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Research paper

Interaction of 31 β-lactam antibiotics with the H^+/peptide symporter PEPT2: analysis of affinity constants and comparison with PEPT1☆

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Abstract

The activity of the renal peptide transporters PEPT2 and PEPT1 determines—among other factors such as metabolic stability in liver and plasma—the circulatory half-life of penicillins and cephalosporins during therapy. This study was initiated to examine systematically the interaction of β-lactam antibiotics with PEPT2. Interaction of 31 cephalosporins and penicillins with the carrier protein was characterized by measuring their ability to inhibit the uptake of [^{14}C]Gly-Sar into renal SKPT cells. Cefadroxil, cefaclor, cyclacillin, cephradine, cephalexin and moxalactam were recognized by PEPT2 with very high affinity comparable to that of natural dipeptides (K_i = 3–100 μM). Ceftibuten, dicloxacillin, amoxicillin, metampicillin, cloxacillin, ampicillin, cefixime, cefamandole, oxacillin and cefmetazole interacted with PEPT2 with medium affinity (K_i = 0.1–5 mM). For the other β-lactam antibiotics studied interaction was very low or not measurable (K_i > 5 mM). The affinity constants of β-lactam antibiotics at rPEPT2 and hPEPT1 are significantly correlated, but the rank orders are not identical. Decisive differences between PEPT1 and PEPT2 recognition of the N-terminal part of the compounds became evident. Moreover, this large data set of affinity constants of β-lactam antibiotics will be useful for structure–transport (binding) analyses of PEPT2.
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Keywords: Peptide transport; β-lactam antibiotics; SKPT cells; Caco-2 cells; PEPT1 and PEPT2

1. Introduction

It is known for many years that the intestinal and renal H^+/peptide cotransporters PEPT1 and PEPT2 accept many cephalosporins and penicillins as substrates [1–5]. The recognition of certain β-lactam antibiotics by the carrier proteins is based on their sterical resemblance to the backbone of physiologically occurring tripeptides. In the kidney both PEPT1 and PEPT2 are expressed in the apical membrane of proximal tubular epithelial cells but in different segments [5,6]. They catalyze active transport of di- and tri-peptides and peptidomimetics such as β-lactam antibiotics from the primary filtrate back to the blood [5,7–14]. The activity of the renal peptide transporters determines—among other factors such as metabolic stability in liver and plasma—the circulatory half-life of penicillins and cephalosporins during therapy.

The human peptide transporters PEPT1 and PEPT2 exhibit only about 50% homology in amino acid sequence [5,10]. PEPT1, present in the epithelial layers of intestine, kidney and extrahepatic bile duct [5] is considered the 'low affinity, high capacity' type H^+/peptide symporter whereas in comparison PEPT2, present mainly in kidney, lung and central nervous system is the 'high affinity, low capacity' type carrier. This view is supported by many data obtained in studies during the last 20 years showing that the affinity constants of dipeptides and many peptidomimetics at both transport proteins differ by a factor of about 15. For example, the very often used reference substrate Gly-Sar displays a K_t value of about 0.8–1.3 mM

☆ Dedicated to the memory of Professor Martin Luckner.

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for PEPT1 and 50–70 μM for PEPT2 [9,13]. In direct comparison, for Ala-Lys K_i values of 0.21 mM at PEPT1 and 13.7 μM at PEPT2 have been measured [13]. Similar ratios were obtained for Ala-Ala, Ala-Asp, Tyr-Arg and many others [5,9,10]. Reviewing the literature, Rubio-Aliaga and Daniel summarize that at PEPT1 the affinity constants of substrates range from 200 to 10,000 μM and at PEPT2—measured under comparable experimental conditions—from 5 to 500 μM [5]. Exceptions are known.

It is generally assumed that β-lactam antibiotics that are substrates for PEPT1 are also substrates for PEPT2. Again, their affinity for PEPT2 is often 10–20-fold higher than for PEPT1. For example, for the prototypical aminocephalosporin cefadroxil Michaelis-Menten constants of 1.1 mM and 50 μM were determined at the heterologously expressed PEPT1 and PEPT2 proteins, respectively [5]. A very important observation, however, has been published 1995 [9] and confirmed by others [14]: The selectivity of PEPT1 and PEPT2 towards β-lactam antibiotics differs significantly. When we studied the substrate recognition pattern of PEPT1 and PEPT2 with cefadroxil and cyclacillin as model substrates for the peptide transporters constitutively expressed in Caco-2 cells (PEPT1) and SKPT cells (PEPT2), cyclacillin was 9-fold more potent than cefadroxil in competing with [^{14}C]Gly-Sar for uptake via PEPT1. Cefadroxil, however, was 14-fold more potent than cyclacillin in competing with the dipeptide for uptake via PEPT2 [9]. When evaluating such differences it has to be kept in mind that Caco-2 cells express human PEPT1 whereas SKPT cells express rat PEPT2 [9]. To rule out that the observed differences in substrate recognition of the peptide transporters between these two cell lines may be due to species differences rather than real differences between PEPT1 and PEPT2, the study was also performed at the cloned human PEPT1 and PEPT2 functionally expressed in HeLa cells and the same result was obtained. In another study, interaction of anionic cephalosporins with hPEPT1 and rPEPT2 was studied in Caco-2 cells and SKPT cells and the results were fully confirmed in experiments with the cloned human intestinal and renal peptide transporters and brush-border membrane vesicles [15]. Hence, the Caco-2/SKPT comparison is a well accepted procedure [9,13,15–17]. The main advantage of this approach is that these cells are tissue-specific epithelial cells expressing the native transporters with the necessary posttranslational modifications. In a previous study [18] we determined in the Caco-2 assay the affinity constants of 23 β-lactam antibiotics for PEPT1 and showed that the route of application for β-lactam antibiotics is mainly determined by their affinity to PEPT1. The data allowed conclusions regarding the structural requirements for β-lactam antibiotics to be recognized by PEPT1. Several studies have been published in recent years comparing 3–10 β-lactam antibiotics with regard to their affinity for PEPT1 and PEPT2 [9,12,14,15,19]. Ideally, the prerequisites for an analysis of structural requirements are (i) a sufficient number of different structures, (ii) determination of inhibition constants reflecting direct single step carrier-compound interaction (and not the transport process) and (iii) a broad range of K_i values measured under identical experimental conditions [20]. The current investigation in SKPT cells was performed to study in a systematic approach the structure–interaction relationship of 31 β-lactam antibiotics and precursors by measuring their affinity to native PEPT2.

2. Material and methods

2.1. Materials

SKPT-0193 Cl.2 cells established from isolated cells of rat renal proximal tubules were provided by U. Hopfer (Case Western Reserve University, Cleveland, OH, USA). The colon carcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell culture media and supplements were purchased from Life Technologies, Inc. (Germany). [Glycine-1-^{14}C]glycylsarcosine (specific radioactivity 52 mCi/mmol) was obtained from Amersham International (UK). Cefadroxil, cefamandole, cephradine, cefaclor, cephalotin, cephalexin, ampicillin, cefapirin, cefmetazole, benzylpenicillin, ceftriaxone, cephaloridine, cefuroxime, cefapirin and cefsulodin were purchased from Sigma (Germany) or ICN (Germany). Cefotaxime, cefodizime and cefpirome were from Hoechst AG (Germany). Cyclacillin, cefixime and ceftibuten were generous gifts from F.H. Leibach (Medical College of Georgia, USA). Cefuroxime-axetil and ceftazidime were gifts from Glaxo Welcome and GlaxoSmithCline, respectively (Germany). Cefepime was from Bristol-Myers Squibb (Germany). All other chemicals were of analytical grade.

2.2. Cell culture

SKPT cells (passage numbers 42–74) were cultured in Dulbecco's Modified Eagle Medium:Nutrient Mixture F12 (Ham) 1:1 and L-glutamine, fetal bovine serum, recombinant insulin, epidermal growth factor, apotransferrin, dexamethasone and gentamicin as described [9,13,16]. Subconfluent cultures were treated 5 min with Dulbecco's phosphate-buffered saline followed by a 2-min incubation with 0.25% trypsin solution. The cells were seeded in 35-mm disposable petri dishes (Becton Dickinson, UK) at a density of 0.8×10^6 cells per dish. The cultures reached confluence within 20 h. Uptake was measured 4 days after seeding [9,13,16]. Caco-2 cells were routinely cultured in Minimum Essential Medium supplemented with 10% fetal bovine serum, gentamicin (45 μg/ml) and 1% nonessential amino acid solution [9,13,18,21]. The uptake measurements were performed on the 6th day after confluence.

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