Mechanisms of Cefadroxil Uptake in the Choroid Plexus: Studies in Wild-Type and PEPT2 Knockout Mice

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ABSTRACT

The choroid plexus uptake of [3H]cefadroxil was studied in peptide transporter 2 (PEPT2) wild-type and null mice as a function of temperature, transport inhibitors, pH, and saturability. At normal pH (7.4) and temperature (37°C), the uptake of 1 μM cefadroxil was reduced by 83% in PEPT2−/− mice as compared with PEPT2+/+ mice (p < 0.001). A further reduction was achieved in null animals by reducing the temperature to 4°C, or by adding saturating concentrations of unlabeled cefadroxil or p-aminohippurate (p < 0.05). Glycylsarcosine coadministration could inhibit the uptake of cefadroxil in PEPT2+/+ mice (p < 0.01) but not PEPT2−/− mice. Although a proton-stimulated uptake of cefadroxil was demonstrated in PEPT2+/+ mice (pH 6.5 versus pH 7.4; p < 0.01), no pH dependence was observed in PEPT2−/− mice. Kinetic parameters for cefadroxil (without p-aminohippurate) in wild-type mice were: Vmax = 5.4 pmol/mg/min, Km = 34 μM, and Ka = 0.0069 μl/mg/min. In null animals, the kinetic parameters of cefadroxil (without p-aminohippurate) were: Vmax = 2.7 pmol/mg/min, Km = 110 μM, and Ka = 0.0084 μl/mg/min. In the presence of p-aminohippurate, only a Ka = 0.010 μl/mg/min was observed. Based on kinetic and inhibitor analyses, it was determined that (under linear conditions), 80 to 85% of cefadroxil’s uptake in choroid plexus is mediated by PEPT2, 10 to 15% by organic anion transporter(s), and 5% by nonspecific mechanisms. These findings demonstrate that PEPT2 is the primary transporter responsible for cefadroxil uptake in the choroid plexus. Moreover, the data suggest a role for PEPT2 in the clearance of peptidomimetics from cerebrospinal fluid.

The mammalian proton-coupled oligopeptide transporter (POT) superfamily currently consists of four members (i.e., PEPT1, PEPT2, PHT1, PHT2) (Herrera-Ruiz and Knipp, 2002). These symporters couple the uphill movement of small peptides with the downhill movement of protons across biological membranes, via an inwardly directed proton gradient and negative membrane potential. Because they can accommodate a wide variety of di- and tripeptides (regardless of size, hydrophobicity, and charge), oligopeptide transporters make good targets for drug delivery strategies. In this regard, they have been shown to transport a number of pharmacologically active agents such as β-lactam antibiotics (e.g., α-amino-containing penicillins and cephalosporins), angiotensin-converting enzyme inhibitors, and nucleoside analog prodrugs (Rubio-Aliaga and Daniel, 2002).

PEPT1 was cloned first from a rabbit intestinal cDNA library (Fei et al., 1994) and was characterized as a low-affinity (i.e., millimolar Km), high-capacity transporter (Daniel, 1996; Leibach and Ganapathy, 1996). PEPT2 was cloned subsequently from a human kidney cDNA library (Liu et al., 1995) and was described as a high-affinity (i.e., micromolar Km), low-capacity transporter (Daniel and Herget, 1997). The two newest members of the POT family were cloned from a rat brain cDNA library and are known as the peptide/histidine transporters PHT1 (Yamashita et al., 1997) and PHT2 (Sakata et al., 2001). These transporters are functionally unique from both PEPT1 and PEPT2 in that they possess an ability to transport the amino acid l-histidine, in addition to di- and tripeptides.

The choroid plexus is the site of the blood-cerebrospinal fluid barrier (BCSFB). The choroid plexus epithelial cells

ABBREVIATIONS: POT, proton-coupled oligopeptide transporter; PEPT, peptide transporter; PHT, peptide/histidine transporter; BCSFB, blood-cerebrospinal fluid barrier; CNS, central nervous system; ACSF, artificial cerebrospinal fluid; MES, 2-(N-morpholino)ethanesulfonic acid; OAT, organic anion transporter.
with their linking tight junctions form a diffusional barrier but also possess an array of transporters that move substrates into or out of the cerebrospinal fluid. With respect to POT expression and activity in the choroid plexus, two transporters should be considered. Specifically, PEPT2 mRNA (Berger and Hediger, 1999), protein (Novotny et al., 2000; Shu et al., 2002), and dipeptide uptake (Teuscher et al., 2000, 2001), along with PHT1 mRNA transcripts (Yamashita et al., 1997), have been reported in this tissue. However, the data suggest that PEPT2, but not PHT1, is expressed and functionally active on the apical membrane of rat choroid plexus epithelial cells in primary culture and may serve a role as an efflux pump for the removal of small peptides from the cerebrospinal fluid (Shu et al., 2002). As a result, PEPT2 is also believed to mediate the efflux of peptidomimetic drugs from the cerebrospinal fluid to the circulating blood supply. If correct, this contention might have significant consequences for drug therapy directed to the central nervous system (CNS).

The recent generation of PEPT2 knockout mice by our laboratory (Shen et al., 2003) and by Rubio-Aliaga et al. (2003) has enabled the examination of the physiologic role and pharmaceutical significance of PEPT2 in the transport of peptides/mimetics in multiple organ systems. In particular, knockout animal models allow one to specifically study the role and relative importance of PEPT2 in choroid plexus and the regional disposition of peptide-like pharmaceuticals in the cerebrospinal fluid. This information is critical since the choroid plexus is believed to be a significant site for the clearance of β-lactam antibiotics from the cerebrospinal fluid (Suzuki et al., 1997). Unfortunately, few if any studies have examined the interaction between aminoccephalosporin drugs and PEPT2 in the choroid plexus. Cefadroxil was chosen for study because it is a model aminoccephalosporin drug and a known PEPT2 substrate (Ries et al., 1994; Boll et al., 1996), and is commercially available as radiolabel. Therefore, the objective of this study was to determine the importance of PEPT2 in the choroid plexus uptake of cefadroxil using whole-tissue preparations isolated from wild-type and PEPT2 null mice. This study also examines the contribution of other transporters in mediating the active uptake of cefadroxil in this tissue. Our findings are novel in demonstrating, for the first time, that PEPT2 is the primary transporter responsible for the choroid plexus uptake of a peptidomimetic drug at the BCSFB.

Materials and Methods

Materials. [3H]Cefadroxil (1 Ci/mmol) and [14C]mannitol (50 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA). [3H]Benzylenpenicillin (21 Ci/mmol) was purchased from Amersham Biosciences Inc. (Piscataway, NJ). Nylon net filters (100-μm) were obtained from Millipore Corporation (Bedford, MA). C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). PEPT2-transgenic mice were generated on a C57BL/6 mouse background and genotyped by reverse transcription-polymerase chain reaction as described by Shen et al. (2003). Glycylsarcosine, cefadroxil, and p-aminohippurate were obtained from Sigma-Aldrich (St. Louis, MO). Hyamine hydroxide was obtained from ICN Pharmaceuticals (Costa Mesa, CA). All other chemicals were obtained from standard sources.

Transport Buffers. Experiments were performed in bicarbonate artificial cerebrospinal fluid (aCSF) or Tris-MES buffers. Bicarbonate-ate aCSF buffers were continuously bubbled with 5% CO2, 95% O2 and contained 127 mM NaCl, 20 mM NaHCO3, 2.4 mM KCl, 0.5 mM KH2PO4, 1.1 mM CaCl2, 0.85 mM MgCl2, 0.5 mM Na2SO4, and 5.0 mM glucose (pH 7.4). Tris-MES buffers were bubbled with 100% O2 and contained 147 mM NaCl, 2.4 mM KCl, 0.5 mM KH2PO4, 1.1 mM CaCl2, 0.85 mM MgCl2, 0.5 mM Na2SO4, 5.0 mM glucose, and 10 mM Tris and/or MES. The pH of these buffers was adjusted with different combinations of Tris and MES to attain pH values between 6.5 and 7.4, with osmolality being held constant.

Cefadroxil Uptake. All uptake experiments were performed in parallel for PEPT2+/− and PEPT2−/− littersmates (6–8 weeks old). Detailed methods for the surgical isolation of the choroid plexus are described in Keep and Xiang (1995) for glutamine uptake. In our experiments, the lateral and fourth ventricle choroid plexuses were isolated from anesthetized (pentobarbital; 65 mg/kg intraperitoneally) mice, weighed, and transferred to bicarbonate aCSF buffer at 37°C. Each sample consisted of pooled plexuses from two animals. A 5-min recovery period was allowed before the beginning of each uptake experiment. After the recovery period, the plexuses were transferred to 0.95 ml of transport buffer with or without drug for 0.5 min. Uptake was then initiated by the addition of 0.05 ml of buffer with approximately 1.0 μCi of [3H]cefadroxil and 0.5 μCi of [14C]mannitol (an extracellular marker). Unless otherwise stated, the uptake was terminated after 1 min by transferring the plexuses to ice-cold buffer and filtering under reduced pressure. The filters were washed three times with cold transport buffer. The filters and choroid plexuses were then soaked in 0.33 ml of 1 M hyamine hydroxide (a tissue solubilizer) for 30 min before the addition of Ecolite (+) scintillation cocktail (ICN Pharmaceuticals) and counting with a dual-channel liquid scintillation counter (Beckman LS 3801; Beckman Coulter, Inc., Fullerton, CA).

The uptake of radiolabeled cefadroxil into choroid plexus, in microliters per milligram of wet tissue weight, was calculated as (Keep and Xiang, 1995):

\[
\text{cefadroxil uptake} = \frac{S_t - S_f - [(M_t - M_f) \times \text{ratio}]}{S_{\text{media}}} \tag{1}
\]

where \( S_t \) is the total substrate (cefadroxil) concentration in the plexus plus filter, \( S_f \) is the filter binding of substrate, and \( S_{\text{media}} \) is the concentration of substrate in the external media. The term \((M_t - M_f) \times \text{ratio}\) is a correction for extracellular space, where \( M_t \) is the total mannitol concentration in the plexus plus filter and \( M_f \) is the filter binding of mannitol. Multiplying the difference between these two parameters by the ratio of [3H]cefadroxil to [14C]mannitol in the external medium provides an estimate of the extracellular content of cefadroxil. The unidirectional flux rate (V) can then be calculated by multiplying cefadroxil uptake by \( S_{\text{media}} \) and dividing by the duration of the experiment. As a negative control, similar experiments were performed with [3H]benzylenpenicillin, a β-lactam antibiotic lacking an α-amino group that is primarily transported by OAT3 in rat choroid plexus (Nagata et al., 2002).

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.

Data Analysis. For most kinetic studies, the concentration-dependent uptake of cefadroxil was best fit to a Michaelis-Menten relationship along with a nonsaturable component:

\[
V = \frac{V_{\text{max}} \times C}{K_m + C} + K_d \times C \tag{2}
\]

where \( V_{\text{max}} \) is the maximal rate of saturable cefadroxil uptake, \( K_m \) is the Michaelis constant, \( K_d \) is the rate constant for nonsaturable processes, and \( C \) is the substrate (cefadroxil) concentration. However, in the presence of p-aminohippurate, the uptake of cefadroxil in PEPT2 null animals was best fit by a nonsaturable component alone.

All data are reported as mean ± S.E. Statistical comparisons were performed using analysis of variance, and post-hoc pairwise compar-
isons were made using Dunnett’s test (GraphPad Prism, v3.0; GraphPad Software Inc., San Diego, CA). A probability of \( p \leq 0.05 \) was considered statistically significant. Linear and nonlinear regression analyses were performed using SigmaPlot 8.0 (SPSS Inc., Chicago, IL) and a weighting factor of unity. The quality of fit was determined by evaluating the coefficient of determination \( (r^2) \) and the coefficient of variation of the parameter estimates, and by visual inspection of the residuals.

**Results**

**Time-Dependent Uptake of Cefadroxil.** The uptake of cefadroxil (0.2 \( \mu \)M) was initially studied in whole choroidplexus tissue isolated from C57BL/6 mice. As shown in Fig. 1, cefadroxil demonstrated a linear uptake for approximately 2 min \((r^2 = 0.989)\). The \( y \)-intercept value was not statistically different from zero \((p = 0.586)\), indicating that nonspecific binding was negligible. Based on these results, an uptake time of 1 min was used for subsequent experiments in wild-type and PEPT2 null animals to maximize radiotracer counts while remaining within the linear region of uptake. Cefadroxil reached a plateau value of 0.28 pmol/mg at 90 min into the experiment. Because the medium concentration of cefadroxil was 0.2 \( \mu \)M, this corresponds to a tissue/medium ratio of 1.4. Thus, accumulation in the choroid plexus was greater than that in the medium, suggesting a concentrative process.

**Temperature-Dependent Uptake of Cefadroxil.** At 37°C, the choroid plexus uptake of 1 \( \mu \)M cefadroxil was reduced by 83% in PEPT2\(^{-/-} \) versus PEPT2\(^{+/+} \) mice \((p < 0.001; \text{Fig. 2})\). Moreover, cefadroxil uptake was reduced by 93% and 68% in PEPT2\(^{+/+} \) and PEPT2\(^{-/-} \) mice, respectively, at 4°C versus 37°C. In contrast, no statistical difference was observed between either genotype at the lower temperature. The data indicate that the large majority of cefadroxil uptake in wild-type animals is governed by PEPT2, with only a small percentage governed by nonspecific, temperature-independent processes. However, these results also suggest that there is yet another active process contributing to cefadroxil uptake in the choroid plexus of PEPT2\(^{-/-} \) mice.

**Effect of Inhibitors on Cefadroxil Uptake.** The choroid plexus uptake of 1 \( \mu \)M cefadroxil (pH 7.4, 37°C) was examined in the presence of potential transport inhibitors (Fig. 3). Saturating concentrations of glycylsarcosine reduced cefadroxil uptake in PEPT2\(^{+/+} \) mice down to the levels observed in PEPT2\(^{-/-} \) mice without inhibitors. In contrast, 5 mM cefadroxil reduced the uptake of radiolabeled drug, in both genotypes, to levels observed at 4°C (Fig. 2). This suggested that there is an active process, in addition to PEPT2, that mediates cefadroxil uptake. To test the hypothesis that an OAT mediates a minor component of cefadroxil uptake in choroid plexus, \( p \)-aminonhippurate was selected as an inhibitor. At 5 mM concentrations, \( p \)-aminonhippurate produced results in null animals that were statistically similar to those of cefadroxil inhibition and reduced temperature, indicating that the residual active uptake of cefadroxil is probably mediated by OAT(s). In wild-type mice, \( p \)-aminonhippurate caused a 15% reduction in cefadroxil uptake \((p > 0.05)\).
pH-Dependent Uptake of Cefadroxil. Because PEPT2-mediated transport is stimulated by a proton gradient, the pH dependence of cefadroxil uptake (1 µM) was investigated in Tris-MES cerebrospinal fluid buffers at pH 7.4 and pH 6.5. Although a proton-stimulated uptake of cefadroxil was demonstrated in PEPT2−/− mice at pH 6.5 versus pH 7.4 (p < 0.01), no pH dependence was observed in PEPT2−/− mice (Fig. 4). The absence of a proton-dependent uptake in PEPT2−/− mice indicates that residual uptake of cefadroxil in choroid plexus is not mediated by another POT family member.

Concentration-Dependent Uptake of Cefadroxil. The uptake kinetics of cefadroxil in choroid plexus was evaluated over the concentration range of 0.5 to 500 µM (pH 7.4, 37°C). As shown in Fig. 5 and Table 1, PEPT2−/− mice demonstrated saturable transport with a Vmax of 5.4 pmol/mg/min, a Km of 34 µM, and a Kd of 0.0069 µmol/min. Thus, under linear conditions, the carrier-mediated component accounted for 96% of cefadroxil's total uptake. The nonsaturable component (0.0069 pmol/mg/min at 1 µM) was similar to the uptake of 1 µM cefadroxil in PEPT2+/+ mice at 4°C (0.0096 pmol/mg/min; Fig. 2). In contrast, PEPT2+/+ mice exhibited a less efficient transport of cefadroxil (i.e., 6-fold lower value for Vmax/Km) with a Vmax of 2.7 pmol/mg/min, a Km of 110 µM, and a Kd of 0.0084 µmol/min. Under linear conditions, the carrier-mediated component accounted for 75% of cefadroxil’s total uptake in PEPT2−/− mice.

To remove the contribution of OAT-mediated uptake, cefadroxil studies were also performed in the presence of 5 mM p-aminohippurate (Fig. 6; Table 1). Kinetic analyses revealed that in PEPT2+/+ mice, the Vmax was 4.1 pmol/mg/min, the Km was 27 µM, and the Kd was 0.0064 µmol/min. In the absence of OAT transport mechanisms, PEPT2−/− mice exhibited a strictly linear uptake in choroid plexus (i.e., no saturable transport and a Kd of only 0.010 µmol/min being observed), with uptake values significantly less than those observed for PEPT2−/− mice in the absence of p-aminohippurate. For ease of comparison, Fig. 7 clearly shows the impact of PEPT2 and/or OAT transporters, along with nonsaturable processes, in the uptake profiles of cefadroxil in the choroid plexus of wild-type and PEPT2 null animals.

Fig. 4. pH dependent uptake of [3H]cefadroxil in the choroid plexuses of PEPT2+/+ and PEPT2−/− mice (1 µM cefadroxil in external medium). Studies were performed at pH 7.4 and pH 6.5 in Tris-MES buffer (37°C). Data are expressed as mean ± S.E. (n = 3). **, p < 0.01 for wild-type animals at pH 6.5 versus pH 7.4.

Fig. 5. Concentration-dependent uptake of [3H]cefadroxil (0.5–500 µM total cefadroxil in external medium) in the choroid plexuses of PEPT2+/+ (○) and PEPT2−/− mice (○). Studies were performed at 37°C in bicarbonate aCSF buffer (pH 7.4) in the absence of p-aminohippurate. Data are expressed as mean ± S.E. (n = 2). Predicted curves were generated using the Vmax, Km and Kd values of Table 1.

Discussion

Few studies have examined the interaction between cephalosporins and PEPT2 in the choroid plexus. In a study by Teuscher et al. (2000) in isolated rat choroid plexus, the aminopeptidase PEPT2 cefadroxil and cephalaxin inhibited the uptake of a model dipeptide, glycylsarcosine, by 85 to 90% when present at 1 mM concentrations. However, two cephalosporins lacking an α-amino functional group, cephaloridine and cephalothin, failed to inhibit glycylsarcosine uptake. A similar finding was reported by Shu et al. (2002) in studies performed in rat choroid plexus epithelial cells in primary culture. These authors found that when probed from the apical membrane, cefadroxil and cephalaxin inhibited about 85% of glycylsarcosine uptake, whereas cephaloridine and cephalothin were without effect. When glycylsarcosine uptake was probed from the basolateral surface, inhibitory effects of the same two aminopeptidase inhibitors were significant but reduced. Cephalosporins lacking a free amino group were unable to inhibit glycylsarcosine uptake from the basolateral side, as shown from the apical side. Although suggestive, these studies (Teuscher et al., 2000; Shu et al., 2002) do not directly demonstrate a POT-mediated mechanism for the uptake of cephalosporins in choroid plexus tissue or cell cultures.

In the present study, choroid plexus uptake of cefadroxil was substantially reduced in PEPT2 null mice as compared with wild-type controls in aCSF buffer under physiological conditions. Although a proton-stimulated uptake was demonstrated in PEPT2−/− mice at pH 6.5 versus pH 7.4, there was no effect of pH on the uptake of drug in PEPT2−/− animals. Based on temperature and inhibitor analyses, we demonstrated that (under linear conditions) the uptake of cefadroxil in choroid plexus tissue was primarily mediated by PEPT2 (80 to 85%) and to a minor extent by OAT transporters (10–15%) and nonspecific mechanisms (5%).

Given the lack of information regarding OAT functionality in the choroid plexus of wild-type and PEPT2 null mice, additional studies were performed with 1 µM benzylpenicillin in aCSF buffer, pH 7.4, at 37°C. As expected for a non-PEPT2 substrate, there was no statistical difference in ben-
component of cefadroxil an important finding because our results show that a minor function was main-
OAT transporter. In this regard, Sweet et al. (2002) reported
ate aCSF buffer (pH 7.4) in the presence of 5 mM
V
were generated using the
zylpenicillin uptake between PEPT2
(wild-type) and PEPT2
(null) mice.

TABLE 1
Choroid plexus uptake kinetics of cefadroxil in PEPT2
(wild-type) and PEPT2
(null) mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Parametera</th>
<th>Absence of PAHb</th>
<th>5 mM PAHb</th>
</tr>
</thead>
</table>
| PEPT2
| V
max (pmol/mg/min) | 5.4 (0.3) | 4.1 (0.3) |
| K
m (μM) | 34 (4) | 27 (4) |
| K
 (μM/mg/min) | 0.0069 (0.0006) | 0.0064 (0.0007) |
| V
max/K
 (μM/mg/min) | 0.16 | 0.15 |
| V
max/K
 (pmol/mg/min) | 2.7 (0.8) | 3.5 (0.8) |
| K
 (μM) | 110 (36) | 110 (36) |
| K
 (μM/mg/min) | 0.0084 (0.0011) | 0.010 (0.0011) |
| V
max/K
 (pmol/mg/min) | 0.025 | 0.025 |

a Parameter estimates (±S.E.) were derived by nonlinear (or linear) regression models. See Figs. 5 and 6 for study conditions.
bPAH, p-aminohippurate.

OAT1–3 transcripts in wild-type rat and mouse choroid plexus, with OAT1 and OAT3 being targeted to the apical membrane of this tissue. The fact that p-aminohippurate is highly transported by OAT1 and OAT3 (K
m < 100 μM), but not OAT2 (Table 1; Sweet et al., 2002), would argue against this latter carrier being involved in the choroid plexus uptake of cefadroxil. As shown in Table 1 of our study, the K
m of 110 μM (determined for PEPT2
 mice in the absence of p-aminohippurate) reflects the affinity of OAT for cefadroxil uptake in choroid plexus whole tissue. In comparison, the low-affinity interactions (i.e., millimolar IC
m or K
values) of cefadroxil in proximal tubule cells stably expressing rat OAT1–3 (Jung et al., 2002; Khamdang et al., 2003) and human OAT1–4 (Takeda et al., 2002; Khamdang et al., 2003) may reflect the different species and experimental systems being utilized.

Kinetic analysis of cefadroxil uptake was confounded by the presence of multiple transport systems in the choroid plexus of PEPT2
 mice. The K
m of 34 μM in this animal model was very close to our best estimate of PEPT2 function, i.e., the K
m of 27 μM in wild-type mice with p-aminohippurate present, since most of cefadroxil’s uptake is via PEPT2. This latter K
m is comparable with values reported for the PEPT2-mediated uptake of cefadroxil in Xenopus oocytes (K
m = 32 μM; Boll et al., 1996) and for cefadroxil in rat renal brush-border membrane vesicles (K
m = 9 μM; Ries et al., 1994). Moreover, the high affinity characteristics of PEPT2 in choroid plexus tissue seem well suited for the efficient removal of drugs, which are generally present at low concentrations in the cerebrospinal fluid relative to plasma. Although there is a lack of published data regarding the concentrations of cefadroxil in cerebrospinal fluid, cefadroxil achieves peak serum concentrations of ≈92 μM (AHFS Drug Information, 2003), and cephalosporins achieve peak cerebrospinal fluid levels ranging from 0 to 55% of peak serum concentrations (Andes and Craig, 1999). Thus, under clinical dosing conditions, PEPT2-mediated transport would be the predominant process responsible for removing cefadroxil from cerebrospinal fluid, when present in this compartment (Fig. 8).

Functional and membrane localization studies suggest that PEPT2 may be the protein that is primarily responsible for subtherapeutic concentrations of some β-lactam antibiotics in the cerebrospinal fluid (Smith et al., 2003). Yet, despite the presence of PEPT2 in the choroid plexus, there are still several cephalosporins that can penetrate the cerebrospinal fluid in sufficient concentrations to be of clinical use for the treatment of bacterial meningitis (Mandell and Petri, 1996; Andes and Craig, 1999). These drugs (e.g., cefuroxime, moxa-lactam, cefotaxime, ceftriaxone, cefepime, cefizoxime) all possess a common structural characteristic, i.e., the absence
of an α-amino group, and are, therefore, unlikely to be PEPT2 substrates. Thus, one can speculate that the aminopeptidase activity of PEPT2 might have reasonable access to the cerebrospinal fluid if it were not being rapidly cleared by PEPT2. This scenario, if true, would result in inadequate cerebrospinal fluid levels of cefadroxil and a clinically ineffective drug for treating bacterial infections in the CNS.

Although PHT1 has been reported in choroid plexus (Yamashita et al., 1997) and PHT2 in brain (Sakata et al., 2001), several factors argue against these POT family members being involved in the uptake of cefadroxil in choroid plexus. For example, in PEP T2 null mice there was no reduction in transport activity by glycylsarcosine, although cefadroxil (self-inhibition) and β-aminohippurate reduced the uptake of radiolabeled drug to levels observed at 4°C. Moreover, a proton-stimulated uptake was noticeably absent in PEPT2−/− animals. These results support our previous studies in which L-histidine had no effect on the uptake of glycylsarcosine in rat choroid plexus whole tissue (Teuscher et al., 2000) or at the apical and basal surfaces of rat choroid plexus epithelial cells in primary culture (Shu et al., 2002). Instead, the peptide/histidine transporters may have a role in the intracellular trafficking of small peptides, as suggested by lysosomal expression studies in rat PHT2-transfected BHK and HEK-293T cells (Sakata et al., 2001) and by studies in PHT1-containing bovine and human retinal pigment epithelium (Ocheltree et al., 2003).

In conclusion, these novel findings have conclusively demonstrated that PEPT2 is the primary transporter responsible for cefadroxil uptake in the choroid plexus. The results further suggest a role for PEPT2 in the clearance of peptide mimetics at the BCFSB, a factor that may alter drug distribution profiles in the cerebrospinal fluid and pharmacologic response. A more efficient delivery of peptide-like pharmacocuticals to the CNS may require specific blocking agents or the design of therapeutic entities with structural characteristics that lack affinity for PEPT2. Future studies will be directed at the in vivo pharmacokinetics and tissue distribution of peptides/mimetics in wild-type and PEPT2 knockout mice. In doing so, the relative importance of this transporter can be more completely understood.

References


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