JPET Fast Forward. Published on June 29, 2005 as DOI: 10.1124/jpet.105.089359

Role and Relevance of PEPT2 in the Kidney and Choroid Plexus: *In Vivo* Studies with Glycylsarcosine in Wild-Type and PEPT2 Knockout Mice

Scott M. Ocheltree, Hong Shen, Yongjun Hu, Richard F. Keep, and David E. Smith

Department of Pharmaceutical Sciences (S.M.O., H.S., Y.H., D.E.S.) and Departments of Neurosurgery and Physiology (R.F.K.), The University of Michigan, Ann Arbor, Michigan

Running title: *In Vivo* Role-Relevance of PEPT2 in Kidney and Choroid Plexus

Corresponding author: David E. Smith, 4302A Upjohn Center, 1310 E. Catherine Street, The University of Michigan, Ann Arbor, MI 48109-0504. Telephone: (734) 647-1431; facsimile: (734) 763-3438; E-mail: smithb@umich.edu

Text: 21 pages

Tables: 2

Figures: 6

References: 35

Abstract: 238 words

Introduction: 660 words

Discussion: words: 1657 words

ABBREVIATIONS: BCSFB, blood-cerebrospinal fluid barrier; CSF, cerebrospinal fluid; GFR, glomerular filtration rate; GlySar, glycylsarcosine; POT, proton-coupled oligopeptide transporter; RT-PCR, reverse transcription-polymerase chain reaction.

Recommended section: Absorption, Distribution, Metabolism, & Excretion

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ABSTRACT

The strategic localization of PEPT2, a proton-coupled oligopeptide transporter, to the apical membrane of epithelial cells in the kidney and choroid plexus suggests that it plays an important role in the disposition of peptides/mimetics in the body. Therefore, the *in vivo* significance of PEPT2 was investigated in wild-type and PEPT2 null mice following an intravenous bolus dose (0.05 μmol/g body weight) of [^{14}C]glycylsarcosine (GlySar). In PEPT2 null mice, the clearance (total and renal) of GlySar was markedly increased (2-fold), resulting in concomitantly lower systemic concentrations. In addition, renal reabsorption was almost abolished and GlySar was eliminated by glomerular filtration. Out of the 46% of GlySar reabsorbed in wild-type mice, PEPT2 accounted for 86% and PEPT1 accounted for 14% of reabsorbed substrate. Analysis of GlySar uptake in kidney sections revealed that PEPT2 was primarily localized in the outer medullary region. Wild-type mice also had greater choroid plexus concentrations of GlySar and a 5-fold greater choroid plexus/CSF ratio as compared to null mice at 60 min. Null mice exhibited a greater CSF/blood ratio at 60 min (0.9 vs. 0.2) and AUC_{CSF}/AUC_{blood} ratio over 60 min (0.45 vs. 0.12), indicating that PEPT2 significantly reduces the exposure of GlySar in CSF. Our *in vivo* results demonstrate that PEPT2 is the predominant peptide transporter in kidney and that it acts as an efflux transporter in choroid plexus. Thus, PEPT2 may have profound effects on the sensitivity and/or toxicity of peptides and peptide-like drugs.

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Introduction

PEPT2, a member of the proton-coupled oligopeptide transporter (POT) family (i.e., PEPT1, PEPT2, PHT1, PHT2), is an electrogenic, sodium-independent symporter that translocates small peptides (i.e., sequence-independent di-and tripeptides) along with protons across biological membranes via an inwardly-directed electrochemical gradient of protons (Herrera-Ruiz and Knipp, 2002). PEPT2 is also important in its ability to transport peptide-like drugs (e.g., aminocephalosporins, angiotensin-converting enzyme inhibitors, antiviral nucleoside prodrugs), endogenous peptidomimetics (e.g., 5-aminolevulinic acid) and neuropeptides (e.g., carnosine) (Rubio-Aliaga and Daniel, 2002; Smith et al., 2004). PEPT2 was initially cloned from a human kidney cDNA library (Liu et al., 1995) and is characterized as a high affinity (i.e., μM K_m), low capacity transporter (Daniel and Rubio-Aliaga, 2003). PEPT2 is widely expressed in a variety of tissues including the kidney, brain, lung, eye and mammary gland (Daniel and Kottra, 2004; Smith et al., 2004). In particular, PEPT2 mRNA and protein are highly expressed in the apical membrane of epithelium in kidney proximal tubule and brain choroid plexus. Despite the high expression of PEPT2, other POT members are present in the kidney (i.e., PEPT1) and brain (i.e., PHT1, PHT2), thereby complicating an accurate assessment of the physiological role and pharmacological significance of PEPT2 in these tissues.

PEPT2 functions in concert with the low affinity (i.e., mM K_m), high capacity transporter PEPT1 (Daniel, 1996; Leibach and Ganapathy, 1996) at the apical membrane of renal proximal tubular epithelium for the efficient reabsorption of peptide-bound amino nitrogen from the glomerular filtrate. It is generally believed that PEPT2 is the predominant POT in kidney responsible for the reabsorption of small peptides and peptide-like drugs (Takahashi et al., 1998;

Smith et al., 1998; Shen et al., 1999), although definitive proof for this assertion is lacking. If true, the activity of PEPT2 in kidney could have significant consequences on the systemic exposure to and renal elimination of peptidomimetics, potentially altering the sensitivity or toxicity to certain therapeutic agents.

The choroid plexus epithelium with linking tight junctions, and array of membrane transporters, forms the blood-cerebrospinal fluid (CSF) barrier (BCSFB). PEPT2 mRNA transcripts (Berger and Hediger, 1999), protein (Novotny et al., 2000, Shu et al., 2002; Shen et al., 2004) and functional activity (Teuscher et al, 2000; 2001), along with PHT1 transcripts (Yamashita et al., 1997), have been reported in rat choroid plexus. PHT2 transcripts have also been found in rat brain (Sakata et al., 2001). Studies in rat choroid plexus epithelial cells in primary culture (Shu et al., 2002; Teuscher et al., 2004) have demonstrated that PEPT2 (as opposed to PHT1 and PHT2) is functionally active on the apical membrane of choroid plexus (i.e., CSF-facing), and that it may play a role in neuropeptide homeostasis. However, *in vivo* experiments in support of this contention are lacking at the present time.

While cellular, molecular and physiological studies have made major contributions toward a mechanistic understanding of PEPT2 structure, function and localization, the experimental approaches are often limited by an *in vitro* design and lack of blood supply, overlapping substrate specificities and contribution of multiple transport systems, some of which are unknown at the time of study. As a result, it is difficult, if not impossible, to define the function of a single specific gene product and its significance in relation to other possible proteins that are present in the tissue or organ of interest. The recent generation of PEPT2 knockout mice by this laboratory (Shen et al., 2003) and others (Rubio-Aliaga et al., 2003) has a provided a unique tool to probe the functional activity of PEPT2 on a multi-organ level under physiological conditions.

With this in mind, we have investigated the role and relative importance of PEPT2 in affecting the *in vivo* pharmacokinetics, tissue distribution and systemic exposure of a hydrolysis/peptidase-resistant synthetic dipeptide, glycylsarcosine (GlySar), in $PEPT2^{+/+}$ (wild-type) and $PEPT2^{-/-}$ (null) mice. Our results are novel in demonstrating, for the first time, that PEPT2 has a substantial influence on the *in vivo* disposition of a dipeptide within the body, particularly the kidney and brain.

Methods

Materials. [Glycine-U-^{14}C]Glycylsarcosine (GlySar; 106 mCi/mmol) and [^{3}H]inulin (17 Ci/mmol) were supplied by Amersham Biosciences (Chicago, IL). [^{3}H]Glycine (30 Ci/mmol) was purchased from Moravek Radiochemicals (Brea, CA). Unlabeled GlySar was obtained from Sigma-Aldrich (St. Louis, MO) and 1 M hyamine hydroxide was purchased from ICN (Irvine, CA). All other chemicals were obtained from standard sources. PEPT2-transgenic mice were generated in-house on a C57BL/6 mouse background and genotyped by polymerase chain reaction, as described by Shen et al. (2003). Animals were housed in a temperature-controlled environment with a 12-hour light, 12-hour dark cycle and given ad libitum access to food and water. All studies were carried out 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.

Pharmacokinetic and Tissue Distribution Studies. Gender-matched PEPT2$^{+/+}$ and PEPT2$^{-/-}$ littermates (6-8 weeks old) were anesthetized with sodium pentobarbital (65 mg/kg i.p.). Mice were then administered [^{14}C]GlySar (5 µCi/mouse plus unlabeled GlySar for a total dose of 0.05 µmol/g body weight) by a tail vein injection (100 µl normal saline). Serial blood samples (5 µl) were collected at 0.25, 1, 2, 5, 10, 20, 30, 45 and 60 min after the intravenous bolus by tail transection. At the end of blood sampling, a CSF sample (~5 µl) was obtained by inserting the tip of a 28G1/2 U-100 insulin syringe (Becton Dickinson; Franklin Lakes, NJ) into the cisterna magna (Carp et al., 1971). The mouse was immediately decapitated and multiple tissues/organs were harvested (i.e., lateral and fourth ventricle choroid plexuses, cerebral cortex, kidney, liver, eye, lung, heart, spleen, small and large intestines, skeletal muscle, thymus, pancreas). Kidney tissue was processed intact for one kidney and after separating the renal

cortex, outer medulla (outer and inner stripe), and inner medulla for the other kidney. Tissue samples were blotted dry, weighed and digested in 0.5 ml of 1 M hyamine hydroxide (a tissue solubilizer) for 24 hr at 37°C. Ecolite(+) liquid scintillation cocktail (ICN; Irvine, CA) was added to the solubilized tissues, CSF and blood samples, and the radioactivity in each sample was measured by a dual-channel liquid scintillation counter (Beckman LS 3801; Beckman Coulter, Inc., Fullerton, CA). [^{3}H]inulin (1 μCi/mouse) was administered intravenously 2 min prior to harvesting the tissues so that GlySar tissue concentrations could be corrected for vascular space of brain (Keep et al., 1999; Ennis et al., 2003) or extracellular space of other tissues.

Corrected tissue concentrations of GlySar (nmol/g wet tissue) were calculated as: C_{tiss}-V•C_b, where C_{tiss} is the uncorrected GlySar tissue concentration (nmol/g), V (ml/g) is the inulin space, and C_b is the GlySar blood concentration (nmol/ml).

Time Course Studies of Tissue Distribution. Since the tissue (or CSF)-to-blood concentration ratio of GlySar may change as a function of time (i.e., a temporal relationship exists), a select number of tissues were harvested during the first hour of dosing. Following sodium pentobarbital anesthesia (65 mg/kg i.p.), gender-matched PEPT2$^{+/+}$ and PEPT2$^{-/-}$ littermates (6-8 weeks old) were administered [^{14}C]GlySar (5 μCi/mouse plus unlabeled GlySar for a total dose of 0.05 μmol/g body weight) by a tail vein injection (100 μl normal saline), and tissue samples were corrected for vascular volume using [^{3}H]inulin. Blood, CSF, choroid plexus, cerebral cortex and kidney samples were obtained at 2, 5 and 15 min after dosing, and processed as described previously (note: 60-min samples were obtained from previous study).

Renal Clearance Studies. Following sodium pentobarbital anesthesia (65 mg/kg i.p.), gender-matched PEPT2$^{+/+}$ and PEPT2$^{-/-}$ littermates (6-8 weeks old) were coadministered [^{14}C]GlySar (5 μCi/mouse plus unlabeled GlySar for a total dose of 0.05 μmol/g body weight)

and [^{3}H]inulin (0.5 μCi/g body weight; 0.00086 μmol/g body weight) by a tail vein injection (100 μl normal saline). Inulin was coadministered in order to estimate the glomerular filtration rate (GFR) of each animal, since inulin is not bound to plasma proteins and is freely filtered, with no secretion or reabsorption by the kidneys (Rowland and Tozer, 1995). Serial blood samples (~25 μl) were collected at 0.25, 1, 2, 5, 10, 20, 30, 45 and 60 min after the intravenous bolus by tail transection. Blood samples were immediately transferred into 0.2 ml thin-wall PCR tubes (United Laboratory Plastics; St. Louis, MO) containing 1 μl of 7.5% potassium EDTA, and then centrifuged at 2,000 x g for 10 min at ambient temperature. The total urine of each animal was aspirated directly from the bladder with a 28G1/2 U-100 insulin syringe at 60 min. Radioactivity in the plasma and urine samples was determined by dual-channel liquid scintillation counting, as described previously.

Plasma Protein Binding Studies. The plasma protein binding of GlySar was determined by ultrafiltration, as described previously (Lepsy et al., 2003). Blank plasma from each genotype was spiked with [^{14}C]GlySar to produce standard concentrations of 10, 100 and 1000 μM, values that span the plasma concentrations observed after an intravenous bolus dose of GlySar (0.05 μmol/g body weight). Subsequently, a 0.5-ml aliquot of each standard was added to a disposable Microcon® YMT-30 centrifugal filter device (Millipore, Billerica, MA), employing an anisotropic hydrophilic membrane that excludes molecules larger than ~30 kDa. The device was capped, equilibrated at 37°C for 15 min in a 35° fixed-angle rotor, and centrifuged for 25 min at 37°C and 1,800 x g. The protein-free ultrafiltrate (~300 μl) was then collected for each sample. Preliminary studies demonstrated a negligible binding of GlySar to the ultrafiltration device and a lack of protein leakage during the ultrafiltration process (data not shown). The unbound

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fraction of GlySar in plasma (fu) was calculated as the ratio of the GlySar concentration in the ultrafiltrate to that in the original plasma standard.

Metabolic Stability Studies. Following sodium pentobarbital anesthesia (65 mg/kg i.p.), gender-matched $PEPT2^{+/+}$ and $PEPT2^{-/-}$ littermates (6-8 weeks old; n=3 per genotype) were administered [^{14}C]GlySar (5 µCi/mouse plus unlabeled GlySar for a total dose of 0.05 µmol/g body weight) by a tail vein injection (100 µl normal saline). Each mouse was separately placed in a Nalgene® metabolic cage with diuresis adapter (Harvard Apparatus; Holliston, MA) for 24 hours. At the end of this period, the diuresis adapter was washed with water and the diluted urine was collected and measured. An aliquot (100 µl) of the diluted urine sample was taken and total recovery of GlySar radioactivity was determined by liquid scintillation counting, as described previously. A second aliquot (100 µl) of the diluted urine sample was pretreated with 200 µl of acetonitrile in a 1.5-ml polypropylene microcentrifuge tube (Safe-Lock; Eppendorf/Brinkman Instruments, Westbury, NY), vortex-mixed for 5 sec, and centrifuged at 2,000 x g for 10 min under ambient conditions. The stability of GlySar was determined by its recovery and the appearance of glycine in the 24-hour urine collection. GlySar and/or glycine were detected in the resultant supernatant using a high-performance liquid chromatography (HPLC) system consisting of a pump (model 616 with 600S controller; Waters, Milford, MA), a Rheodyne® injector port (Rohnert Park, CA) with 100 µl sample loop, a reversed-phase column (5-µm, Hypersil ODS C-18, 4.6 mm x 250 mm; Alltech Associates Inc., Deerfield, IL) and a radiochemical detector (FLO-ONE 515TR Series Flow Scintillation Analyzer; Perkin Elmer Life and Analytical Sciences, Boston, MA). The mobile phase was composed of 0.01 M phosphate buffer (pH 2.0) and 0.1% heptafluorobutyric acid, and isocratically pumped at 1 ml/min under ambient conditions. Retention times for glycine and GlySar were approximately 4.8 and 9.8

min, respectively. Peaks were identified by injecting known standards of radiolabeled glycine and GlySar.

Pharmacokinetics of GlySar Blood Concentrations. Blood concentration vs. time data for GlySar were best described by a 2-compartment open model with first-order elimination and a weighting factor of unity (WinNonlin v4.1; Pharsight Inc., Mountain View, CA):

$$C_b = C_1 \bullet e^{-\lambda_1 t} + C_2 \bullet e^{-\lambda_2 t}$$

where C_b is the GlySar blood concentration at time t, and C_1 and C_2 are the coefficients associated with the λ_1 and λ_2 exponents, respectively, where $\lambda_1 > \lambda_2$. The quality of the fit was determined by evaluating the coefficient of determination (r^2), the standard error of parameter estimates, and by visual inspection of the residuals. The following standard pharmacokinetic parameters were then generated by WinNonlin:

$$V_1 = D / (C_1 + C_2)$$

$$Vd_{ss} = D \bullet (C_1/\lambda_1^2 + C_2/\lambda_2^2) / (C_1/\lambda_1 + C_2/\lambda_2)^2$$

$$AUC = C_1/\lambda_1 + C_2/\lambda_2$$

$$CL = D / (C_1/\lambda_1 + C_2/\lambda_2)$$

$$K_{10} = CL / V_1$$

$$t_{1/2} = 0.693 / \lambda_2$$

$$MRT = Vd_{ss} / CL$$

where D is the intravenous bolus dose of GlySar, V_1 is the volume of the central compartment, Vd_{ss} is the volume of distribution steady state, AUC is the area under the blood concentration-time curve, CL is the total blood clearance, k_{10} is the elimination rate constant from the central compartment, $t_{1/2}$ is the terminal half-life, and MRT is the mean residence time.

Renal Pharmacokinetics of GlySar Plasma Concentrations. The renal clearance (CL_R) of GlySar (or inulin, GFR) was calculated as:

$$CL_R \text{ (or GFR)} = Ae^{60} / AUC^{60}$$

where Ae^{60} is the amount of GlySar (or inulin) excreted unchanged in the urine over 60 min and AUC^{60} is the area under the GlySar (or inulin) plasma concentration-time curve from 0-60 min (determined noncompartmentally by WinNonlin). In the absence of tubular secretion (of which there is no evidence for GlySar), the renal clearance of GlySar can be expressed as (Levy, 1980):

$$CL_R = fu \bullet GFR \bullet (1 - F)$$

where fu is the fraction of GlySar unbound in plasma and F is the fraction of available dipeptide that is reabsorbed. The excretion ratio of GlySar (ER), which normalizes CL_R for any differences in protein binding (fu) and functional nephron mass (GFR), was calculated as:

$$ER = CL_R / (fu \bullet GFR) = 1 - F$$

Based on the sequential expression of PEPT1 and PEPT2 in the proximal tubule (Smith et al., 1998; Shen et al., 1999), this equation can be transformed, by taking into account the individual contributions of peptide transporters, such that:

$$CL_R = fu \bullet GFR \bullet (1 - F_1) \bullet (1 - F_2)$$

where F_1 is the fraction of available dipeptide that is reabsorbed by PEPT1 and F_2 is the fraction of available dipeptide that is reabsorbed by PEPT2. Since F_2=0 in null mice, and having experimentally determined values for CL_R, fu and GFR, an estimate of F_1 can be made in the $PEPT^{-/-}$ mice. An estimate of F_2 can then be made in the $PEPT2^{+/+}$ mice since all remaining values are known (i.e., CL_R, fu and GFR experimentally determined and F_1 assumed to be unchanged in the wild-type mice). The relative contribution of each transporter was calculated as %PEPT1 = 100•(F_1/F) and %PEPT2 = 100•[F2•(1-F1)/F], where %PEPT1 is the percent of

reabsorbed GlySar that occurs via PEPT1 and %PEPT2 is the percent of reabsorbed GlySar that occurs via PEPT2.

Statistics. All data are reported as mean ± SE. Statistical comparisons were performed with ANOVA (GraphPad Prism, v3.0; GraphPad Software, Inc., San Diego, CA) in which a probability of $p \leq 0.05$ was considered statistically significant.

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Results

Pharmacokinetics of GlySar. As shown in Fig. 1, the blood concentrations of GlySar were significantly lower in PEPT2$^{-/-}$ mice as compared to PEPT2$^{+/+}$ control animals. A summary of the pharmacokinetic parameters for each genotype is presented in Table 1. While it appears that PEPT2 did not influence volume of distribution (central compartment volume, V_1 or steady-state volume, Vd_{ss}), the total blood clearance (CL) and central compartment elimination rate constant (K_{10}) of GlySar were about 2-fold greater in PEPT2 null mice, resulting in a 2-fold reduction in area under the blood concentration-time curve (AUC). Less dramatic differences were observed in the terminal half-life ($t_{1/2}$) and mean residence time (MRT) of GlySar, since these parameters reflect distribution (which does not change) as well as elimination (which does change).

Tissue Distribution of GlySar at 60 Minutes. Fig. 2 displays the concentrations of GlySar, 60 min following an intravenous bolus, in a variety of tissues, CSF and blood. As evident, PEPT2 deletion had a significant effect on the ability of these tissues to accumulate dipeptide. As a result, GlySar concentrations were significantly reduced in the kidney (4.5-fold), lung (2.8-fold), spleen (2.4-fold), choroid plexus (1.9-fold), eye (1.8-fold), liver (1.6-fold) and cerebral cortex (1.4-fold) of PEPT2$^{-/-}$ mice. Because kidney was the tissue demonstrating the highest concentration of GlySar in wild-type animals and the most dramatic difference between genotypes, the functional presence of PEPT2 was further probed by examining concentration differences in three distinct anatomical segments. As shown in Fig. 3A, GlySar concentrations were significantly reduced in all three kidney segments of PEPT2 null mice (as compared to wild-type animals), especially in the outer medulla where concentrations were the greatest and an 85% reduction was observed. However, when the data were corrected for differences in blood

concentrations, the outer medulla was the only region of kidney to demonstrate a statistically significantly difference between genotypes in the tissue/blood concentration ratio of GlySar (Fig. 3B). When the data were not adjusted for wet tissue weight, the outer medulla accounted for 66% of the total GlySar uptake by the kidney (data not shown).

In contrast to tissue and blood, PEPT2$^{-/-}$ mice had a 2-fold greater concentration of GlySar in CSF, despite having significantly lower blood concentrations (Fig. 2). As a result, the CSF/blood concentration ratio of GlySar was 4-fold greater in PEPT2 null animals as compared to wild-type controls ($p<0.001$; Fig. 4B). In the absence of PEPT2, the CSF/blood ratio was approximately 0.9, indicating that GlySar can achieve CSF concentrations roughly equivalent to that observed in blood. However, when PEPT2 is functionally present, GlySar's access to the CSF is dramatically limited (i.e., CSF/blood ratio of 0.2). While a modest 1.4-fold increase was observed in the cerebral cortex/blood concentration ratio of GlySar in PEPT2$^{-/-}$ mice (i.e., 0.28 in wild-type vs. 0.39 in PEPT2 null animals; $p<0.01$; Fig. 4D), no differences were observed between genotypes in the choroid plexus/blood ratio (i.e., 0.62 in wild-type vs. 0.68 in PEPT2 null animals; $p>0.50$; Fig. 4C). Nevertheless, a substantial difference was observed in the choroid plexus/CSF concentration ratio of GlySar, where PEPT2$^{+/+}$ mice had a 5-fold greater value (i.e., 4.2 in wild-type vs. 0.8 in PEPT2 null animals; $p<0.01$).

Time Course of GlySar Tissue Distribution. The tissue concentrations and tissue/blood concentration ratios of GlySar are shown over one hour following an intravenous bolus injection of dipeptide (Fig. 4). In general, PEPT2$^{+/+}$ mice exhibited greater tissue concentrations of GlySar in the kidney (Fig. 4A), choroid plexus (Fig. 4C) and cerebral cortex (Fig. 4D) than PEPT2$^{-/-}$ mice over the entire study period. In contrast, the wild-type animals had reduced levels of GlySar in CSF (Fig. 4B). While these significant differences were reduced (kidney) or absent

(choroid plexus) when tissue concentrations were corrected for differences in blood concentrations, the differences were magnified when tissue/blood ratios were compared between genotypes for CSF and reversed when tissue/blood ratios were compared between genotypes for cerebral cortex. In this regard, there was almost a 4-fold greater exposure of GlySar in CSF, relative to systemic blood levels, for PEPT2$^{-/-}$ vs. PEPT2$^{+/+}$ mice (AUC_{CSF}/AUC_{blood} ratios of 0.45 vs. 0.12, respectively; $p<0.001$). In addition, while cerebral cortex concentrations of GlySar were low (compared to these other tissues) and relatively constant, the tissue/blood ratio of GlySar steadily increased over time for both genotypes and was significantly greater in PEPT2 null mice.

Renal Clearance of GlySar. Plasma concentration-time profiles of GlySar and inulin are shown in Figs. 5A and 5B, respectively, following their coadministration by intravenous bolus injection. While the plasma levels of GlySar were significantly reduced in PEPT2$^{-/-}$ mice (as shown before in Fig. 1 for blood), the inulin plasma concentrations were similar, indicating that GFR was unchanged between genotypes. Since urine samples were obtained in these studies, along with estimates of plasma protein binding, an analysis of the renal tubular transport mechanisms of GlySar was possible, as shown in Table 2 and Fig. 6. The binding of GlySar to plasma proteins was negligible (fu < 10%) and no difference was observed between wild-type and PEPT2 null mice. There was also no statistical difference between genotypes in the urinary excretion of GlySar over 60 min (i.e., 0.57 μmol in wild-type vs. 0.68 μmol in PEPT2 null animals; $p>0.10$), where about 60% of the dipeptide dose was recovered in the urine unchanged. However, the renal clearance of GlySar was increased by 2-fold in PEPT2$^{-/-}$ mice as compared to PEPT2$^{+/+}$ animals (i.e., CL_R of 0.11 ml/min in wild-type vs. 0.24 ml/min in PEPT2 null mice; $p=0.020$) and, as a result, its excretion ratio was also substantially increased (i.e., ER of 0.54 in

wild-type vs. 0.94 in PEPT2 null mice; p=0.012). Thus, 46% of the available GlySar dose was reabsorbed by the kidney in PEPT2$^{+/+}$ animals (F=0.46), accounting for the 2-fold lower renal clearance for this genotype. Moreover, out of the 46% of GlySar reabsorbed in wild-type mice, PEPT2 accounted for 86% and PEPT1 accounted for 14% of reabsorbed substrate. In contrast, the renal clearance of GlySar in PEPT2$^{-/-}$ mice was almost identical to that of GFR. With an excretion ratio of 0.94, it appears that GlySar is almost exclusively filtered in PEPT2 null animals with only 6% of the available dipeptide being reabsorbed by the kidney (F=0.06). Taken together, these results explain the 2-fold difference in systemic exposure profiles observed between genotypes.

Metabolic Stability of GlySar. HPLC with radiochemical detection, coupled to liquid scintillation counting spectrometry, demonstrated that approximately 90% of GlySar was recovered in the urine 24 hours after an intravenous bolus dose of dipeptide, and that 95% of the sample was intact GlySar (i.e., < 5% hydrolysis to glycine) for both genotypes. Therefore, GlySar instability was not a confounding issue in these studies, and no further correction of the data was necessary.

Discussion

The present study examined the *in vivo* disposition of a model dipeptide substrate, GlySar, in wild-type and PEPT2 null mice. The absence of PEPT2 caused profound changes in the pharmacokinetics, tissue distribution and systemic exposure of GlySar. In particular, PEPT2 was essential for the efficient renal tubular reabsorption of GlySar (i.e., F=0.46 in wild-type vs. F=0.06 in null mice) and in dramatically limiting the exposure of GlySar in CSF. This study provides, to our knowledge, the first direct *in vivo* evidence that PEPT2 is the predominant peptide transporter in kidney and that it acts as an efflux transporter in the choroid plexus.

Functional analysis of GlySar uptake in kidney sections revealed that PEPT2 is localized primarily in the outer medullary region. This result coincides with previously reported RT-PCR and immunolocalization studies detailing the differential expression of PEPT1 and PEPT2 along the rat proximal tubule (Smith et al., 1998; Shen et al., 1999). In rat, PEPT1 was expressed in the early region of the proximal tubule (i.e., S1 segment of pars convoluta in renal cortex) while PEPT2 was expressed in the latter regions of the proximal tubule (i.e., S2/S3 segments of pars recta in deep cortex and outer stripe of outer medulla). Although the direct immunolocalization of PEPT1 and PEPT2 proteins in mouse kidney has not been performed, indirect localization studies in PEPT2 knockout mice (using β-galactosidase expression and fluorophore-conjugated dipeptide accumulation) suggested that PEPT1 and PEPT2 are also sequentially distributed in mouse proximal tubule (Rubio-Aliaga et al., 2003).

The sequential nature for peptide/mimetic processing in the proximal tubule, first by PEPT1 and then by PEPT2, was the basis for the model used to estimate the relative contribution of each POT in the renal reabsorption of GlySar. Renal pharmacokinetic studies revealed that out of the

46% of GlySar reabsorbed by the kidney in wild-type mice, PEPT2 accounted for 86% and PEPT1 accounted for 14% of reabsorbed substrate. Combining these results with GlySar renal uptake (Figs. 3B and 4A) and renal clearance data in null mice (Fig. 6) clearly showed that PEPT2 is the dominant peptide transporter in kidney. However, it is important to note that fractional reabsorption (F), including the fractional reabsorption by each transporter (F_1 and F_2), may vary depending on GlySar concentrations in the glomerular filtrate. In this study, GlySar produced plasma concentrations of about 10-400 μM. Although the concentrations and composition of plasma di/tripeptides are unknown, animal studies suggest that about 10-30% of circulating amino acids in plasma might be peptide bound (Daniel and Rubio-Aliaga, 2003). Moreover, the highest plasma concentration of a known individual dipeptide (i.e., cysteinylglycine) is about 50-70 μM in humans (Pastore et al., 1998). Because GlySar is not secreted and has negligible binding in plasma, the filtrate concentrations of GlySar are likely to approximate that in plasma. If true, the average filtrate concentrations of GlySar would be around its estimated K_m value (i.e., 50-100 μM) determined in rat renal brush border membrane vesicles (Tiruppathi et al., 1990; Takahashi et al., 1998) and the SKPT-0193 renal cell line (Brandsch et al., 1995; Shu et al., 2001) and, thus, favor transport by PEPT2. The low affinity, high capacity PEPT1 transporter may be responsible for handling higher di- and tripeptide concentrations, whereas PEPT2 dominates at lower, more physiologically relevant substrate concentrations. It is worth emphasizing the dual importance of inulin in this study, since inulin not only provided an estimate of GFR in each animal, but also served as a negative control between genotypes, validating the pharmacokinetic and tissue distribution data for GlySar.

Rubio-Aliaga and coworkers (2003) illustrated the importance of PEPT2 in the renal uptake of D-[^{3}H]Phe-Ala by demonstrating that only 35% of the dipeptide remained in kidney

homogenates prepared from PEPT2 null mice (as compared to wild-type animals) after an intraperitoneal injection of compound. Those studies were limited, however, by examining kidney concentrations at a single time point and by not characterizing the impact of PEPT2 on the renal clearance of dipeptides from the systemic circulation. Renal clearance of a compound is a function of glomerular filtration (fu•GFR), tubular secretion and tubular reabsorption. When tubular reabsorption occurs (and tubular secretion is absent), renal clearance is less than fu•GFR and the excretion ratio (ER) is less than unity. Therefore, an ER of 0.54 in wild-type mice indicates that 46% of the available GlySar dose is reabsorbed (i.e., F=0.46). In contrast, PEPT2 null mice had a 2-fold greater renal clearance of GlySar and almost complete abolishment of renal tubular reabsorption (i.e., ER=0.94 and F=0.06), indicating that GlySar is almost exclusively filtered by the kidney (i.e., $CL_R \approx$ GFR). The urinary excretion of GlySar was not statistically different between genotypes over the 60-min collection period (Ae^{60}). This finding is consistent with the relationship: $Ae^{60} = CL_R \bullet AUC^{60}$, in which equal and opposite changes (2-fold) were observed in CL_R and AUC^{60} between wild-type and PEPT2 null mice. For a compound in which the kidney is the only route of elimination, no difference in urinary excretion is expected.

The partitioning of GlySar in red blood cells relative in that in plasma (i.e., C_{rbc}/C_p) was estimated at 0.19 for each genotype and was calculated using AUC_{blood}/AUC_{plasma} ratios (0.61 in $PEPT2^{+/+}$ mice vs. 0.62 in $PEPT2^{-/-}$ mice) and hematocrit determinations (0.47 in $PEPT2^{+/+}$ mice vs. 0.48 in $PEPT2^{-/-}$ mice). The relatively minor partitioning of GlySar in mouse red blood cells is consistent with a study by Lochs et al. (1990), who reported the *in vitro* uptake of trace amounts of dipeptides by human red blood cells. As a result, red blood cells do not appear to play an appreciable role in the clearance of GlySar from plasma. Therefore, when these results

are combined with the lack of metabolism for GlySar (less than 5% over 24 hours based on urinary excretion data), it was concluded that renal PEPT2 activity determines the overall clearance and systemic exposure of similar peptides.

Although $PEPT2^{+/+}$ mice had greater choroid plexus GlySar concentrations, compared to $PEPT2^{-/-}$ mice, no differences were found in the corresponding tissue-to-blood concentration ratios at 60 min post-dose. However, PEPT2 is located at the interface of choroid plexus epithelium and CSF and, as a result, the tissue-to-CSF concentration ratios were more informative. We observed that wild-type mice had a 5-fold greater choroid plexus/CSF ratio, suggesting that while PEPT2 is responsible for the cellular uptake of GlySar from CSF, a significant accumulation of GlySar in CSF occurs in the protein's absence. Reinforcing this finding were the 2-fold greater CSF concentrations, and 4-fold greater CSF/blood and AUC_{CSF}/AUC_{blood} ratios of GlySar in $PEPT2^{-/-}$ mice, indicating that PEPT2 activity at the BCSFB reduces the exposure of GlySar in CSF. Thus, it was concluded that PEPT2 operates *in vivo* as an efflux transporter at the BCSFB, as suggested previously from *in vitro* studies on the choroid plexus uptake of GlySar and 5-aminolevulinic acid (Ocheltree et al., 2004a), carnosine (Teuscher et al, 2004) and cefadroxil (Ocheltree et al., 2004b) in transgenic mice. This conclusion is in complete accordance with the current model of peptide/mimetic trafficking at the BCSFB (Smith et al., 2004). Moreover, these results suggest that PEPT2 could have important implications for the distribution of peptide-like drugs (e.g., aminocephalosporins) in CSF, a factor potentially altering pharmacological response.

The role of PEPT2 in brain (i.e., excluding the choroid plexus) currently remains unclear. Shen and coworkers (2004) recently showed that PEPT2 protein is found extensively throughout the rat brain, with strongest expression in the cerebral cortex. However, the lower and less

dramatic differences in cerebral cortex concentrations between genotypes suggest that these changes may be slower relative to the more rapid changes in CSF or blood, thereby complicating the accurate assessment of PEPT2 in this tissue. The role of PEPT2 in cerebral cortex will need to be further investigated, perhaps following intracerebroventricular administration of peptide.

Because no pathological phenotype was observed during our initial development and validation of PEPT2-deficient mice (Shen et al, 2003), it is possible that other POT family members may be upregulated as a compensatory response to PEPT2 gene disruption. This was further investigated by performing semi-quantitative PCR (i.e., POT/GAPDH ratio) on kidney samples obtained from $PEPT2^{+/+}$ and $PEPT2^{-/-}$ mice (n=12 per genotype, equal gender). In this study, we found that PEPT1, PHT1 and PHT2 mRNA were not very different between the two genotypes, with enhancement ratios (null/wild type) of 1.33, 0.99 and 1.04, respectively. These findings are in agreement with Rubio-Aliaga et al. (2003) who found that renal PEPT1 protein and PHT1 mRNA levels were not increased in PEPT2 knockout mice. In a more limited study (i.e., a single pooled sample), we found that PHT1 mRNA levels were not different in the choroid plexuses (1.04 enhancement ratio) and brain (0.96 enhancement ratio) of wild type vs. PEPT2 null animals, and that PEPT1 and PHT2 mRNA were not detectable. This is consistent with PEPT1 protein being absent in rat choroid plexus and brain (Shen et al., 2004), although PHT2 mRNA was detected faintly in rat brain (Sakata et al., 2001). Taken as a whole, it appears that PEPT2 null mice do not exhibit an adaptive upregulation in the expression level of related POT genes (i.e., as least in kidney and brain).

In conclusion, our results are unique in demonstrating that under physiologic *in vivo* conditions PEPT2 is the predominant oligopeptide transporter in kidney and that it operates as an efflux transporter in choroid plexus. Moreover, renal PEPT2 alters the systemic concentrations

of GlySar, which provide the "driving force" for distribution of dipeptide to important tissues and fluids (e.g., brain and CSF). The combined effort of PEPT2 in the kidney and brain may potentially influence the sensitivity or toxicity to peptide-like drugs. As a result, the rational design of new peptide-based pharmaceuticals may need to take into account the affinity of these agents for PEPT2 as well as the protein's expression in targeted tissues. Future studies will be directed at understanding the affect of PEPT2 on the pharmacokinetics and pharmacodynamics of neuropeptides and peptidomimetic drugs.

References

Berger UV and Hediger MA (1999) Distribution of peptide transporter PEPT2 mRNA in the rat nervous system. *Anat Embryol* **199**:439-449.

Brandsch M, Brandsch C, Prasad PD, Ganapathy V, Hopfer U and Leibach FH (1995) Identification of a renal cell line that constitutively expresses the kidney-specific high affinity H+/peptide cotransporter. *FASEB J* **9**:1489-1496.

Carp RI, Davidson AI, and Merz PA (1971) A method for obtaining cerebrospinal fluid from mice. *Res Vet Sci* **12**:499.

Daniel H (1996) Function and molecular structure of brush border membrane peptide/H+ symporters. *J Membr Biol* **154**:197-203.

Daniel H and Rubio-Aliaga I (2003) An update on renal peptide transporters. *Am J Physiol* **284**:F885-F892.

Daniel H and Kottra G (2004) The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. *Pflugers Arch* **447**:610-618.

Ennis SR, Novotny A, Xiang J, Shakui P, Masada T, Stummer W, Smith DE, and Keep RF (2003) Transport of 5-aminolevulinic acid between blood and brain. *Brain Res* **959**:226-234.

Herrera-Ruiz D and Knipp GT (2002) Current perspectives on established and putative mammalian oligopeptide transporters. *J Pharm Sci* **92**:691-714.

Keep RF, Si X, Shakui P, Ennis SR and Betz AL (1999) Effect of amiloride analogs on DOCA-salt-induced hypertension in rats. *Am J Physiol* **276**:H2215-H2220.

Leibach FH and Ganapathy V (1996) Peptide transporters in the intestine and the kidney. *Annu Rev Nutr* **16:**99-119.

Lepsy CS, Guttendorf RJ, Kugler AR and Smith DE (2003) Effects of organic anion, cation, and dipeptide transport inhibitors on cefdinir in the isolated perfused rat kidney. *Antimicrob Agents Chemother* **47**:689-696.

Levy G (1980) Effect of plasma protein binding on renal clearance of drugs. *J Pharm Sci* **69**:482-483.

Liu W, Liang R, Ramamoorthy S, Fei Y-J, Ganapathy ME, Hediger MA, Ganapathy V, and Leibach FH (1995) Molecular cloning of PEPT 2, a new member of the H^+/peptide cotransporter family, from human kidney. *Biochim Biophys Acta* **1235**:461-466

Lochs H, Morse EL, and Adibi SA (1990) Uptake and metabolism of dipeptides by human red blood cells. *Biochem J* **271**:133-137.

Novotny A, Xiang J, Stummer W, Teuscher NS, Smith DE, and Keep R (2000) Mechanisms of 5-aminolevulinic acid uptake at the choroid plexus. *J Neurochem* **75**:321-328.

Ocheltree SM, Shen H, Hu Y, Xiang J, Keep RF, and Smith DE (2004a) Role of PEPT2 in the choroid plexus uptake of glycylsarcosine and 5-aminolevulininc acid: Studies in wild-type and null mice. *Pharm Res* **21**:1680-1685.

Ocheltree SM, Shen H, Hu Y, Xiang J, Keep RF, and Smith DE (2004b) Mechanisms of cefadroxil uptake in the choroid plexus: Studies in wild-type and PEPT2 knockout mice. *J Pharm Exp Ther* **308**: 462-467.

Pastore A, Massoud R, Motti C, Lo Russo A, Fucci G, Cortese C, and Federici G (1998) Fully automated assay for total homocysteine, cysteine, cysteinylglycine, glutathione, cysteamine, and 2-mercaptopropionylglycine in plasma and urine. *Clin Chem* **44**:825-832.

Rowland M and Tozer TN. In Clinical Pharmacokinetics: Concepts and Applications, 3rd Edition. Lea & Febiger, Philadelphia, PA, 1995, pp 170-172.

Rubio-Aliaga I and Daniel H (2002) Mammalian peptide transporters as targets for drug delivery. *Trends Pharmacol Sci* **23**:434-440.

Rubio-Aliaga I, Frey I, Boll M, Groneberg D, Eichinger HM, Balling R, and Daniel H (2003) Targeted disruption of the peptide transporter Pept2 gene in mice defined its physiological role in the kidney. *Mol Cell Biol* **23**:3247-3252.

Sakata K, Yamashita T, Maeda M, Moriyama Y, and Shimada S (2001) Cloning of a lymphatic peptide/histidine transporter. *Biochem J* **356**:53-60.

Shen H, Smith DE, Keep RF and Brosius III FC (2004) Immunolocalization of the proton-coupled oligopeptide transporter PEPT2 in developing rat brain. *Mol Pharm* **1**:248-256.

Shen H, Smith DE, Keep RF, Xiang J, and Brosius III FC (2003) Targeted disruption of the PEPT2 gene markedly reduces dipeptide uptake in choroid plexus. *J Biol Chem* **278**:4786-4791.

Shen H, Smith DE, Yang T, Huang YG, Schnermann JB and Brosius III FC (1999) Localization of PEPT1 and PEPT2 proton-couple oligopeptide transporter mRNA and protein in rat kidney. *Am J Physiol* **276**:F658-F664.

Shu C, Shen H, Teuscher NS, Lorenzi PJ, Keep RF, and Smith DE (2002) Role of PEPT2 in peptide/mimetic trafficking at the blood-cerebrospinal fluid barrier: Studies in rat choroid plexus epithelial cells in primary culture. *J Pharmacol Exp Ther* **301**:820-829.

Shu C, Shen H, Hopfer U, and Smith DE (2001) Mechanism of intestinal absorption and renal reabsorption of an orally active ACE inhibitor: Uptake and transport of fosinopril in cell cultures. *Drug Metab Dispos* **29**:1307-1315.

Smith DE, Johanson CE and Keep RF (2004) Peptide and peptide analog transport systems at the blood-CSF barrier. *Adv Drug Deliv Rev* **56**:1765-1791.

Smith DE, Pavlova A, Berger UV, Hediger MA, Yang T, Huang YG and Schnermann JB (1998) Tubular localization and tissue distribution of peptide transporters in rat kidney. *Pharm Res* **15**:1244-1249.

Takahashi K, Nakamura N, Terada T, Okano T, Futami T, Saito H and Inui KI (1998) Interaction of beta-lactam antibiotics with H+/peptide cotransporters in rat renal brush-border membranes. *J Pharmacol Exp Ther* **286**:1037-1042.

Teuscher NS, Novotny A, Keep RF, and Smith DE (2000) Functional evidence for the presence of PEPT2 in rat choroid plexus: Studies with glycylsarcosine. *J Pharmacol Exp Ther* **294**:494-499.

Teuscher NS, Keep RF, and Smith DE (2001) PEPT2-mediated uptake of neuropeptides in rat choroid plexus. *Pharm Res* **18**:807-813.

Teuscher NS, Shu C, Xiang J., Keep RF and Smith DE (2004) Carnosine uptake in rat choroid plexus primary cell cultures and choroid plexus whole tissue from PEPT2 null mice. *J Neurochem* **89**:375-382.

Tiruppathi C, Ganapathy V, and Leibach FH (1990) Kinetic evidence for a common transporter for glycylsarcosine and phenylalanylprolylalanine in renal brush border membrane vesicles. *J Biol Chem* **265**:14870-14874.

Yamashita T, Shimada S, Guo W, Sato K, Kohmura E, Hayakawa T, Takagi T, and Tohyama M (1997) Cloning and functional expression of a brain peptide/histidine transporter. *J Biol Chem* **272**:10205-10211.

Footnotes

This study was supported in part by Grants R01 GM035498 (to D.E.S.), and R01 NS034709 and P01 HL018575 (to R.F.K.) from the National Institutes of Health. S.M.O. was supported by an American Foundation for Pharmaceutical Education PreDoctoral Fellowship and the Pharmacological Sciences Training Program of the National Institutes of Health (Grant T32 GM007767).

Reprint requests: David E. Smith, 4302A Upjohn Center, 1310 E. Catherine Street, The University of Michigan, Ann Arbor, MI 48109-0504. Telephone: (734) 647-1431; facsimile: (734) 763-3438; E-mail: smithb@umich.edu

Figure Legends

Fig. 1. Blood concentrations of GlySar as a function of time in PEPT2$^{+/+}$ (wild-type) and PEPT2$^{-/-}$ (null) mice after an intravenous bolus dose of dipeptide (0.05 μmol/g body weight). Data are expressed as mean ± SE (n=12-14).

Fig. 2. Tissue, CSF and blood concentrations of GlySar in PEPT2$^{+/+}$ (wild-type) and PEPT2$^{-/-}$ (null) mice, 60 min after an intravenous bolus dose of dipeptide (0.05 μmol/g body weight). Data are expressed as mean ± SE (n=5-10). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with wild-type mice.

Fig. 3. (A) Whole kidney, renal cortex, outer medulla and inner medulla concentrations of GlySar in PEPT2$^{+/+}$ (wild-type) and PEPT2$^{-/-}$ (null) mice, 60 min after an intravenous bolus dose of dipeptide (0.05 μmol/g body weight). (B) Corresponding tissue-to-blood concentration ratios of GlySar. Data are expressed as mean ± SE (n=4). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with wild-type mice.

Fig. 4. Time course of GlySar concentrations (or tissue-to-blood ratios) in the (A) kidney, (B) cerebrospinal fluid, (C) choroid plexus and (D) cerebral cortex of PEPT2$^{+/+}$ (wild-type) and PEPT2$^{-/-}$ (null) mice after an intravenous bolus dose of dipeptide (0.05 μmol/g body weight). Data are expressed as mean ± SE (n=4-10). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with wild-type mice.

Fig. 5. Plasma concentrations of (A) GlySar (0.05 µmol/g body weight) and (B) inulin (0.00086 µmol/g body weight) in $PEPT2^{+/+}$ (wild-type) and $PEPT2^{-/-}$ (null) mice after coadministration by intravenous bolus injection. Data are expressed as mean ± SE (n=4). Note the dual importance of inulin in serving as an estimate of GFR, as well as a negative control between genotypes.

Fig. 6. Renal clearance of GlySar in $PEPT2^{+/+}$ (wild-type) and $PEPT2^{-/-}$ (null) mice after an intravenous bolus dose of dipeptide (0.05 µmol/g body weight). The estimated GFR was approximately 0.25 ml/min for both genotypes and is indicated with a dashed line. Data are expressed as mean ± SE (n=4). *$p < 0.05$ compared with wild-type mice.

JPET Fast Forward. Published on June 29, 2005 as DOI: 10.1124/jpet.105.089359
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TABLE 1

Pharmacokinetics of GlySar Blood Concentrations in $PEPT2^{+/+}$ and $PEPT2^{-/-}$ Mice

Parameter[a]	$PEPT2^{+/+}$	$PEPT2^{-/-}$
V_1 (ml)	4.3 (0.3)[b]	4.6 (0.4)
Vd_{ss} (ml)	10.2 (0.7)	12.3 (0.8)
AUC (μmol • min/ml)	3.91 (0.27)	1.97 (0.09)***
CL (ml/min)	0.29 (0.02)	0.57 (0.05)***
k_{10} (min^{-1})	0.074 (0.005)	0.136 (0.012)***
$t_{1/2}$ (min)	25.0 (0.9)	16.8 (1.0)***
MRT (min)	33.2 (1.3)	22.3 (1.3)***
r^2	0.998 (0.001)	0.997 (0.001)

[a] V_1 is the volume of the central compartment; Vd_{ss} is the volume of distribution steady-state; AUC is the area under the blood concentration-time curve, CL is the total blood clearance, K_{10} is the elimination rate constant from the central compartment; $t_{1/2}$ is the terminal half-life; MRT is the mean residence time; r^2 is the coefficient of determination.

[b] Data represented as mean ± SE (n=12-14).

***$p < 0.001$ compared with $PEPT2^{+/+}$ (wild-type) mice.

JPET Fast Forward. Published on June 29, 2005 as DOI: 10.1124/jpet.105.089359
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TABLE 2

Renal Pharmacokinetics of GlySar Plasma Concentrations in $PEPT2^{+/+}$ and $PEPT2^{-/-}$ Mice

Parameter[a]	$PEPT2^{+/+}$	$PEPT2^{-/-}$
CL_R (ml/min)	0.11 (0.03)[b]	0.24 (0.03)*
GFR (ml/min)	0.23 (0.08)	0.27 (0.03)
fu	0.93 (0.02)	0.94 (0.02)
fe^{60}	0.56 (0.06)	0.66 (0.02)
ER	0.54 (0.05)	0.94 (0.10)*
F	0.46 (0.05)	0.06 (0.10)*
F_1	0.06	0.06 (0.10)
F_2	0.43 (0.05)	0
%PEPT1	14 (2)	100
%PEPT2	86 (2)	0

[a] CL_R is the renal clearance of GlySar; GFR is the glomerular filtration rate (of inulin); fu is the unbound fraction of GlySar in plasma; fe^{60} is the fraction of GlySar excreted unchanged in the urine over 60 min ($Ae^{60}/Dose_{iv}$); ER is the excretion ratio of GlySar; F is the fraction of available GlySar that is reabsorbed; F_1 is the fraction of available GlySar that is reabsorbed by PEPT1; F_2 is the fraction of available GlySar that is reabsorbed by PEPT2; %PEPT1 is the percent of reabsorbed GlySar that occurs via PEPT1; %PEPT2 is the percent of reabsorbed GlySar that occurs via PEPT2.

[b] Data represented as mean ± SE (n=4).

*$p < 0.05$ compared with $PEPT2^{+/+}$ (wild-type) mice.

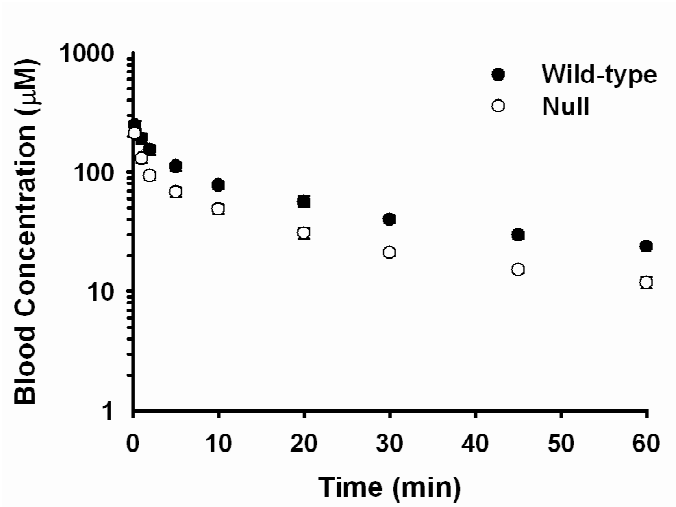
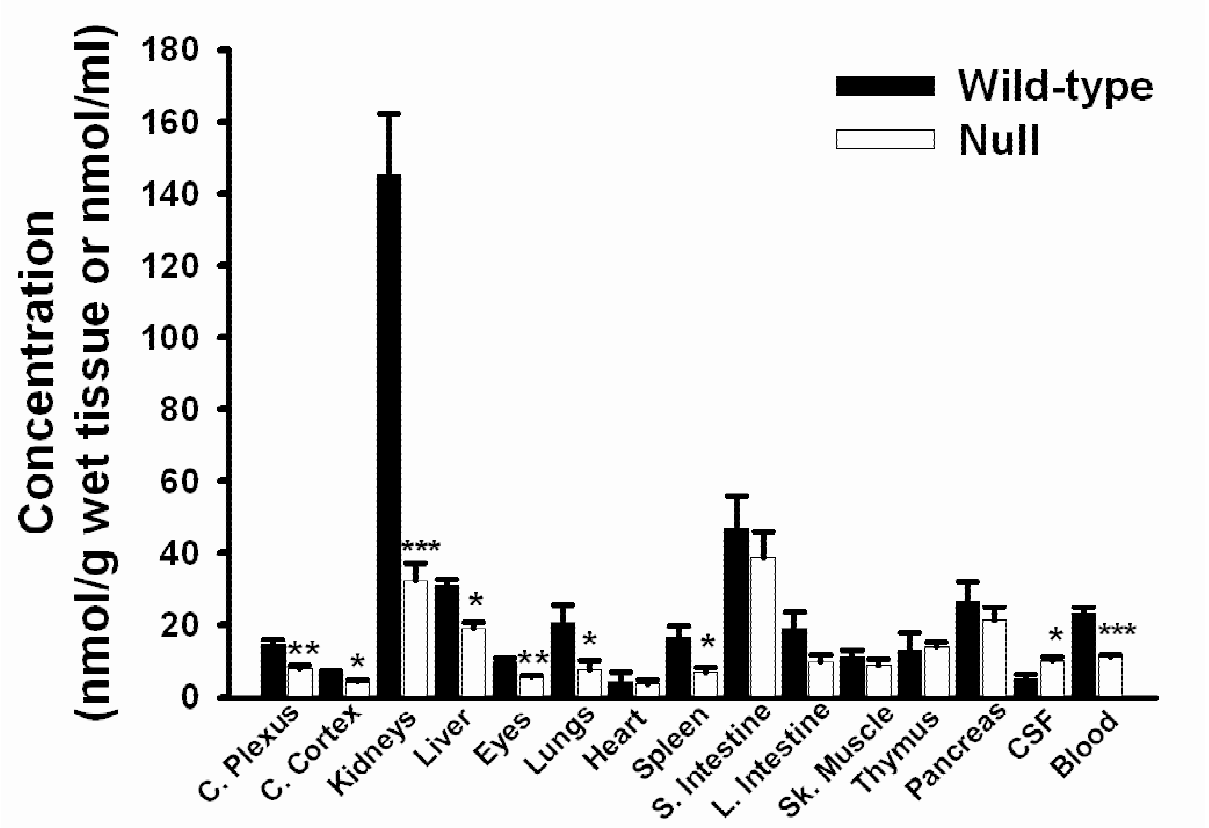


Figure 1



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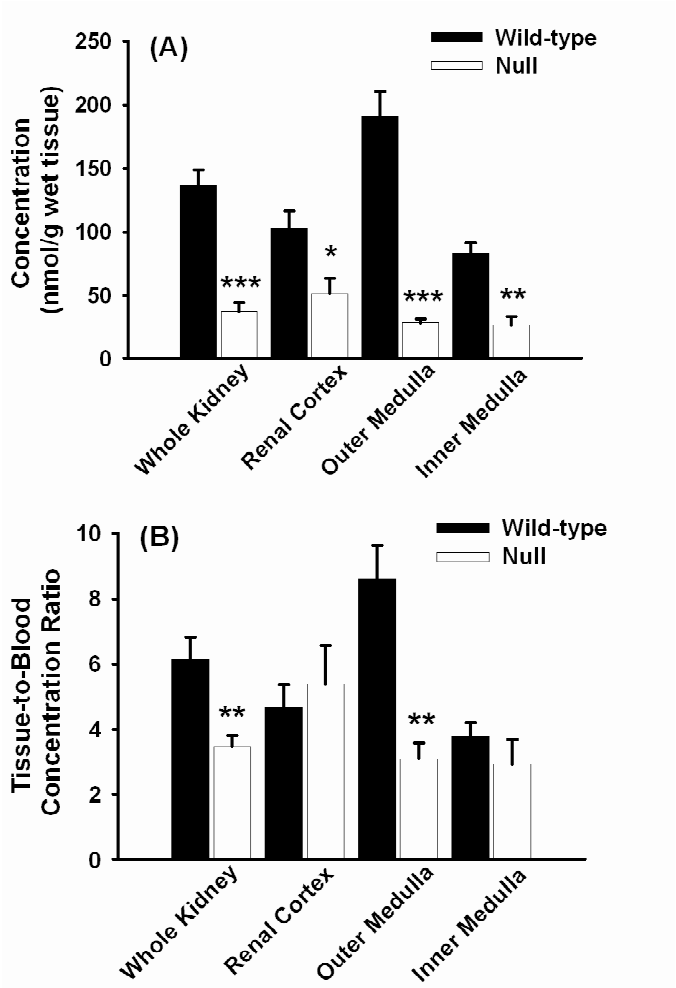

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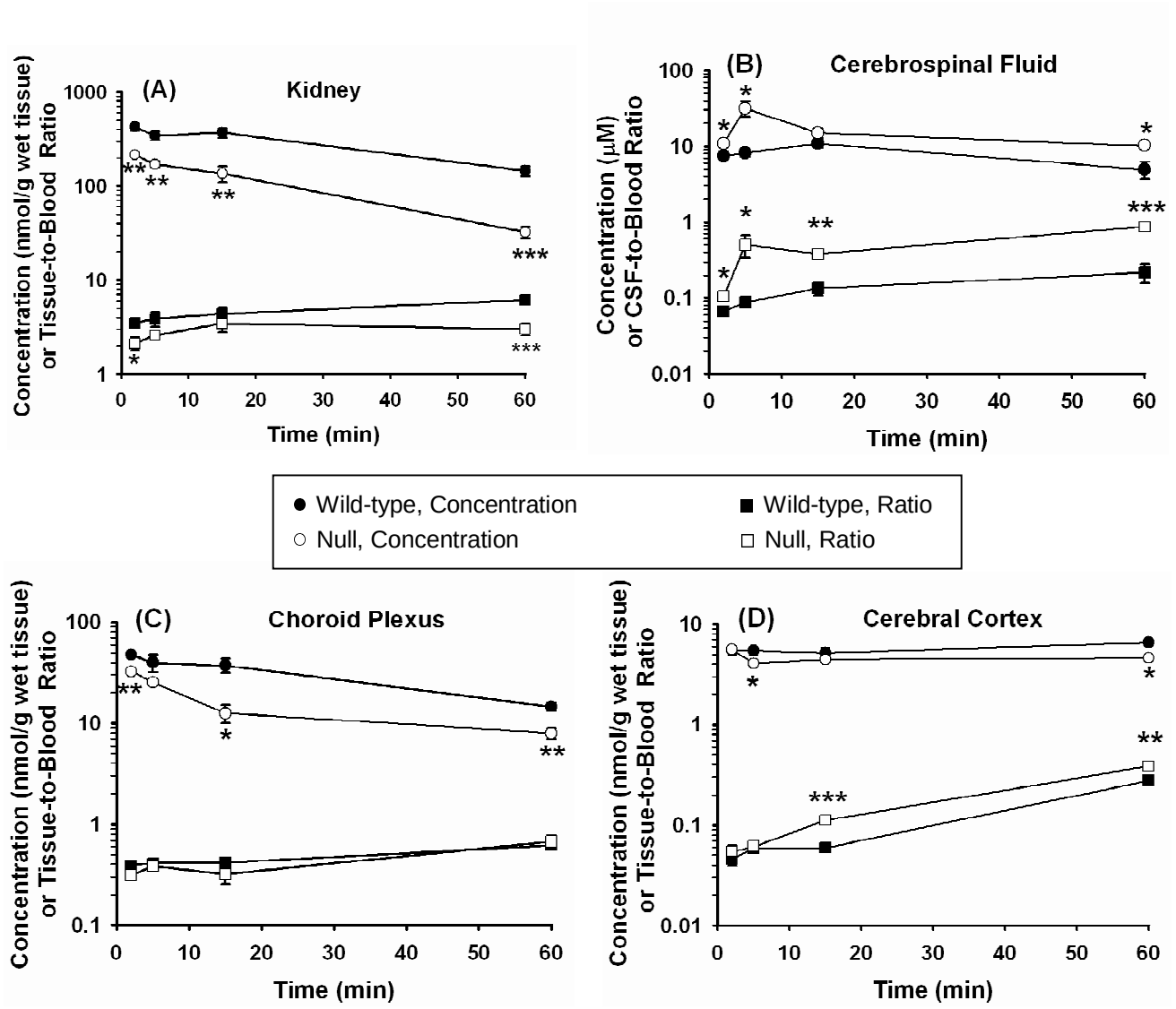

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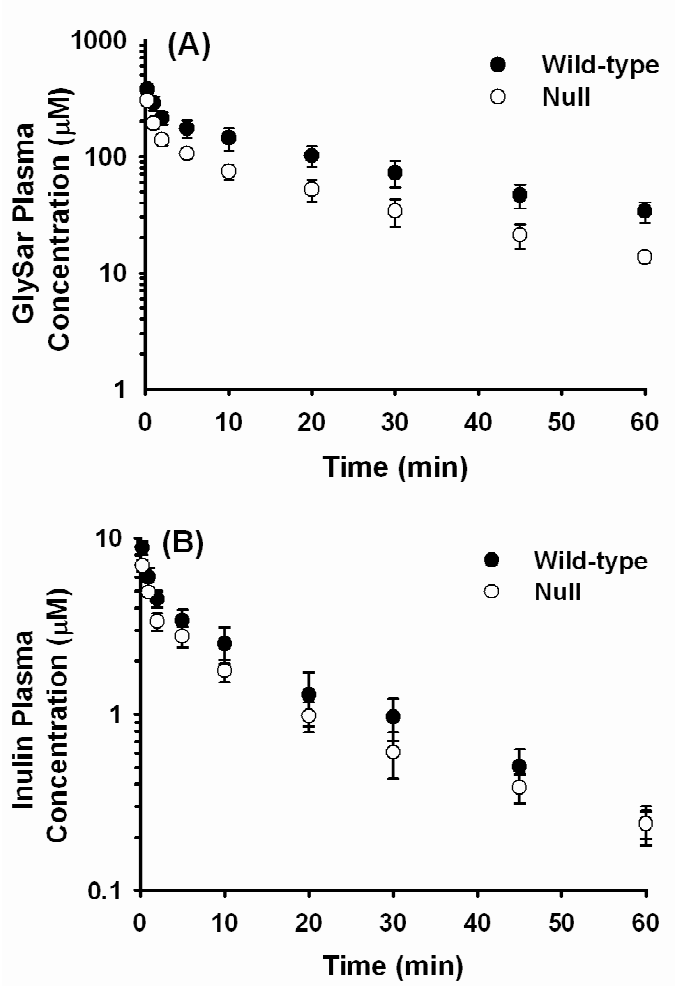

Figure 5

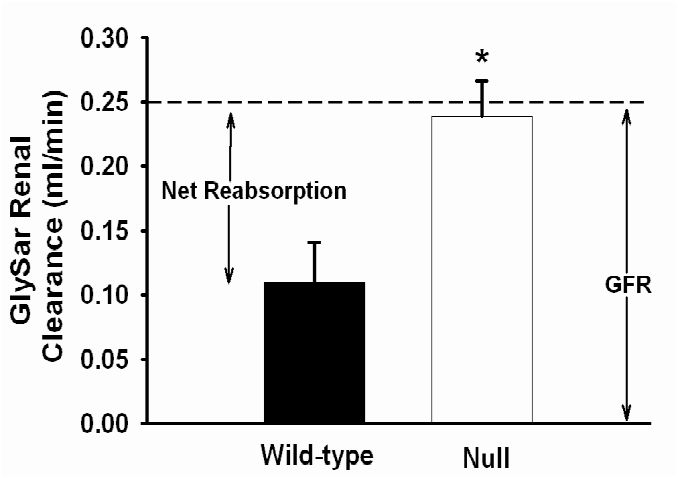

Figure 6