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

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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