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Influence of peptide transporter 2 (PEPT2) on the distribution of cefadroxil in mouse brain: A microdialysis study

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ABSTRACT

Peptide transporter 2 (PEPT2) is a high-affinity low-capacity transporter belonging to the proton-coupled oligopeptide transporter family. Although many aspects of PEPT2 structure-function are known, including its localization in choroid plexus and neurons, its regional activity in brain, especially extracellular fluid (ECF), is uncertain. In this study, the pharmacokinetics and regional brain distribution of cefadroxil, a β-lactam antibiotic and PEPT2 substrate, were investigated in wildtype and *Pept2* null mice using *in vivo* intracerebral microdialysis. Cefadroxil was infused intravenously over 4 h at 0.15 mg/min/kg, and samples obtained from plasma, brain ECF, cerebrospinal fluid (CSF) and brain tissue. A permeability-surface area experiment was also performed in which 0.15 mg/min/kg cefadroxil was infused intravenously for 10 min, and samples obtained from plasma and brain tissues. Our results showed that PEPT2 ablation significantly increased the brain ECF and CSF levels of cefadroxil (2- to 2.5-fold). In contrast, there were no significant differences between wildtype and *Pept2* null mice in the amount of cefadroxil in brain cells. The unbound volume of distribution of cefadroxil in brain was 60% lower in *Pept2* null mice indicating an uptake function for PEPT2 in brain cells. Finally, PEPT2 did not affect the influx clearance of cefadroxil, thereby, ruling out differences between the two genotypes in drug entry across the blood-brain barriers. These findings demonstrate, for the first time, the impact of PEPT2 on brain ECF as well as the known role of PEPT2 in removing peptide-like drugs, such as cefadroxil, from the CSF to blood.

1. Introduction

Proton-coupled oligopeptide transporters (POTs) move di-/tripeptides and peptidomimetics across biologic membranes down an electrochemical membrane gradient, thereby playing an important role in the absorption, distribution and elimination of substrates in the body [1]. Among the four mammalian POTs, peptide transporter 2 (PEPT2, also known as SLC15A2) is a high-affinity and low-capacity transporter. It is widely expressed in brain, kidney, lung, eye and mammary gland [2,3]. The functional activity of PEPT2 has been studied using a variety of substrates including the synthetic dipeptide glycylsarcosine (GlySar) [4–6], endogenous peptidomimetics (e.g., 5-aminolevulinic acid) [7,8], neuropeptides (e.g., carnosine and kyotorphin) [9–11], as well as peptide-like drugs (e.g., cefadroxil) [12,13]. In kidney, PEPT2 is expressed at the apical membrane of proximal tubule epithelial cells where it plays an important role in the reabsorption of substrates from urine, thereby limiting renal clearance [14].

PEPT2 is also expressed at the apical membrane of choroid plexus epithelial cells (CSF-facing), the site of the blood-cerebrospinal fluid barrier (BCSFB), where it facilitates substrate efflux from CSF to blood, thus reducing substrate distribution in CSF [15]. According to an immunolocalization study in rat brain, PEPT2 is distributed in brain parenchyma, particularly at the plasma membrane of neural cells (neonates and adults) and astrocytes (neonates only) [16]. As an uptake transporter in brain cells, PEPT2 plays a role in the homeostasis of neuropeptides and the distribution of peptide-like therapeutics within brain

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parenchyma. Furthermore, our previous studies in wildtype and *Pept2* null mice indicate that PEPT2 has a profound influence on the neurological effects of its substrates in the central nervous system (CNS). For instance, PEPT2 reduces the neurotoxicity of 5-aminolevulinic acid [7] and the anti-nociceptive effect of kyotorphin [10]. However, in these two studies, the effect of PEPT2 in ECF is inferred since our study design did not allow direct measurement of this biological fluid.

In addition to small peptides, PEPT2 is able to transport peptide-like drugs that have similar structures to the backbones of di- or tripeptides (e.g., cephalosporins, angiotensin-converting enzyme inhibitors) as well as antiviral nucleoside prodrugs [17]. Among such drugs, cefadroxil is a first-generation cephalosporin with a broad spectrum of antibacterial activity, high PEPT2 affinity, and favorable biological stability [18,19]. Thus, cefadroxil serves as a good model compound to study the role and relevance of PEPT2 in the disposition of peptide-like drugs. Comparing wildtype and *Pept2* null mice, Shen et al. [12] found that PEPT2 was almost entirely responsible for the renal reabsorption of cefadroxil. Moreover, the CSF/blood concentration ratio was higher (6- to 7-fold) in the *Pept2* null mice, indicating the CSF-to-blood efflux function of PEPT2 at the BCSFB.

Intracerebral microdialysis is the only method *in vivo* that allows for the direct measurement of drug concentrations in ECF [20,21]. Microdialysis also has the advantage of enabling repeated sampling without fluid loss on freely moving animals. In our previous microdialysis study in rats [22], an attempt was made to investigate the role of PEPT2 on cefadroxil disposition in ECF and CSF by functional ablation of PEPT2 via competitive inhibition of cefadroxil transport using intraventricular infusion of the dipeptide Ala-Ala. Unfortunately, the results were negative, probably because of the biological instability of Ala-Ala *in vivo* [23]. Compared to inhibition studies, *Pept2* null mice may be a better tool to specifically study the effects of PEPT2 on cefadroxil brain distribution.

We hypothesize that PEPT2 ablation will impact the disposition of cefadroxil in the brain extracellular fluid (ECF), the site of action of many neuroactive agents. With this in mind, the primary objective of this study was to determine, using *in vivo* intracerebral microdialysis, the pharmacokinetics and regional brain distribution of cefadroxil in wildtype and *Pept2* null mice following a 4-h intravenous infusion. The secondary objective was to calculate the permeability-surface area product of cefadroxil in mice, as a measure of the impact of PEPT2 on drug transport from plasma to brain (i.e., influx clearance), following a 10-min intravenous infusion.

2. Material and methods

2.1. Chemicals

Cefadroxil and cefadroxil-D4 were purchased from Sigma-Aldrich (St. Louis, MO). [^{3}H]Cefadroxil (0.57 Ci/mmol; 97.6% purity) was obtained from Moravek Inc (Brea, CA) and [^{14}C]dextran 70,000 (1.4 mCi/g) was obtained from American Radiolabeled Chemicals (ARC, St. Louis, MD). Methanol and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of analytical grade or better. Ultrapure water was obtained using the Milli-Q Reference Water Purification System (Millipore, Billerica, MA). Perfusion fluid for microdialysis consisted of Ringer's solution, which contained 145 mM NaCl, 0.6 mM KCl, 1.0 mM $MgCl_2$, and 1.2 mM $CaCl_2$ in 2 mM phosphate buffer, pH 7.4.

2.2. Animals

Pept2 null mice (*Pept2*$^{-/-}$) with >99% C57BL/6 genetic background were developed previously in our laboratory [24]. Male wildtype (*Pept2*$^{+/+}$) and *Pept2* null mice (12–16 weeks) were bred in-house and maintained in a temperature- and humidity-controlled environment with 12-h light/dark cycles and unlimited access to food and water (Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI). All procedures in this study 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 and were approved by the University of Michigan Committee on Use and Care of Animals.

2.3. Animal surgery

Mice were placed on a heating pad to maintain body temperature and anesthetized by 2% isoflurane inhalation together with 0.5 L/min oxygen. Once fully anesthetized, a 3-fr polyurethane cannula (fused with a 2-fr polyurethane tip) was inserted into the right jugular vein for cefadroxil infusion and a 2-fr polyurethane cannula (fused with a 1-fr polyurethane tip) was inserted into the left carotid artery for blood sampling. To avoid clotting, a locking solution of 500 IU/mL heparin and 50% glycerol was used to fill the arterial catheter. The two vessel catheters were passed subcutaneously to the upper back of mice and then fixed to a silicone cup sutured to the skin.

Following catheter insertion, the mouse was placed on a stereotaxic frame equipped with an anesthesia mask (Stoelting, Wood Dale, IL). A guide cannula was implanted into the right brain striatum (coordinates: 0.6 mm anteroposterior, −1.8 mm lateral and −2.0 mm dorsoventral) and then fixed to the skull with two anchor screws and dental cement. A pre-emptive dose of buprenorphine (0.08 mg/kg) was administered subcutaneously and additional doses were given every eight hours for one day after surgery. The mouse was allowed to recover for 5–6 days prior to experimentation. One day before microdialysis, the mouse was moved to an infusion cage (Harvard Apparatus, MA, US) where it could move freely, and have access to food and water. At the same time, the dummy in the guide cannula was replaced by a CMA7 microdialysis probe containing a 2-mm cuprophane membrane and 6000 Dalton cut-off (CMA, Stockholm, Sweden).

2.4. Microdialysis study design

Microdialysis was initiated 1.5 h prior to drug infusion (i.e., stabilization period) and maintained during the infusion of cefadroxil. During this time, Ringer's solution was perfused through the microdialysis probe at 0.5 μL/min using a CMA 402 pump (Stockholm, Sweden). Cefadroxil (6 mg/mL in saline) was then infused intravenously at a constant rate of 0.15 mg/min/kg for 4 h using a Harvard Apparatus 22 pump (Holliston, MA, US). During the infusion, microdialysis samples were collected every 20 min using a CMA 142 microfraction collector (Stockholm, Sweden) and kept on ice at 4 °C. Blood samples (≈10 μL each) were harvested from the carotid artery at 5, 10, 20, 40, 60, 80, 100, 120, 180, and 240 min after initiating the intravenous drug infusion. Plasma was obtained by centrifuging the blood at 10,600*g* for 5 min. Before terminating the cefadroxil infusion, a combination of ketamine (120 mg/kg) and xylazine (10 mg/kg) was administered intraperitoneally to anesthetize the mouse so that CSF could be collected quickly from the cisterna magna. The mouse was then decapitated and the brain divided into left and right cortex (including hippocampus), left and right basal ganglia (including striatum), and cerebellum. Samples were weighed and stored at -80 °C until analysis (as were the microdialysis and plasma samples). If blood was observed in CSF or a hemorrhage was found in brain tissue, then those samples were discarded.

The CMA7 microdialysis probes were calibrated by determining the relative recovery of [^{3}H]cefadroxil in three mice using *in vivo*