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Influence of Fed-Fasted State on Intestinal PEPT1 Expression and *In Vivo* Pharmacokinetics of Glycylsarcosine in Wild-Type and *Pept1* Knockout Mice

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Abstract

Purpose—To determine if fasting would affect the intestinal expression and *in vivo* functional activity of PEPT1 as determined after oral dosing of the dipeptide glycylsarcosine (GlySar).

Methods—Systemic exposure and tissue distribution studies were performed in wild-type and *Pept1* knockout mice, under fed and fasted conditions, following both intravenous and oral doses of [¹⁴C]GlySar at 5 nmol/g body weight. Intestinal PEPT1 expression was evaluated by real-time PCR and immunoblot analyses.

Results—We found that expression of PEPT1 protein in the small intestine was increased ~2-fold in wild-type mice during fasted as compared to fed conditions. In agreement, systemic exposure and peak plasma concentrations of orally administered GlySar were 40 and 65% greater, respectively, in wild-type mice during fasted vs. fed state. No significant differences were observed between fed and fasted animals during PEPT1 ablation. Tissue distribution of GlySar was unchanged after oral dosing for all four treatment groups.

Conclusions—As little as 16 hr of fasting can cause significant upregulation of PEPT1 protein expression in the small intestine, which then translates into a significant increase in *in vivo* oral absorption of GlySar.

Keywords

fed-fasted state; glycylsarcosine; intestinal PEPT1; knockout mice; oral absorption

INTRODUCTION

Cloning of the proton-coupled oligopeptide transporter (POT) from rabbit, designated as PEPT1 (SLC15A1), provided the first molecular evidence for the functional activity of intestinal peptide transport (1). The protein contains 12 putative transmembrane domains with both the C- and N-termini localized inside the cell. The first four transmembrane regions from the amino terminus, and domains 7 to 9, are responsible for PEPT1's substrate affinity and unique features (2). PEPT1 is classified as a low-affinity, high-capacity transporter and demonstrates a broad specificity for substrates of different molecular size, polarity, charge and conformation (3, 4). It is expressed abundantly in all regions of the small intestine with little or no expression in the large intestine (5); it is also expressed at much lower levels in the kidney (6). In contrast, the POT member PEPT2 (SLC15A2) is

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classified as a high-affinity, low-capacity transporter and is highly expressed in the kidney (6) along with brain (7), lung and mammary gland (3, 4). Both PEPT1 and PEPT2 exert their effects at the luminal surface of intestinal and renal epithelia, respectively, where they absorb and reabsorb di/tripeptides, mimetics and peptide-like drugs. Two other POT members, PHT1 (SLC15A4) (8) and PHT2 (SLC15A3) (9) have also been identified in small intestine, but their expression and function have yet to be clearly elucidated. Regardless, they appear not to have relevance at the plasma membrane of enterocytes (5, 10).

It has been reported that PEPT1 is regulated by a number of factors including the alteration of proton-motive force, food intake, hormones, pathological state, some pharmacologic agents, and diurnal rhythm (3, 4). Given its importance in absorbing peptide-bound nitrogen from the diet and gastrointestinal secretions, as well as pharmacological agents, the role of nutritional status in PEPT1 regulation is of particular interest. For example, several studies have shown that various degrees of fasting (i.e., from 1-4 days) result in an upregulation of PEPT1 mRNA and protein in small intestine, along with functional activity (11-13). However, the functional activity of PEPT1 was evaluated using *in vitro* models of rat jejunal brush border membrane vesicles (11) and Ussing chambers lined with intestinal tissue sheets (12), *in situ* rat perfusion models using a closed loop method (12), and Caco-2 cell cultures (13). None of these studies investigated the effect of fasting/starvation on the oral absorption of a PEPT1 substrate under physiological *in vivo* conditions. In one study, the pharmacokinetics of an oral β -lactam antibiotic, ceftibuten, was evaluated in fed and 4-day fasted rats after oral and intravenous drug administration (14). Although its systemic exposure after oral dosing was significantly increased during fasting, the results were confounded by dispositional changes, as indicated by substantial reductions in the total clearance of ceftibuten in fasted rats after intravenous dosing.

Food may alter the systemic availability of drugs by a multitude of mechanisms that act on the drug's physicochemical properties and gastrointestinal environment (15, 16). Such mechanisms might include changes in drug solubility, permeability, and metabolism via delayed gastric emptying, stimulated bile and/or splanchnic blood flow, increased pH and fluid volume in the gut, and altered luminal drug concentrations. While the literature is quite extensive concerning drug, meal and formulation interactions, much less is known about how food (or fasting) affects intestinal transporters and transporter-mediated absorption. With this in mind, our primary objective was to determine if fasting would affect the intestinal expression and *in vivo* functional activity of PEPT1 as determined after oral dosing of the model dipeptide glycylsarcosine (GlySar). Our secondary objective was to examine if fasting affected the small intestinal transit times, and if histological differences were present in mouse small intestine as a function of diet or genotype.

MATERIALS AND METHODS

Materials

[¹⁴C]GlySar (106 mCi/mmol) was purchased from Amersham Biosciences (Chicago, IL) and [³H]dextran 70,000 (265 mCi/gm) from American Radiolabeled Chemicals (St. Louis, MO). *Pept1* knockout mice were generated on a C57BL/6 mouse background as described previously (17). Unlabeled GlySar was obtained from Sigma-Aldrich (St. Louis, MO). Diet LD 101, obtained from TestDiet (Richmond, IN), arrived as a dry powder and was reconstituted according to the manufacturer's instructions. Hyamine hydroxide was obtained from ICN Pharmaceuticals (Costa Mesa, CA). All other chemicals were obtained from standard sources.

Fed-Fasted Study Design

All mouse studies adhered to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised in 1985), and were approved by the Institutional Animal Care and Use Committee of the University of Michigan, Ann Arbor. Wild-type and *Pept1* knockout mice (gender- and weight-matched, 6 to 8 weeks of age) were used in the proposed studies under fed and fasted conditions. The mice were kept in a temperature-controlled environment, with 12-hr light and dark cycles, and received a standard diet of solid chow and water *ad libitum* (Unit for Laboratory Animal medicine, University of Michigan, Ann Arbor, MI). Four-days prior to conducting the fed-fasted experiments, solid chow was changed to the micro-stabilized rodent liquid diet mix - LD101 (18). For three of these days, all mice received this liquid diet. On the fourth day, one cohort of mice was maintained on diet LD 101 (i.e., fed group) while in another cohort the liquid diet was replaced with water at 5 pm (i.e., fasted group). Mouse experiments were then performed at 9 am the next day, representing a 16-hr fast for those mice on water.

Real-Time PCR Analysis

Following intra-peritoneal anesthesia with sodium pentobarbital (65 mg/kg body weight), the small intestine and colon of mice were opened longitudinally and the mucus layer was gently scraped from the duodenal, jejunal, ileal, and proximal and distal colonic segments using a glass slide. Total RNA was then isolated according to the manufacturer's protocol using TriZol reagent (Invitrogen, Carlsbad, CA). After genomic DNA was removed from the total RNA by DNase I treatment (Ambion, Austin, TX), first strand cDNA was synthesized by reverse-transcriptase III (Invitrogen, Carlsbad, CA). PEPT1 and house-keeping gene 18s rRNA primers were designed with Primer Express 3.0 software (Applied Biosystems, Foster City, CA) and synthesized by Integrated DNA Technologies (Coralville, IA). The forward and reverse primers for PEPT1 were CTTGGAGCCACCACAATGG and ACAGAATTCATTGACCACGATGA, respectively; the forward and reverse primers for 18s rRNA were GGCGTCCCCCACTTCTTA and GGGCATCACAGACCTGTTATTG, respectively. The thermal profile for real-time PCR was one cycle at 50°C × 2 min, one cycle at 95°C × 10 min, 40 cycles at 95°C × 15 sec, and 60°C × 1 min. Relative abundance of PEPT1 transcripts was calculated based on the Ct cycles and normalized for 18s rRNA.

Immunoblot Analysis

Total protein from the mucus layers of mouse small intestine and colon was resolved on 7.5% SDS-PAGE, transferred to a PVDF membrane, and blotted with specific polyclonal rabbit anti-mouse PEPT1 antisera (raised against the COOH- terminal region, KGIGKENPYSSLEPVSQTNM; Lampire Biological Laboratories, Pipersville, PA) (1:1000 dilution) (5, 17). After the membrane was washed three times with TBS-T, it was incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA) (1:3000 dilution). To control for loading efficiency, β -actin from the same membrane was blotted with a mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) (1:1000 dilution) and then incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA) (1:3000 dilution). The membrane-bound antibodies were detected with Immobilon Western Chemiluminescent Substrate (Millipore, Billerica, MA).

In Vivo Pharmacokinetic Studies

Following intra-peritoneal anesthesia with sodium pentobarbital (65 mg/kg body weight), mice were administered a bolus dose of [¹⁴C]GlySar (5 nmol/g body weight) by tail vein injection. Serial blood samples were then collected at 0.25, 1, 5, 15, 30, 60, 90 and 120 min after dosing. For oral studies, [¹⁴C]GlySar (5 nmol/g body weight) was administered to

conscious mice and serial samples collected at 5, 15, 30, 45, 60, 90, 120, 240 and 360 min. After oral gavage, the mice were returned to their cages in between blood sampling where they had free access to water. For both routes of administration, blood samples (15 to 20 μ l) were obtained via tail transections and the plasma harvested.

Tissue distribution studies were performed at the end of blood sampling for both the intravenous and oral experiments with [14 C]GlySar, as described above. However, an intravenous bolus of [3 H]dextran 70,000 (2 μ Ci/mouse) was administered 2 min prior to collecting the last blood sample so that tissue concentrations could be corrected for the extracellular space of GlySar. As demonstrated for albumin, 2 min was a suitable time for estimating intravascular volume (19). Once this blood sample was obtained mice were immediately decapitated and multiple tissues/organs were harvested (i.e., cerebral cortex, eye, lung, heart, liver, stomach, duodenum, jejunum, ileum, colon, spleen, kidney, and skeletal muscle). Tissue samples were blotted dry, weighted, and digested in 0.33 ml of 1 M hyamine hydroxide (a tissue solubilizer) at 37°C. A 20- μ L aliquot of 33% H₂O₂ was added to each solubilized tissue followed by Ecolite (+) liquid scintillation cocktail (MP Biomedicals). Radioactivity in the tissue and plasma samples was detected by a dual-channel liquid scintillation counter (Beckman LS 3801; Beckman Coulter, Fullerton, CA). Corrected tissue concentrations of GlySar (nmol/g of wet weight) were calculated as $C_{\text{tiss}} - DS \cdot C_b$ where C_{tiss} is the uncorrected GlySar tissue concentration (nmol/g), DS is the dextran space (ml/g), and C_b is the concentration of GlySar in blood (nmol/ml).

Small Intestinal Transit

Mice were administered a meal of 10% activated charcoal in 5% gum arabic solution (Sigma, St. Louis, MO), by oral gavage, at a volume of 10 μ l/g body weight. At 30 min, the animals were sacrificed by cervical dislocation after brief anesthesia. The stomach and small intestine were separated from the greater omentum and then removed. The distance traveled by the leading edge of the charcoal suspension and the total length of small intestine (i.e., from the pylorus to ileocecum) were measured, and the small intestinal transit (SIT) calculated as (20): $\text{SIT (\%)} = 100 \times \text{charcoal distance} / \text{small intestine length}$.

Histological Analysis

Swiss rolls were prepared from freshly obtained duodenal, jejunal, ileal and colonic mouse segments (21), fixed overnight in 10% formaldehyde at 4°C, and then transferred to 70% (v/v) ethanol. The samples were stained with hematoxylin and eosin by the Comprehensive Cancer Center and analyzed by the Pathology Cores for Animal Research at the University of Michigan.

Data Analysis

Pharmacokinetic parameters after both intravenous and oral dosing of [14 C]GlySar were determined using a noncompartmental approach (WinNonlin, v5.2; PharSight, Mountain View, CA). Data were reported as mean $\pm$ SE. Statistical differences between two treatment groups were determined using a two-sample student's t-test. For multiple treatment groups, statistical differences were determined using a one-way analysis of variance and Tukey method. All statistical analyses were performed by Prism v5.0 (GraphPad Software, Inc., San Diego, CA). A p value ≤ 0.05 was considered statistically significant.

RESULTS

Effect of 16-Hr Fast on PEPT1 Expression in Intestines

In wild-type mice, mRNA expression of PEPT1 was most abundant in the jejunum and ileum, then duodenum, with low level expression in the colon (Fig. 1). Fasting had little

effect on PEPT1 mRNA in the intestines, with minor reductions being observed in the duodenum and distal colon. In contrast, protein expression of PEPT1 was increased more than 2-fold in the duodenum and jejunum and about 1.5-fold in the ileum of fasted mice (Fig. 2). Protein expression in proximal and distal colon was undetectable in both fed and fasted states.

Effect of 16-Hr Fast on Systemic Exposure and Tissue Distribution of GlySar after Intravenous Dosing

As shown in Fig. 3, the plasma concentration-time curves of intravenously administered GlySar were very similar in wild-type and *Pept1* knockout mice under both fed and fasted conditions. Pharmacokinetic analysis of the curves showed that GlySar disposition was not different between the fed and fasted states in each genotype (Table I). Likewise, there was no difference between wild-type and *Pept1* knockout mice under fed conditions. However, significant reductions were observed in the volume of distribution steady-state (V_{dss}), mean residence time (MRT), the terminal half-life ($T_{1/2}$) of GlySar in *Pept1* knockout mice under fasted conditions; clearance (CL) was unchanged in all treatment groups.

Significant differences in the tissue distribution of GlySar, two hours after intravenous dosing, were evident even though blood concentrations of dipeptide were not different as a function of genotype and diet (Fig. 4). These differences included a greater distribution of GlySar in the ileum of wild-type fasted vs. fed mice, a reduced distribution of GlySar in the lung, liver, stomach, duodenum, colon, and spleen of *Pept1* knockout fasted vs. fed mice, and a reduced distribution of GlySar in the liver, stomach, duodenum, jejunum, and ileum of *Pept1* knockout vs. wild-type animals during fasted conditions. The fact that kidney levels were not different among the four treatment groups probably explains the lack of significant differences in GlySar clearance since the renal route dictates how dipeptide is eliminated from the body.

Effect of 16-Hr Fast on Absorption and Tissue Distribution of GlySar after Oral Dosing

The plasma concentration-time profiles of GlySar in wild-type and *Pept1* null mice, during fed and fasted conditions, are displayed in Fig. 5. A summary of the pharmacokinetic parameters for these four treatment groups is presented in Table II. Area under the plasma concentration-time curves ($AUC_{0-360 \text{ min}}$) of GlySar were significantly lower in *Pept1* knockout mice than wild-type animals, regardless of diet, reflecting the reduced intestinal absorption of dipeptide during PEPT1 ablation. For the same reason, no difference was observed in the $AUC_{0-360 \text{ min}}$ of GlySar in fed vs. fasted *Pept1*-deficient mice. However, in wild-type mice, fasted animals had a significantly greater $AUC_{0-360 \text{ min}}$ where the increased intestinal absorption of GlySar reflected the upregulation of PEPT1 protein in all three regions of small intestine (see Fig. 2). The same trends and conclusions were true for peak plasma concentrations (C_{max}) of GlySar. In contrast, no differences were observed between the four treatment groups in the time to reach peak plasma concentrations (T_{max}) or in the terminal half-life ($T_{1/2}$) of dipeptide. Likewise, the tissue distribution of GlySar was unchanged when sampled six hr after oral dosing (Fig. 6).

Effect of 16-Hr Fast on Small Intestinal Transit Time

A charcoal meal was administered orally as a method to compare the small intestinal transit times of wild-type and *Pept1* knockout mice in the fed-fasted state. As observed in Fig. 7, the leading edge of the charcoal suspension advanced to about 75% of small intestine 30 min after the meal was given. The 75% estimate for SIT was independent of genotype as well as diet.

Histological Examination of Intestines

Small intestinal and colonic segments of wild-type and *Pept1* knockout mice, under fed and fasted conditions, were evaluated for mucosal height and composition, lamina propria lymphoplasmacytic inflammatory cells, degree of mitotic activity, submucosal and muscular thickness, appearance, and overall morphology (n=6). No significant histological or morphological differences were observed in the animals as a function of genotype or diet. Representative examples of jejunum histology are shown for wild-type and *Pept1* knockout null mice during the fed (Fig. 8) and fasted (Fig. 9) states.

DISCUSSION

The transcription factors Sp1 (22) and Cdx2 (23) are functionally and physically associated with one another, thereby, resulting in the basal transcriptional regulation and intestine-specific expression of human PEPT1. Moreover, the peroxisome proliferator-activated receptor PPAR plays a critical role in fasting-induced intestinal PEPT1 expression (13). These studies and others (3, 4) have provided a molecular understanding of PEPT1 regulation. However, there are no studies that demonstrate unambiguously the upregulation of intestinal PEPT1 expression during fasted conditions and a concomitant increase in the *in vivo* intestinal absorption of a PEPT1 substrate following oral dosing.

In the present study, wild-type mice were compared to *Pept1* knockout animals after oral and intravenous administrations of GlySar in both the fed and fasted state. We found that: 1) expression of PEPT1 protein in small intestine was increased about 2-fold in wild-type mice during fasted vs. fed conditions, 2) systemic exposure and peak plasma concentrations of orally administered GlySar were 40 and 65% greater, respectively, in wild-type mice during fasted vs. fed conditions; no significant differences were observed between fed and fasted animals during PEPT1 ablation; 3) GlySar clearance was unaffected by genotype or the fed-fasted state; 4) tissue distribution of GlySar was unchanged after oral dosing for all four treatment groups; and 5) no differences were observed in small intestinal transit time or histology for all treatment groups.

Most studies investigating the effect of fasting on PEPT1 expression and functional activity have been performed over a 2- to 4-day period in rats (12-14). Because mice have smaller body weights than rats, and because drug absorption studies are usually performed in mammals (especially humans) after an overnight fast, we chose 16 hr as the fasting time in our study design. Our compound of interest, GlySar, was dissolved in isotonic saline solution for both intravenous and oral administrations. To minimize the potential for solid food effects during the oral absorption of GlySar (e.g., changes in gastric emptying and intestinal motility), regular chow was changed to a liquid diet during the fed studies. The liquid diet (LD 101) provides the same proportion of lipid, carbohydrate, and protein as solid rat chow, and is formulated for rodents where solid diets are not appropriate. As reported previously, similar weight gains were observed in mice fed this liquid formulation compared to standard solid chow (18, 24). It remains to be elucidated if solid food, as compared to a liquid diet, might alter PEPT1 intestinal expression and activity.

Differences in systemic exposure (i.e., $AUC = F \cdot Dose / CL$) after the oral dosing of GlySar could be due to differences in intestinal absorption (F) and/or total clearance (CL). To test if diet might cause dispositional changes in clearance, an intravenous dose of GlySar was administered to wild-type and *Pept1* null mice during fed and fasted conditions. As demonstrated in Table I and Fig. 3, the clearance of GlySar was unchanged as a function of diet as well as genotype, thereby, ruling out this possible explanation for the increased AUC of orally administered GlySar in fasted vs. fed wild-type mice. This finding was consistent with an earlier study in which PEPT1 protein levels in kidney were unaltered between fed

and 4-day fasted rats (14). Moreover, it was reported that PEPT1 accounted for only 16% of the renal reabsorption of GlySar when studied in wild-type and *Pept2* null mice (25). That study and others (26, 27) demonstrated conclusively that renal PEPT1 was a minor mechanism in the elimination of GlySar and other peptide/mimetic substrates from the body.

Aside from regulating transporter expression, food can change the absorption characteristics of a drug by a variety of mechanisms including the alteration of gastrointestinal residence time. To investigate this possibility as well as the potential for genotypic differences, a charcoal suspension was given orally to mice and the small intestinal transit times were evaluated over 30 min. We found that the charcoal had advanced to 75% of the small intestinal length in wild-type and *Pept1* knockout mice, and that the intestinal transit times were not influenced by fed-fasted conditions (Fig. 7). Moreover, no histological or morphological differences were observed in small intestinal and colonic segments of the mice, regardless of genotype or dietary treatment (Figs. 8 and 9). Finally, other POT family members (i.e., PEPT2, PHT1 and PHT2) were not upregulated in the small intestine, colon, or kidney during PEPT1 ablation (17) and, as a result, were not responsible for any differences between treatment groups in the systemic exposure of GlySar.

No differences were observed between wild-type and *Pept1* knockout mice, regardless of dietary state, in the tissue distribution of GlySar after oral dosing (Fig. 6). This finding was particularly surprising in small intestine given that PEPT1 protein is abundantly expressed in the duodenum, jejunum and ileum of mice (5). However, whole gut transit times of about 200 min have been reported in mice (28) and, as a result, the 6-hr tissue samples were probably more reflective of systemic distribution than absorption effects since. Thus, a sampling time around the $T_{\max}$ value would have been more suited for evaluating the influence of PEPT1 on intestinal GlySar distribution.

In fed-state mice, no differences were observed between wild-type and *Pept1* knockout mice in the tissue distribution of GlySar 120 min after intravenous bolus dosing (Fig. 4). In contrast, fasted-state *Pept1* knockout mice showed reduced concentrations of GlySar in the liver, stomach, duodenum, jejunum and ileum as compared to wild-type animals. The latter result is more difficult to reconcile since PEPT1 is expressed solely on the lumen-facing apical membrane of enterocytes and would not be expected to affect the intestinal distribution of GlySar following intravenous injection. However, GlySar accumulation will depend in general upon the rate at which the dipeptide enters, leaves, and is retained by the tissue. Little is known about the precise localization and function of peptide/histidine transporters PHT1 and PHT2 in the intracellular milieu of small intestine (29, 30). Even less is known about how peptides/mimetics efflux from enterocytes into blood, although functional studies suggest a facilitative transporter is operative at the basolateral membrane (31). Although speculative, up-regulation of peptide/histidine transporters in the first case and/or of facilitative basolateral transporters in the second case, during fasted-state conditions, might serve to lower the intestinal accumulation of GlySar in *Pept1* knockout mice. An up-regulation of PEPT1, during fasting, might also lower the accumulation of GlySar in liver since functional studies in rat have supported its presence in lysosomes (32) while immunohistochemical studies have located this POT protein to the apical membrane of mouse bile duct epithelial cells (33). Obviously, such regulatory changes would have to be demonstrated experimentally.

Using everted jejunal rings in wild-type and *Pept1* null mice, PEPT1 was previously reported by our laboratory to account for about 80% of the intestinal uptake of GlySar (10). In contrast, the magnitude of change in this study was more modest, with *Pept1* knockout mice having systemic exposures of GlySar that were 65% and 52% of wild-type animals, respectively, during fed and fasted states. This finding highlights the fact that *in vitro*

studies, although valid mechanistically, may not represent what happens under physiologic *in vivo* conditions. Although speculative, we believe that GlySar takes advantage of the small intestine's residual length and residence times so that other mechanisms become more important (e.g., passive permeability, paracellular flux) in the absence of PEPT1 (paper in review).

In concluding, drug-diet interactions are very complex with a variety of possible outcomes. Nevertheless, our studies clearly demonstrate that as little as 16 hr of fasting can cause a significant upregulation of PEPT1 protein expression in the small intestine which then translates into a significant increase in the *in vivo* oral absorption of GlySar. Future studies will be directed at the oral absorption of pharmacologically active PEPT1 substrates (e.g., cefadroxil and valacyclovir) under various fed-fasted conditions.

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Figure 1.

PEPT1 mRNA expression in small intestinal and colonic segments of wild-type (+/+) mice during fed and fasted conditions. mRNA expression in the duodenum of fed mice was considered the control group and was arbitrarily assigned a value of unity. mRNA expression in other treatment groups was scaled to the control value. Data are reported as mean $\pm$ SE (n=6). Statistical differences between fed and fasted conditions for each tissue segment were determined using a two-sample student's t-test; * $p \leq 0.05$.

Figure 2.

PEPT1 protein expression in small intestinal and colonic segments of wild-type mice during fed and fasted conditions. For each tissue, protein expression in fed mice was considered the control group and was arbitrarily assigned a value of unity. Protein expression in fasted mice was scaled to the control value. Data are reported as mean $\pm$ SE (n=6). Statistical differences between fed and fasted conditions for each tissue segment were determined using a two-sample student's t-test; **p $\leq$ 0.01 and ***p $\leq$ 0.001. Relative protein expression levels in small intestinal and colonic segments have been reported previously (see reference 5, Fig 7).

Figure 3. Plasma concentration-time profiles of [^{14}C]GlySar during fed and fasted conditions in wild-type (+/+) and Pept1 knockout (-/-) mice after 5 nmol/g intravenous bolus doses of dipeptide. Data are reported as mean $\pm$ SE (n=6).

Figure 4.

Tissue distribution of [^{14}C]GlySar during fed and fasted conditions in wild-type (+/+) and Pept1 knockout (-/-) mice, 120 min after 5 nmol/g intravenous bolus doses of dipeptide. Data are reported as mean $\pm$ SE (n=6). One-way ANOVA and Tukey method of multiple comparisons were performed to test for differences among treatment groups: ^ARepresents significant differences between fed and fasted conditions in wild-type mice; ^Brepresents significant differences between fed and fasted conditions in Pept1 knockout mice; ^Crepresents significant differences between wild-type and Pept1 knockout mice in fed conditions; and ^Drepresents significant differences between wild-type and Pept1 knockout mice in fasted conditions.

Figure 5. Plasma concentration-time profiles of [^{14}C]GlySar during fed and fasted conditions in wild-type (+/+) and Pept1 knockout (-/-) mice after 5 nmol/g oral doses of dipeptide. Data are reported as mean $\pm$ SE (n=6).

Figure 6.

Tissue distribution of [¹⁴C]GlySar during fed and fasted conditions in wild-type (+/+) and Pept1 knockout (-/-) mice, 360 min after 5 nmol/g oral doses of dipeptide. Data are reported as mean ± SE (n=6). No statistical differences were observed using one-way ANOVA.

Figure 7.

Small intestinal transit, as determined at 30 min by a charcoal meal, during fed and fasted conditions in wild-type (+/+) and *Pept1* knockout (-/-) mice. Data are reported as mean $\pm$ SE (n=6). No statistical differences were observed using one-way ANOVA.

FED CONDITIONS

Figure 8. Histology of the jejunum during fed conditions in wild-type (+/+) mice (**a**, **b**) and Pept1 knockout (-/-) mice (**c**, **d**). Sections were stained with hematoxylin and eosin, and examined by light microscopy. In (**a**) and (**c**), the bar = 1 mm; in (**b**) and (**d**), the bar = 100 μm.

FASTED CONDITIONS

Figure 9. Histology of the jejunum during fasted conditions in wild-type (+/+) mice (**a**, **b**) and Pept1 knockout (-/-) mice (**c**, **d**). Sections were stained with hematoxylin and eosin, and examined by light microscopy. In (**a**) and (**c**), the bar = 1 mm; in (**b**) and (**d**), the bar = 100 μm.

Table I

Pharmacokinetics of [^{14}C]GlySar During Fed and Fasted Conditions in Wild-Type and *Pept1* Knockout Mice after 5 nmol/g Intravenous Bolus Doses of Dipeptide

Parameter	Wild-Type		<i>Pept1</i> Knockout	
	Fed	16-Hr Fast	Fed	16-Hr Fast
CL ($\mu\text{l}/\text{min}$)	216 $\pm$ 19	253 $\pm$ 21	223 $\pm$ 13	221 $\pm$ 11
Vdss (ml)	8.6 $\pm$ 1.2	13.2 $\pm$ 2.0 ^D	8.1 $\pm$ 0.8	6.9 $\pm$ 0.6 ^D
MRT (min)	33.5 $\pm$ 1.8	39.1 $\pm$ 2.0 ^D	32.9 $\pm$ 1.6	29.0 $\pm$ 1.6 ^D
T _{1/2} (min)	44.6 $\pm$ 5.5	59.1 $\pm$ 6.3 ^D	37.9 $\pm$ 2.6	34.1 $\pm$ 2.4 ^D

Data are represented as mean $\pm$ SE (n = 6).

CL, total plasma clearance; Vdss, volume of distribution steady-state; MRT, mean residence time; T_{1/2}, terminal half-life.

One-way ANOVA and Tukey method of multiple comparisons were performed to test for differences among treatment groups: ^ARepresents significant differences between fed and fasted conditions in wild-type mice; ^Brepresents significant differences between fed and fasted conditions in *Pept1* knockout mice; ^Crepresents significant differences between wild-type and *Pept1* knockout mice in fed conditions; and ^Drepresents significant differences between wild-type and *Pept1* knockout mice in fasted conditions.

Table II

Pharmacokinetics of [^{14}C]GlySar During Fed and Fasted Conditions in Wild-Type and *Pept1* Knockout Mice after 5 nmol/g Oral Doses of Dipeptide

Parameter	Wild-Type		<i>Pept1</i> Knockout	
	Fed	16-Hr Fast	Fed	16-Hr Fast
AUC _{0-360 min} ($\mu\text{M}\cdot\text{min}$)	415 $\pm$ 11 ^{A,C}	581 $\pm$ 46 ^{A,D}	268 $\pm$ 8 ^C	302 $\pm$ 13 ^D
C _{max} (μM)	1.67 $\pm$ 0.03 ^{A,C}	2.80 $\pm$ 0.10 ^{A,D}	1.15 $\pm$ 0.07 ^C	1.21 $\pm$ 0.03 ^D
T _{max} (min)	47.5 $\pm$ 4.6	52.5 $\pm$ 3.4	45.0 $\pm$ 3.9	55.0 $\pm$ 7.4
T _{1/2} (min)	350 $\pm$ 16	410 $\pm$ 47	374 $\pm$ 31	401 $\pm$ 47

Data are represented as mean $\pm$ SE (n = 6).

AUC_{0-360 min}, area under the plasma concentration-time curve from 0 to 360 min; C_{max}, peak plasma concentration; T_{max}, time to reach peak plasma concentration; T_{1/2}, terminal half-life.

One-way ANOVA and Tukey method of multiple comparisons were performed to test for differences among treatment groups: ^ARepresents significant differences between fed and fasted conditions in wild-type mice; ^Brepresents significant differences between fed and fasted conditions in *Pept1* knockout mice; ^Crepresents significant differences between wild-type and *Pept1* knockout mice in fed conditions; and ^Drepresents significant differences between wild-type and *Pept1* knockout mice in fasted conditions.