at pH 6.0–6.5) but not in *Pept1* null mice; (3) the uptake kinetics of GlySar in wild-type mice was of low affinity ($K_m = 10$ mM) and the PEPT1-mediated process accounted for about 75% of the total uptake under linear conditions; and (4) GlySar uptake was specific in wild-type mice as demonstrated by extensive inhibition studies. Although the K_m of GlySar in mouse PEPT1 cRNA-injected *Xenopus* oocytes[13] differed from the K_m in the present study (0.6 mM vs. 10 mM, respectively), this probably reflects differences in experimental design (i.e., oocytes vs. mouse intestine). There may be added complexity due to the presence of a diffusional gradient in jejunal preparations (and thus higher K_m values) that is lacking in cell experiments. Still, taken as a whole, these findings clearly show that under the *in vitro* conditions presented here, PEPT1 is the predominant mechanism by which dipeptides (and presumably peptide-like drugs) are absorbed in the small intestine.

The relevance of our findings will depend upon many factors including how well mouse PEPT1 faithfully represents the transport properties of human PEPT1. In this regard, both species exhibit similarities in pH and membrane potential uptake, substrate specificity, and substrate affinity of cloned PEPT1.[13,19] Moreover, both species express PEPT1 in the apical membrane of epithelial cells lining the small intestine (i.e., duodenum, jejunum, and ileum), with little or no expression in colon.[20,21] Finally, both species have comparable expression levels of PEPT1 in small intestine.[9,22] As a result, we believe that the mouse model will provide results that are mechanistically valid with respect to intestinal PEPT1 in humans. It should also be noted that compensatory responses to PEPT1 ablation were not evident in the null animals.[11] PEPT2 protein was not aberrantly expressed in the small intestine of *Pept1* null mice, and PHT1/PHT2 mRNA expression did not differ between genotypes in this tissue.

In our studies, the everted jejunal rings consisted of intact tissue, with mucosa being exposed to the drug solution and submucosa and serosa adhering to the glass rod. Although a pH-dependent uptake of GlySar was observed in wild-type mice (Fig. 4), the magnitude of change in everted jejunal rings was somewhat lower than what was typically observed in cell culture preparations such as Caco-2 cells.[23] This finding probably reflects the ability of intact tissue to better self-regulate its microenvironment in the presence of bulk fluid insult. As demonstrated in rat jejunum *in vivo*, the effective pH at the surface of intestinal cells varied only from 6.0 to 6.5 when the luminal pH was raised from pH 5.0 to 7.4.[24] It is unlikely that tissue viability can explain the difference because incubation times were so short (i.e., 20 s) relative to the cutoff time (i.e., 10 min) advocated for this technique.[25]

The specificity of GlySar uptake was ensured by the wide variety of potential inhibitors examined in wild-type and *Pept1* null mice (Fig. 6). In wild-type animals, PEPT1-mediated transport of GlySar in jejunum was demonstrated by its significantly reduced uptake by dipeptides, aminocephalosporins, ACE inhibitors, and the antiviral prodrug ester valacyclovir. In contrast, a contribution of peptide/histidine transporters such as PHT1 and PHT2 can be discounted because L-histidine had no effect on GlySar uptake. Likewise, GlySar uptake was not altered when the dipeptide was coincubated with other amino acids, acidic and cationic substrates, and the antiviral active drug acyclovir. Surprisingly, we did observe some inhibition of GlySar uptake by valacyclovir in *Pept1* null mice. In addition to PEPT1, valacyclovir is transported by the amino acid transporter $ATB^{0,+}$ (SLC6A14)[26] and inhibited by the organic anion *p*-aminohippuric acid and the organic cation quinine.[27] However, it is unlikely that anionic and cationic transporters are involved in the jejunal uptake of GlySar because neither SITS nor TEA inhibited its uptake in wild-type and *Pept1* null mice. It is also unlikely that $ATB^{0,+}$ is involved in the uptake of GlySar because this transporter is expressed primarily in the large intestine and is dependent on a transmembrane sodium gradient that was not observed in our studies. Moreover, GlySar uptake was not inhibited by glycine, a

prototypical substrate for $ATB^{0,+}$, in either genotype. Hydrolysis of valacyclovir to acyclovir in the intestinal lumen is possible but, even in this instance, would not cause this "apparent" discrepancy because the uptake of GlySar in both genotypes was unaffected by acyclovir. As a result, the reduced uptake of GlySar by valacyclovir during PEPT1 ablation was probably due to an artifact of the experiment or our committing a Type I error because of the relatively small sample size.

A final point concerns the nature of the non–PEPT1-mediated uptake of GlySar in everted jejunal rings (see Fig. 2). As discussed previously, PEPT1 accounted for about 80% of the total uptake as shown by substantial reduction of GlySar in *Pept1* null versus wild-type mice at 37°C (i.e., about 20% residual uptake). At 4°C, the residual uptake of GlySar was only about 6% to 10% in the two genotypes. As a result, the difference in uptake between *Pept1* null mice at 37°C and the mice at 4°C is about 10% to 14%, which represents a temperature-dependent, PEPT1-independent process. Although speculative, the temperature dependency observed in the current study is consistent with an endocytic uptake of GlySar as reported earlier in ARPE-19 cells.[28] To confirm this belief, experiments that specifically address this issue will be needed (e.g., those using endocytic inhibitors). GlySar uptake by HPT-1 may also be possible. However, there is almost nothing known about the structure–function relationship of this putative transporter, including whether or not it transports GlySar.[2]

In conclusion, we have characterized for the first time the intestinal uptake properties of GlySar, as a function of driving force, saturability, and specificity, in the jejunum of wild-type and *Pept1* null mice. Taken as a whole, the results demonstrate that PEPT1 accounts for the great majority of dipeptide uptake (about 80%) in the intestine. The results suggest that variability in intestinal PEPT1 expression should have a dramatic effect on the oral absorption of peptides and peptide-like drugs.

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