Impact of Genetic Knockout of PEPT2 on Cefadroxil Pharmacokinetics, Renal Tubular Reabsorption, and Brain Penetration in Mice

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ABSTRACT:

The aim of this study was to examine the role of PEPT2, a proton-coupled oligopeptide transporter of the SLC15 family, on the disposition of the antibiotic cefadroxil in the body, particularly the kidney and brain. Pharmacokinetic, tissue distribution, and renal clearance studies were performed in wild-type and PEPT2 null mice after intravenous bolus administration of [3H]cefadroxil at 1, 12.5, 50, and 100 nmol/g body weight. Studies were also performed in the absence and presence of probenecid and quinine. Cefadroxil disposition kinetics was clearly nonlinear over the dose range studied (1–100 nmol/g), which was attributed to both saturable renal tubular secretion and reabsorption of the antibiotic. After an intravenous bolus dose of 1 nmol/g cefadroxil, PEPT2 null mice exhibited a 3-fold greater total clearance and 3-fold lower systemic concentrations of drug compared with wild-type animals. Renal clearance studies further demonstrated that the renal reabsorption of cefadroxil was almost completely abolished in PEPT2 null versus wild-type mice (3% versus 70%, p < 0.001). Of the 70% of cefadroxil reabsorbed in wild-type mice, PEPT2 accounted for 95% and PEPT1 accounted for 5% of reabsorbed substrate. Tissue distribution studies indicated that PEPT2 had a dramatic effect on cefadroxil tissue exposure, especially in brain where the cerebrospinal fluid (CSF)-to-blood concentration ratio of cefadroxil was 6-fold greater in PEPT2 null mice compared with wild-type animals. These findings demonstrate that renal PEPT2 is almost entirely responsible for the reabsorption of cefadroxil in kidney and that choroid plexus PEPT2 limits the exposure of cefadroxil (and perhaps other aminocapachalosporins) in CSF.

Proton-coupled oligopeptide transporters (POTs) not only deliver peptide-bound amino nitrogen to cells but may also have a significant influence on the pharmacokinetics and pharmacodynamics of peptide-like drugs (Daniel and Kottra, 2004; Smith et al., 2004). To date, four POTs have been identified in a variety of mammalian species including human, rabbit, rat, and mouse (i.e., PEPT1, PEPT2, PHT1, PHT2). Among these mammalian POTs, PEPT2 (also known as SLC15A2) consists of 729 amino acids with an apparent molecular mass of 85 kDa, which is reduced to 81 kDa after deglycosylation. PEPT2 is specifically expressed on the apical (luminal) membrane of epithelial cells of the proximal tubule in the kidney and transports a broad range of peptide-like compounds (e.g., cefadroxil, enalapril, bestatin, valacyclovir, 5-amino-levulinic acid) (Daniel and Kottra, 2004; Smith et al., 2004). Because of its high expression in kidney and high affinity for transport, it has been suggested that PEPT2 plays a primary role in renal reabsorption of some drugs (Takahashi et al., 1998; Shen et al., 1999). However, definitive in vivo evidence for this assertion is lacking.

PEPT2 is thought to play an important role in the exposure and therapeutic outcome of peptide-like drugs in the central nervous system (CNS). Using immunohistochemistry, our group has recently demonstrated expression of PEPT2 in the apical membrane [cerebrospinal fluid (CSF)-facing] of rat choroid plexus epithelial cells, suggesting a possible role for PEPT2 in facilitating the efflux of substrates from CSF to blood (Shen et al., 2004). Primary cultures of rat choroid plexus epithelial cells, a model of the blood-CSF barrier, also express PEPT2 on their apical surface (Shu et al., 2002). These cultures allow one to study the apical-to-basal and basal-to-apical permeability of drugs. Our results indicate a vectorial apical-to-basal directed and saturable transport of PEPT2 substrates like glycylsarcosine (GlySar), carnosine, and cefadroxil (Shu et al., 2002; Teuscher et al., 2004; Shen et al., 2005), suggesting that PEPT2 facilitates drug efflux from CSF to blood. However, in vivo experiments are needed to confirm this contention.

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ABBREVIATIONS: POT, proton-coupled oligopeptide transporter; CNS, central nervous system; CSF, cerebrospinal fluid; ER, excretion ratio; GFR, glomerular filtration rate; GlySar, glycylsarcosine; OAT, organic anion transporter; OCT, organic cation transporter; AUC, area under the plasma concentration-time curve; CL, clearance; ANOVA, analysis of variance.
Cefadroxil is an aminopenicillin anti-infective agent with a broad spectrum of antibacterial activity. Clinical studies showed that cefadroxil is completely absorbed in the small intestine and distributed extensively to body tissues and fluids including kidney, lung, liver, muscle, and tonsils (Quintiliani, 1982; Tanrisever and Santella, 1986). In addition, in vitro and in vivo studies have shown that cefadroxil is not metabolized (Tanrisever and Santella, 1986; AHFS, 2006). Despite its favorable stability, the residence time of cefadroxil in vivo is relatively short (half-life ~1.5 h in human). Rapid renal excretion is responsible for the short sojourn in vivo. Like other cephalosporin antibiotics, the elimination of cefadroxil is markedly decreased in patients with impaired renal function, resulting in a significant increase in the drug’s elimination half-life (Leroy et al., 1982). The renal secretion of most cephalosporins is believed to be mediated by organic anion transporters (OATs) located on the basolateral membrane of the proximal tubule. Whereas PEPT1 is expressed in the early part of the proximal tubule (pars convoluta), PEPT2 is expressed further along the proximal tubule (pars recta). Moreover, like other β-lactam antibiotic, cefadroxil may fail in the treatment of CNS infections, including meningitis, because of poor penetration across the blood-brain barrier and blood-CSF barrier. It is reasonable to speculate that PGTs and OATs in the choroid plexus might act as drug efflux proteins, resulting in subtherapeutic levels of antibiotics in CSF (Shen et al., 2005).

In vivo experiments that demonstrate the role and relevance of PEPT2 in affecting the disposition of β-lactam antibiotics in the body have yet to be performed. With this in mind, we examined the pharmacokinetics and tissue distribution of cefadroxil in wild-type and PEPT2 null mice. Our results are novel in demonstrating for the first time, to our knowledge, that PEPT2 has substantial impact on the in vivo disposition of an aminopenicillin antibiotic drug, cefadroxil, particularly within the kidney and brain.

Materials and Methods

Chemicals. [1H]Cefadroxil (3.8 Ci/mmol), [14C]carboxyl-inulin (1.3 mCi/g), and [3H]carboxyl-dextran (mol. wt. 70,000) (1.1 mCi/g) were purchased from Moravek Radiochemicals (Brea, CA). Radiochemical purity of all compounds was >98% as determined by reversed-phase high-performance liquid chromatography. Unlabeled cefadroxil (mol. wt. = 363.4) was obtained from Sigma-Aldrich (St. Louis, MO) and hyamine hydroxide was purchased from Sigma (St. Louis, MO) and large intestines, skeletal muscle, testes, and ovary were collected. Samples were weighed and then solubilized with 1 M hyamine hydroxide, as described by the manufacturer (MP Biomedicals). After solubilization, the level of radioactivity was determined by liquid scintillation analysis using Spectrolyte 9000 scintillation cocktail (Packard Instruments Co., Downers Grove, IL). where $C_{\text{tiss, corr}}$ and $C_{\text{tiss}}$ are concentrations of cefadroxil before and after correction for vascular space, respectively.

Animals. Animal 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. Wild-type (PEPT2+/−) and PEPT2 null (PEPT2−/−) mice (>99% C57BL/6 genetic background) between 8 and 15 weeks of age were used for these studies. Animals were kept in a temperature-controlled environment with a 12-h light, 12-h dark cycle and received a standard diet and water ad libitum (Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI).

Pharmacokinetic Studies with Cefadroxil. In all cases, gender- and weight-matched mice were fasted overnight before the onset of each experiment. Cefadroxil was dissolved in normal saline and administered to the mice in aqueous solution. Following sodium pentobarbital anesthesia (50 mg/kg i.p.), mice received cefadroxil through a tail vein injection (5 μg/k g body weight). Blood samples were collected (~15 μl) via tail nicks at 0.25, 1, 2, 5, 15, 30, 45, 60, 90, and 120 min after the intravenous bolus dose. Heparinized blood samples were centrifuged immediately at 2000 g for 10 min to obtain plasma. Urine samples were collected, as indicated in the experiments below. The radioactivity of plasma and urine samples was measured by a dual-channel liquid scintillation counter (Beckman LS 3801; Beckman Coulter, Inc., Fullerton, CA).

Metabolic stability of cefadroxil. [1H]Cefadroxil was administered intravenously to both genotypes at a dose of 50 nmol/g body weight (i.e., 0.5 μCi/g). Each animal was then placed in a metabolic cage with a diuresis adapter (Nalge Nune International, Naperville, IL) and urine was collected for 24 h. After several washes of the diuresis adapter with water, the diluted urine was collected and analyzed by reversed-phase high-performance liquid chromatography. The system consisted of a Waters 515 pump (Waters, Milford, MA), a Rhodyne injector port (Rhodyne, Rohnert Park, CA), and a Packard 500TR radiochemical detector (PerkinElmer Life and Analytical Sciences, Boston, MA). Cefadroxil was separated on a Discovery 5-μm (C18) column, 250 × 4.6 mm (Supelco, Bellefonte, PA), preceded by a 6-μm Bondapak C18 guard column (Waters). Isoelectric elution was performed by mixing acetonitrile and water (6:94) at a constant flow rate of 1.0 ml/min. Peaks were recorded and integrated using FLO-ONE software for Windows Analysis (PerKinElmer Life and Analytical Sciences, version 3.61). The retention time of cefadroxil was 4.8 min.

Dose dependence of cefadroxil. [1H]Cefadroxil was administered intravenously to both genotypes at doses of 1, 12.5, 50, and 100 nmol/g body weight (i.e., 0.4, 4.5, 18.2, and 36.4 μg/kg); serial blood samples were collected over 2 h and the plasma was harvested.

Renal clearance of cefadroxil. [1H]Cefadroxil was administered intravenously to both genotypes at a dose of 1 nmol/g body weight along with [14C]Dextran (mol. wt. 70,000) (0.25 μCi/mouse) for the determination of GFR. Serial blood samples were then drawn over 2 h and the plasma was harvested. Urine was collected directly from the bladder at 120 min.

Inhibition of cefadroxil renal secretion by probenecid or quinine. [1H]Cefadroxil (1 nmol/g body weight) and [14C]Dextran (30.8 μg/kg) were administered intravenously to both genotypes. Three treatments of probenecid or quinine (at either 70 or 700 mg/kg) were administered subcutaneously at 60 min before, at the same time, and at 60 min after the [1H]Cefadroxil and [14C]Dextran injections. Serial blood samples were obtained over 2 h and urine was collected from the bladder at end of blood sampling (i.e., at ~2 h).

Tissue distribution of cefadroxil. Wild-type and PEPT2 null mice were injected with [1H]Cefadroxil (1 nmol/g body weight) through the tail vein. [14C]Dextran (mol. wt. = 70,000) (0.25 μCi/mouse) was administered intravenously 5 min before harvesting the tissues so that cefadroxil tissue concentrations could be corrected for the vascular space. A blood sample was obtained 120 min after intravenous administration of cefadroxil, after which a CSF sample was taken from the cisterna magna by puncture of the atlantooccipital membrane with a 28-gauge needle. The mouse was immediately decapitated, and tissue samples from choroid plexuses (i.e., lateral and fourth ventricles), cerebral cortex, whole kidney, liver, eye, lung, heart, spleen, small and large intestines, skeletal muscle, testes, and ovary were sampled. Samples were weighed and then solubilized with 1 M hyamine hydroxide, as described by the manufacturer (MP Biomedicals). After solubilization, the level of radioactivity was determined in tissue homogenates after mixing with Ecolite (+) liquid scintillation cocktail (MP Biomedicals).

Corrected tissue concentrations of cefadroxil ($C_{\text{tiss, corr}}$, nmol/g wet tissue) were calculated (Keep et al., 1999; Ocheltree et al., 2005) as

$$C_{\text{tiss, corr}} = C_{\text{tiss}} - DS \cdot C_{p}$$

where $C_{\text{tiss}}$ is the uncorrected cefadroxil tissue concentration (nmol/g), DS is the dextran space (i.e., blood vascular space) in the tissue (ml/g), and $C_{p}$ is the cefadroxil blood concentration (nmol/ml).

Plasma Protein Binding of Cefadroxil. The plasma unbound fraction of cefadroxil in wild-type and PEPT2 knockout mice was measured based on the ultrafiltration method reported by Ocheltree et al. (2005), with minor modification. In brief, mouse plasma samples were spiked with cefadroxil to produce standard concentrations of 0.01, 0.1, 1, and 10 μM, A 1 μM cefadroxil plasma sample was also spiked with probenecid (0.1, 1, and 10 mM). After incubation at 37°C for 15 min, plasma samples (500 μl) were loaded into Microcon...
YMT-30 devices and centrifuged at 1800g for 25 min at 37°C. Approximately 60% of the volume was recovered in the ultrafiltrate containing the unbound cefadroxil. The unbound fraction in plasma samples was calculated by dividing the ultrafiltrate cefadroxil concentration by that in the original plasma standard. Negligible binding of cefadroxil to the ultrafiltration device and lack of protein leakage during the ultrafiltration procedure were demonstrated by preliminary studies (data not shown).

Data Analysis. Pharmacokinetics of cefadroxil plasma concentrations. Concentration-time curves, \( C(t) \), were fit to a two-compartment model with a weighting factor of unity (WinNonlin version 5.0.1; Pharsight Inc., Mountain View, CA) such that

\[
C(t) = C_1 \cdot e^{-kt_1} + C_2 \cdot e^{-kt_2}
\]  

(2)

Quality of the fit was judged by evaluating the standard error of parameter estimates and the coefficient of determination \( r^2 \), and by the visual inspection of residual plots. Area under the plasma concentration-time curve (AUC; equivalent to AUC_{0–120}) total body clearance (CL), elimination rate constant from the central compartment \( \left( K_{el} \right) \), volume of the central compartment \( \left( V_1 \right) \), volume of distribution at steady state \( \left( V_d \right) \), terminal half-life \( \left( t_{1/2} \right) \), and mean residence time \( \left( MRT \right) \) were then determined by standard methods.

Renal clearance of cefadroxil. The renal clearance of cefadroxil (CL_{R}) and inulin (GFR) were calculated as

\[
\text{CL}_{R} = \frac{A_{ce} \cdot CL_{ce}}{AUC_{ce} \cdot AUC_{in}}
\]  

(3)

\[
\text{GFR} = \frac{A_{inulin} \cdot CL_{inulin}}{AUC_{inulin} \cdot AUC_{inulin}}
\]  

(4)

where \( A_{ce} \) (or \( A_{inulin} \)) is the cumulative amount of unchanged cefadroxil or inulin excreted in urine over 120 min. AUC_{ce} (or AUC_{inulin}) is the area under the cefadroxil or inulin plasma concentration-time curve from 0 to 120 min, as determined by the trapezoidal rule.

Since cefadroxil undergoes glomerular filtration, tubular secretion (Bins and Mattie, 1988), and tubular reabsorption (Garcia-Carbonell et al., 1993) in the kidney, and given the sequential expression of PEPT1 and PEPT2 in the renal proximal tubule (Smith et al., 1998; Shen et al., 1999), the drug's renal clearance can be represented as

\[
\text{CL}_{R} = F_{R} \cdot (\text{GFR} + CL_{ce} \cdot (1 - F_{i})
\]  

(5)

where \( F_{R} \) is the fraction of cefadroxil unbound in plasma, \( CL_{ce} \) is the secretion clearance, \( F_{i} \) is the fraction of available drug that is reabsorbed in which \( F_{R} \) is the fraction of available drug reabsorbed by PEPT1 and \( F_{R} \) is the fraction of available drug reabsorbed by PEPT2.

Since \( F_{R} = 0 \) in PEPT2 null mice and \( CL_{ce} = 0 \) in the presence of saturating concentrations of probenecid, knowing the experimental values for renal clearance of cefadroxil, as well as \( F_{R} \) and \( GFR \), will allow the direct calculation of \( F_{R} \) in PEPT2−/− mice. \( F_{R} \) can then be estimated directly in wild-type mice treated with cefadroxil plus probenecid (\( CL_{ce} = 0 \)) since all other parameters will be known (i.e., \( CL_{R}, F_{R}, \text{GFR}, \) and \( F_{R} \)).

The relative contribution of each transporter can be calculated as

\[
\%\text{PEPT1} = (F_{R}(F_{P})) \times 100 \quad \text{and} \quad \%\text{PEPT2} = [F_{R} \times (1 - F_{i})/F_{i}] \times 100
\]  

(6)

where \%PEPT1 and \%PEPT2 are the percentage of reabsorbed cefadroxil handled by PEPT1 and PEPT2, respectively.

The excretion ratio of cefadroxil (ER) was calculated as

\[
\text{ER} = \frac{\text{CL}_{R}(F_{R} \cdot \text{GFR})}{\text{CL}_{R}(F_{R} \cdot \text{GFR})}
\]  

(7)

Statistical analysis. Data are reported as mean ± S.E. To test for statistically significant differences among multiple treatments for a given parameter, one-way analysis of variance (ANOVA) was performed. When the \( F \) ratio showed that there were significant differences among treatments, the Dunnnett or Tukey method of multiple comparisons was used to determine which treatments differ. All statistical analyses were performed using Prism version 4.0 (GraphPad Software, Inc., San Diego, CA).

Results

Metabolic Stability of Cefadroxil. Over 90% of cefadroxil radio-label was recovered in the urine 24 h after intravenous administration of the drug. No significant metabolism was found, in that 96.8% and 95.0% of the urine sample was recovered as intact cefadroxil for wild-type and PEPT2 null mice, respectively. These results were consistent with the reported lack of metabolism for cefadroxil (Tanrisever and Santella, 1986; AHFS, 2006). Therefore, cefadroxil instability was not a confounding issue in these studies and no further correction of the data was necessary.

Cefadroxil Dose-Dependent Pharmacokinetics. A preliminary pharmacokinetic analysis was performed in wild-type and PEPT2 null mice after administering intravenous doses of cefadroxil over a 1 to 100 nmol/g dose range (Table 1). Although clearance of cefadroxil increased steadily in PEPT2−/− animals (1.5-fold) as the dose increased, the change was not statistically different. In addition, terminal half-life and volume of distribution steady-state values were relatively constant in these animals. Although the results suggest an “apparent” linearity in the clearance of cefadroxil in wild-type mice, it is more likely that statistical differences are not achieved because of a mitigating effect of renal tubular secretion (which would tend to reduce the clearance at higher doses) on renal tubular reabsorption (which would tend to increase the clearance at higher doses).

In contrast, cefadroxil clearance decreased in PEPT2−/− animals (2-fold) as the dose increased from 1 to 100 nmol/g. The slower clearance at higher doses implies a capacity-limited elimination process and probably reflects that of renal tubular secretion. In PEPT2−/− mice, no significant differences were found in the volume of distribution at steady state and terminal half-life of cefadroxil, although the latter term increased monotonically (1.5-fold) with higher dose.

In Fig. 1, plasma concentration-time curves of cefadroxil are shown for PEPT2+/+ and PEPT2−/− mice after intravenous administration of drug over the 100-fold dose range. When the 100 nmol/g dose was given, only minor differences were observed between genotypes in the plasma profiles of drug (Fig. 1A). However, cefadroxil levels were substantially different between PEPT2+/+ and PEPT2−/− animals.

### TABLE 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Parameter</th>
<th>1</th>
<th>12.5</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPT2+/+</td>
<td>CL (ml/min)</td>
<td>0.302 ± 0.026</td>
<td>0.377 ± 0.099</td>
<td>0.393 ± 0.023</td>
<td>0.461 ± 0.057</td>
</tr>
<tr>
<td></td>
<td>( t_{1/2} ) (min)</td>
<td>30.4 ± 3.2</td>
<td>26.0 ± 9.1</td>
<td>25.9 ± 8.7</td>
<td>26.5 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>( V_d ) (ml)</td>
<td>11.9 ± 1.9</td>
<td>13.7 ± 2.6</td>
<td>12.5 ± 2.6</td>
<td>10.8 ± 5.2</td>
</tr>
<tr>
<td>PEPT2−/−</td>
<td>CL (ml/min)</td>
<td>0.921 ± 0.098</td>
<td>0.643 ± 0.148</td>
<td>0.509 ± 0.070</td>
<td>0.480 ± 0.096</td>
</tr>
<tr>
<td></td>
<td>( t_{1/2} ) (min)</td>
<td>19.5 ± 1.5</td>
<td>24.8 ± 3.4</td>
<td>26.6 ± 6.9</td>
<td>29.2 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>( V_d ) (ml)</td>
<td>15.7 ± 1.7</td>
<td>12.7 ± 3.3</td>
<td>14.0 ± 3.6</td>
<td>13.2 ± 4.5</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) compared with the control group at 1 nmol/g cefadroxil.
when the 1 nmol/g dose was administered (Fig. 1D). These results suggest that the pharmacokinetics of cefadroxil are nonlinear and, in this case, probably reflect a change in renal tubular reabsorption. Because the 1 nmol/g dose showed the largest difference in cefadroxil plasma concentrations between genotypes, this dose was studied in more detail (Table 2).

### Table 2: Pharmacokinetics of cefadroxil after a 1 nmol/g intravenous bolus dose of drug

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PEPT2&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>PEPT2&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (min·μmol/l)</td>
<td>67.9 ± 11.7</td>
<td>21.9 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>0.30 ± 0.03</td>
<td>0.92 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;y&lt;/sub&gt; (ml)</td>
<td>3.3 ± 0.2</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>K&lt;sub&gt;10&lt;/sub&gt; (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.09 ± 0.01</td>
<td>0.33 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μM)</td>
<td>6.3 ± 1.0</td>
<td>7.0 ± 1.2</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>30.4 ± 3.2</td>
<td>19.5 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean residence time (min)</td>
<td>31.4 ± 1.5</td>
<td>19.8 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V&lt;sub&gt;dss&lt;/sub&gt; (ml)</td>
<td>11.9 ± 1.9</td>
<td>15.2 ± 1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.01, <sup>b</sup>p < 0.001 compared with PEPT2<sup>+/+</sup> (wild-type) mice.

Effect of Probenecid and Quinine on Cefadroxil Pharmacokinetics. Cefadroxil plasma concentration versus time plots, in the absence and presence of probenecid (a classic inhibitor of OATs), are shown in Fig. 2 (A and B). Administration of cefadroxil plus 70 mg/kg probenecid resulted in greater plasma concentrations of cefadroxil in both genotypes than after administration of cefadroxil alone. In contrast, quinine [a classic inhibitor of organic cation transporters (OCTs)] had little effect on cefadroxil disposition (Fig. 2C). We also evaluated cefadroxil pharmacokinetics with coadministration of probenecid at a dose of 700 mg/kg. Cefadroxil plasma concentrations after coadministration with probenecid at the 70 and 700 mg/kg doses were not significantly different in either genotype, strongly suggesting that the renal secretion clearance of cefadroxil was completely abolished when probenecid was given at the lower probenecid dose.

Renal Clearance of Cefadroxil. As shown in Table 3 (and Figs. 1D, and 2, A and B), PEPT2 deletion and probenecid coadministration caused profound changes in the pharmacokinetics and plasma concentration versus time profiles of cefadroxil. In contrast, inulin plasma concentrations were similar in all studies, indicating that GFR was unchanged as a function of genotype or probenecid treatment (Fig. 2D). Neither PEPT2 gene deletion nor treatment with probenecid had an effect on the extent of protein binding by cefadroxil, with approximately 80% of the drug being unbound in plasma. Approximately 55 to 60% of the administered dose of cefadroxil was excreted in the urine over 2 h after an intravenous bolus dose of 1 nmol/g in both wild-type and PEPT2 null mice; the urinary recovery of cefadroxil was somewhat lower in probenecid-treated mice (40–45%), which most likely reflects saturation of renal tubular secretion and the slower excretion of drug.

It was observed (Table 3) that cefadroxil renal clearance increased by 3-fold in PEPT2<sup>−/−</sup> versus PEPT2<sup>+/+</sup> mice (i.e., 0.554 versus 0.182 ml/min after cefadroxil alone and 0.215 versus 0.063 ml/min when the 1 nmol/g dose was administered (Fig. 1D). These results suggest that the pharmacokinetics of cefadroxil are nonlinear and, in this case, probably reflect a change in renal tubular reabsorption.
after cefadroxil plus probenecid), but that cefadroxil renal clearance decreased by 3-fold in probenecid-treated versus untreated animals (i.e., 0.063 versus 0.182 ml/min in PEPT2+/− mice and 0.215 versus 0.554 ml/min in PEPT2−/− mice). When cefadroxil renal clearance was normalized for fraction unbound and functional nephron mass (i.e., GFR), the excretion ratio in wild-type mice was 0.95, suggesting that neither renal secretion nor reabsorption of the drug had occurred (i.e., filtration was the sole mechanism of renal excretion). However, the excretion ratio of cefadroxil was substantially greater in PEPT2 null mice (i.e., ER of 2.68), indicating a net secretory mechanism for drug clearance. In the absence of renal tubular secretion (i.e., in the presence of probenecid), the excretion ratio of cefadroxil was significantly reduced for both genotypes. Thus, the excretion ratio fell from 0.95 to 0.30 in wild-type mice and from 2.68 to 0.97 in PEPT2 null animals (Fig. 3). Renal clearance studies further demonstrated that the tubular reabsorption of cefadroxil was almost completely abolished in PEPT2 null mice compared with wild-type animals (i.e., 3% versus 70%, respectively; p < 0.001). Of the 70% of cefadroxil reabsorbed in wild-type mice, PEPT2 accounted for 95% and PEPT1 accounted for 5% of reabsorbed substrate (Table 3).

**Tissue Distribution of Cefadroxil at 120 Min.** Figure 4A shows the distribution of cefadroxil in selected tissues at 120 min postadministration. A comparison of PEPT2 null mice to wild-type controls clearly demonstrates that PEPT2 has a major effect on the tissue distribution of cefadroxil. In particular, mice with an intact PEPT2 gene had 21 times more cefadroxil in kidney than in PEPT2-deficient animals. Except for CSF, the concentration of cefadroxil in other tissues and blood was 2 to 8 times greater in wild-type than in PEPT2 null mice. Because the blood concentration of cefadroxil was significantly different between genotypes, tissue concentrations of drug were also corrected by their corresponding values in blood (Fig. 4B). The tissue-to-blood concentration ratio in kidney was significantly lower in PEPT2−/− mice than that in PEPT2+/− mice (approximately 3-fold); however, the concentration ratio in CSF was significantly higher in PEPT2 null animals (approximately 6- to 7-fold). No statistically significant differences were observed in the blood-corrected ratios of other tissues, including the choroid plexus and cerebral cortex. However, when drug concentrations in these two brain tissues were normalized by CSF concentration (Fig. 4C), PEPT2−/− mice had significantly lower ratios than those in PEPT2+/− mice (12-fold and 3-fold in choroid plexus and cerebral cortex, respectively).

**Discussion**

The antibacterial activity of an antibiotic depends on its exposure at pharmacological target sites and, as such, it is important to characterize the molecular mechanism(s) that affects the pharmacokinetics and tissue distribution of cephalosporins. Cefadroxil has a significantly slower rate of excretion and a more persistent concentration in blood and tissue, compared with other cephalosporins, which is a pharmacokinetic advantage in terms of its antibacterial activity (Tanriser and Santella, 1986). Nevertheless, the exact mechanism for the delivery and removal of cefadroxil from tissue has not yet been fully elucidated. In this study, we examined the in vivo function of the proton-coupled oligopeptide transporter PEPT2 by analyzing the disposition of cefadroxil in wild-type and PEPT2 knockout mice. Our data clearly show that PEPT2 had an overwhelmingly predominant role in the renal tubular reabsorption of cefadroxil. The decreased renal reabsorption in PEPT2 null mice resulted in substantially decreased levels of cefadroxil in plasma and tissues, indicating that this protein can reduce the systemic and tissue exposure of drugs that are PEPT2 substrates. PEPT2 also affected the efflux of cefadroxil from CSF into choroid plexus, thereby having a major effect on drug
Renal pharmacokinetics of cefadroxil after a 1 nmol/g intravenous bolus dose of drug

Values represent the mean ± S.E. (n = 5–7). Statistics were conducted by ANOVA followed by Tukey’s test. For a given parameter, means with the same superscript letter are not significantly different (α = 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PEPT2+/−</th>
<th>PEPT2-/−</th>
<th>PEPT2+/− + Probenecid</th>
<th>PEPT2-/− + Probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLr (ml/min)</td>
<td>0.182 ± 0.002 A</td>
<td>0.554 ± 0.016 A</td>
<td>0.063 ± 0.004 C</td>
<td>0.215 ± 0.008 B</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>0.235 ± 0.004 A</td>
<td>0.258 ± 0.007 A</td>
<td>0.249 ± 0.024 A</td>
<td>0.278 ± 0.012 B</td>
</tr>
<tr>
<td>k_M</td>
<td>0.82 ± 0.01 A</td>
<td>0.80 ± 0.01 A</td>
<td>0.80 ± 0.02 A</td>
<td>0.79 ± 0.01 B</td>
</tr>
<tr>
<td>k_E</td>
<td>0.55 ± 0.02 A</td>
<td>0.60 ± 0.02 A</td>
<td>0.41 ± 0.01 A</td>
<td>0.43 ± 0.01 C</td>
</tr>
<tr>
<td>ER</td>
<td>0.95 ± 0.02 A</td>
<td>2.68 ± 10 B</td>
<td>0.90 ± 0.03 A</td>
<td>0.97 ± 0.03 A</td>
</tr>
<tr>
<td>F</td>
<td>0.70 A</td>
<td>0.03 B</td>
<td>0.70 ± 0.03 A</td>
<td>0.03 ± 0.03 B</td>
</tr>
<tr>
<td>F1</td>
<td>0.03 A</td>
<td>0.03 B</td>
<td>0.03 A</td>
<td>0.03 A</td>
</tr>
<tr>
<td>F2</td>
<td>0.69 A</td>
<td>100 B</td>
<td>0.69 ± 0.03 A</td>
<td>100 B</td>
</tr>
<tr>
<td>%PEPT1</td>
<td>4.6 ± 0.2 A</td>
<td>0.0 A</td>
<td>4.5 ± 0.2 A</td>
<td>0.0 A</td>
</tr>
<tr>
<td>%PEPT2</td>
<td>95.4 A</td>
<td>0.0 A</td>
<td>95.4 ± 0.2 A</td>
<td>0.0 A</td>
</tr>
</tbody>
</table>

fe120, the fraction of administered cefadroxil excreted unchanged in urine over 120 min.

Cefadroxil undergoes three mechanisms of renal excretion, namely, glomerular filtration, renal tubular secretion, and renal tubular reabsorption. The latter two processes could become saturated at higher plasma concentrations of drug. Although cefadroxil and other β-lactam aminocephalosporin antibiotics are secreted by organic anion transporters located in the basolateral membrane of the proximal tubule (i.e., OAT1 and OAT3 in mouse), the drugs are reabsorbed by brush-border membrane POTs (i.e., PEPT1 and PEPT2) in the proximal tubule (Terada et al., 1997; Takeda et al., 2002; Luckner and Brandsch, 2005; Shitara et al., 2005). In comparison to the relatively high affinity of cefadroxil for PEPT2 (i.e., $K_m = 10-40 \mu M$, as noted before), the affinity of cefadroxil for rodent OATs appears to be much lower, with $K_i$ and $K_m$ values in the millimolar range (Jung et al., 2002; Khamdang et al., 2003). Therefore, it appears likely that the PEPT2-mediated reabsorption of cefadroxil may exhibit more satura-bility than the OAT1/3-mediated secretion of drug at therapeutic plasma concentrations.

In the renal clearance experiments, PEPT2-mediated reabsorption of cefadroxil was knocked out by gene deletion, whereas the drug’s tubular secretion was functionally knocked out by probenecid, a potent inhibitor of rodent OAT1 and OAT3 (Shitara et al., 2005). In contrast, quinone, a potent inhibitor of rodent OCT1 and OCT2 (Shitara et al., 2005), had no effect on the renal tubular secretion of cefadroxil, indicating a lack of organic cation transport in the uptake process. As a result, only filtration and PEPT1-associated reabsorption of cefadroxil remained intact in the PEPT2-/−/− animals with probenecid present. The fact that the excretion ratio of cefadroxil was almost unity (i.e., ER 0.97) suggests that PEPT1 played a very minor role in the drug’s renal reabsorption. When these results are combined with the fractional reabsorption calculations (Table 3), it is clear that PEPT2 is the dominant peptide transporter in kidney. Of the 70% of cefadroxil reabsorbed in PEPT2+/−/− mice, 95% of this value was reabsorbed by PEPT2 and only 5% by PEPT1. In a previous study by our laboratory (Ocheltree et al., 2005), we reported that of the 46% of GlySar reabsorbed in wild-type mice, PEPT2 accounted for 86% and PEPT1 accounted for 14% of reabsorbed substrate. There is little doubt that PEPT2 is much more important than PEPT1 in the reabsorption of both cefadroxil and GlySar. However, it is less obvious why the fractional reabsorption of GlySar ($F = 0.46$) is lower than the total plasma clearance of cefadroxil in wild-type mice at increasing dose levels of drug (Table 1), a finding consistent with saturable tubular reabsorption. This observation agrees with other pharmacokinetic studies in which an increase in the plasma clearance of cefadroxil was observed following high doses of drug in both human and rat (Garrigues et al., 1991; Garcia-Carbonell et al., 1993).
that of cefadroxil ($F_t = 0.70$). One possible explanation is that cefadroxil was studied at plasma concentrations of $\leq 10 \mu M$ (this study), whereas GlySar was studied at plasma concentrations $\leq 500 \mu M$ (Ocheltree et al., 2005), concentrations at which GlySar may be experiencing capacity-limited renal tubular reabsorption. Regardless, it should be appreciated that cefadroxil plasma concentrations produced at the $1 \text{nmol/g}$ dose (i.e., approximately $0.01–10 \mu M$) in this study are clinically relevant, since they are in the minimal inhibitory concentration range of most bacteria (Courtie and Drugeon, 1983).

Strikingly higher concentrations of cefadroxil were observed in all the tissues of wild-type mice compared with PEPT2-deficient animals, suggesting a dramatic effect of PEPT2 on the tissue distribution of drug (Fig. 4A). The shift in concentration between genotypes could be due to differences in systemic drug concentrations or cellular uptake. To rule out differences being due to systemic exposure alone, the tissue concentrations of cefadroxil for each genotype were corrected by their corresponding blood concentrations. When this correction was made, only the kidney (3-fold reduction in null mice) and CSF (6- to 7-fold increase in null mice) had statistically significant differences in their tissue-to-blood concentration ratios between genotypes (Fig. 4B). These findings substantiate our hypothesis that the renal reabsorption and CSF efflux of cefadroxil are mediated by PEPT2, a protein that is absent from the apical membranes of renal proximal tubular and choroid plexus epithelial cells in PEPT2$^{-/-}$ mice (Shen et al., 2003). Although the tissue-to-blood concentration ratios in choroid plexus and cerebral cortex were not significantly different between genotypes, the tissue-to-CSF concentration ratios were substantially lower in PEPT2$^{-/-}$ mice (i.e., 12-fold for choroid plexus and 3-fold for cerebral cortex; Fig. 4C). These findings suggest that the CNS exposure of cefadroxil is largely controlled by PEPT2's functional activity in brain and, in particular, its role in effluxing drug from CSF into choroid plexus.

Recently, the PEPT2 gene was found to be polymorphically expressed in humans with single nucleotide polymorphisms (Pinsonneau et al., 2004; Terada et al., 2004). The GlySar transport activity of variant R57H was completely abolished (Terada et al., 2004). Genetic deficiencies for the gene may have both positive and negative consequences for drug therapy. In the case of a PEPT2 deficiency, reduced uptake of drugs into the kidney may result in a decreased efficacy of drugs that have their therapeutic action in the kidney. On the other hand, reduced uptake of drugs into the kidney could be beneficial for drugs that have adverse effects in the kidney. Moreover, the loss of PEPT2 expression in choroid plexus may have significant influences on CSF and brain concentrations of peptides and peptidyl-like drugs. On the basis of our results, we expect that humans with a deficiency in PEPT2 will have impaired renal reabsorption of some drugs and that this may result in decreased systemic exposure. We also expect that genetic deficiencies may reduce the clearance of some drugs from CSF to blood. Therefore, it is important to test whether or not these transport differences will translate into pharmacological or toxicological phenotypes in our transgenic mouse colony. Moreover, it will be interesting to determine whether polymorphisms in the human PEPT2 genes also correlate with altered drug disposition and dynamics in patients. If our findings can indeed be extrapolated to humans, the PEPT2 knockout mouse model will provide a unique tool for predicting and explaining peptide/mimetic sensitivity and/or toxicity, which may ultimately help in the development of new peptide-based pharmaceuticals.

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