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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.

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

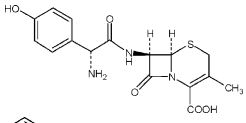
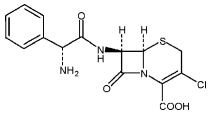
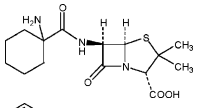
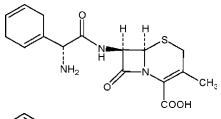
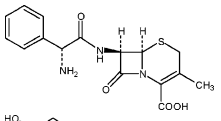
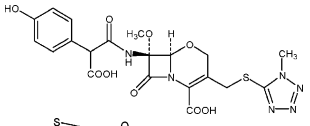
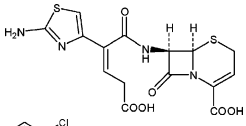
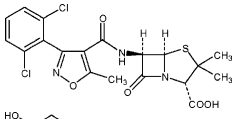
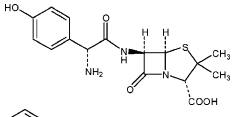
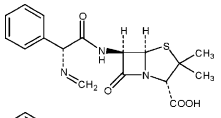
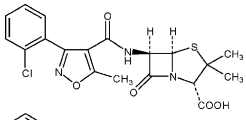
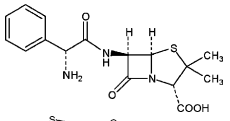
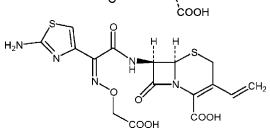
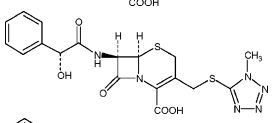
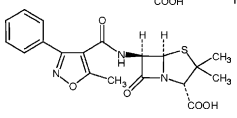
2.1. Materials

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

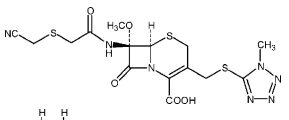
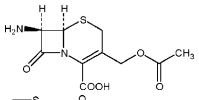
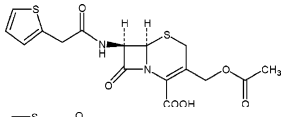
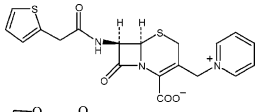
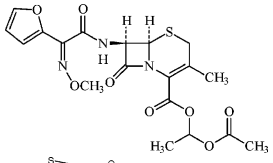
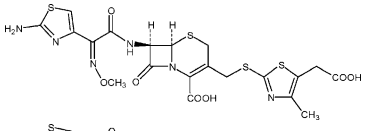
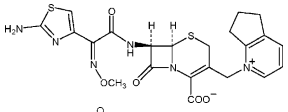
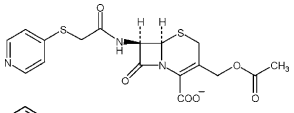
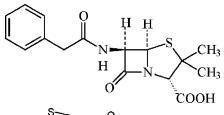
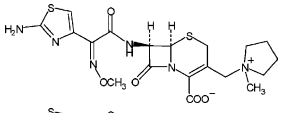
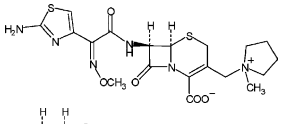
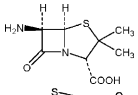
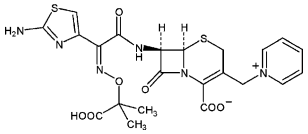
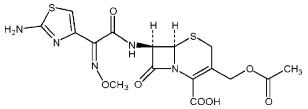
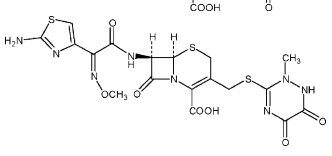
SKPT cells (passage numbers 42–74) were cultured in Dulbecco's Modified Eagle Medium:Nutrient Mixture F12 (Ham) 1:1 and L-glutamine, fetal bovine serum, recombinant insulin, epidermal growth factor, apotransferrin, dexamethasone and gentamicin as described [9,13,16]. Subconfluent cultures were treated 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.

Table 1
Affinity constants of 31 β-lactam antibiotics and precursors at rPEPT2 measured in SKPT cells and hPEPT1 measured in Caco-2 cells

Substance	Structure	$K_{i\ PEPT2}$ (mM)	$Rank_{K_i}$ at PEPT2	$K_{i\ PEPT1}$ (mM)	$Rank_{K_i}$ at PEPT1	$K_{i\ PEPT1}/K_{i\ PEPT2}$
Cefadroxil		0.003 ± 0.001	1	7.2 ± 0.8[a]	4	2400
Cefaclor		0.029 ± 0.004	2	> 10(≈ 11)[a,b]	8	380
Cyclacillin		0.044 ± 0.013	3	0.50 ± 0.05[a]	2	11
Cephradine		0.065 ± 0.006	4	9.8 ± 1.2[a]	7	150
Cephalexin		0.075 ± 0.017	5	14.4 ± 2.4[a]	15	190
Moxalactam		0.087 ± 0.016	6	12.3 ± 0.6	12	140
Ceftibuten		0.28 ± 0.01	7	0.34 ± 0.03[a]	1	1
Dicloxacillin		0.42 ± 0.03	8	7.2 ± 0.9	5	17
Amoxicillin		0.43 ± 0.02	9	> 10 (≈ 25)[b]	20	60
Metampicillin		0.73 ± 0.13	10	12.7 ± 1.1	13	17
Cloxacillin		0.95 ± 0.17	11	3.0 ± 1.0	3	3
Ampicillin		1.3 ± 0.2	12	14.5 ± 1.7[a]	16	11
Cefixime		2.6 ± 1.9	13	12 ± 2[a]	10	5
Cefamandole		2.8 ± 1.1	14	8.1 ± 0.8[a]	6	3
Oxacillin		3.3 ± 0.9	15	12.0 ± 0.6	11	4

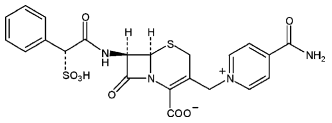
(*continued on next page*)

Table 1 (*continued*)

Substance	Structure	$K_{i\ PEPT2}$ (mM)	Rank$_{K_i}$ at PEPT2	$K_{i\ PEPT1}$ (mM)	Rank$_{K_i}$ at PEPT1	$K_{i\ PEPT1}/K_{i\ PEPT2}$
Cefmetazole		4.3±0.9	16	28±3[a]	22	7
7-Aminocephalosporanic acid		4.9±1.8	17	>10(≈15)[b]	17	3
Cephalothin		8.3±1.1	18	>10 (≈14)[a,b]	14	2
Cephaloridine		8.5±0.8	19	>30 (≈100)[a,b]	30	12
Cefuroxime-Axetil		>3 (≈9)	20	>5 (≈12)[a,b]	9	1
Cefodizime		9.0±2.9	21	22±3[a]	19	2
Cefpirome		9.5±3.1	22	>30 (≈45)[a,b]	26	5
Cephapirin		>10 (≈11)[b]	23	>10 (≈20)[a,b]	18	2
Benzylpenicillin		11.1±3.7	24	>30 (≈40)[a,b]	24	4
Cefepime		11.4±1.0	25	>30 (≈70)[a,b]	29	6
Cefuroxime		12.6±5.4	26	26±4[a]	21	2
6-Amino-penicillanic acid		19±12	27	>30 (≈50)[b]	27	3
Ceftazidime		>10 (≈18)[b]	28	>10 (≈40)[a,b]	23	2
Cefotaxime		20±10	29	>30 (≈50)[a,b]	28	3
Ceftriaxone		>20 (≈28)[b]	30	>30 (≈40)[a,b]	25	1

(*continued on next page*)

Table 1 (*continued*)

Substance	Structure	$K_{i\ PEPT2}$ (mM)	$Rank_{K_i}$ at PEPT2	$K_{i\ PEPT1}$ (mM)	$Rank_{K_i}$ at PEPT1	$K_{i\ PEPT1}/K_{i\ PEPT2}$
Cefsulodin		>30 (≈55)[b]	31	>30 (≈150)[a,b]	31	3

K_i-values of [^{14}C]Gly-Sar uptake inhibition were calculated from inhibition curves shown in Fig. 1 A–F. $n=4$.

[a] From Ref. [18].

[b] K_i-values extrapolated beyond measurement range because of limited solubility of compounds or low inhibition. See Fig. 1A–F. (this study) and Fig. 1A and B in Ref. [18] for maximal substrate concentration used.

2.3. Transport studies

Uptake of [^{14}C]Gly-Sar was initiated after washing the cells for 30 s by adding 1 ml of uptake medium [9,13,16,18,21]. The buffer contained 25 mM Mes/Tris (pH 6.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, 10 μM [^{14}C]Gly-Sar and unlabeled β-lactam antibiotics or dipeptides (0–31.6 mM or concentration of maximal solubility, respectively). If necessary, the pH of the solutions was corrected before preparing the required dilutions. After incubation for 10 min, the cells were quickly washed with ice-cold buffer, solubilized in 1 ml of Igepal® Ca-630 solution (0.5% v/v, Sigma Aldrich Chemie, Germany) and prepared for liquid scintillation spectrometry. Protein was determined according to the method of Bradford. The non-saturable component of [^{14}C]Gly-Sar uptake (diffusion, adherent radioactivity: 11% in Caco-2 cells, 5% in SKPT cells) was determined by measuring the uptake of [^{14}C]Gly-Sar in the presence of excess amount of unlabeled Gly-Sar (SKPT: 10 mM, Caco-2: 31.6 mM).

2.4. Data analysis

Experiments were done in duplicate and each experiment was repeated two to three times. Results are given as means ± SE. IC_{50} values (i.e. concentration of the unlabeled compound necessary to inhibit 50% of carrier-mediated [^{14}C]Gly-Sar uptake) were determined by non-linear regression [18,21]. Inhibition constants (K_i) were calculated from IC_{50} values using K_t values of Gly-Sar uptake of 112 μM (SKPT, this study) and 0.83 mM (Caco-2, Ref. 21).

3. Results and discussion

SKPT cells are currently the best native cell culture model for PEPT2 studies available [9,15–17]. There is no human kidney cell line known to express PEPT2 but the results obtained in SKPT cells are very often confirmed using the cloned human PEPT2 functionally expressed in HeLa and other cells [9,15]. In the present study, we first confirmed kinetic constants of substrate uptake under the current experimental conditions. The uptake of the hydrolytically stable reference dipeptide Gly-Sar into confluent monolayers of SKPT cells is driven uphill by an inwardly directed proton gradient and mediated by a single transport system, PEPT2 [9,15–17]. The Gly-Sar uptake was characterized by kinetic parameters of K_t (Michaelis-Menten constant) = 112 ± 4 μM and V_{max} (maximal velocity of transport) = 2.7 ± 0.03 nmol/(10 min per mg pf protein). Moreover, we determined the inhibiton constant (K_i) of alanylalanine vs. [^{14}C]Gly-Sar uptake as a measure for its affinity to PEPT2. The kinetic constants of Gly-Sar uptake and the K_i value of alanylalanine of 6 ± 2 μM agree very well with results obtained earlier [9,13,16].

We then applied the competition assay to determine the affinity constants of 31 β-lactam antibiotics and precursors for PEPT2. The chemical structures of the compounds used are given in Table 1. The inhibition curves of [^{14}C]Gly-Sar uptake are shown in Fig. 1A–F. From these curves, the K_i values listed in Table 1 were derived by non-linear regression analysis. Compounds in the table were sorted according to decreasing affinity (column $Rank_{K_i}$ at PEPT2). Cefadroxil, cefaclor, cyclacillin, cephradine, cephalexin and moxalactam were recognized by PEPT2 with affinity constants comparable to those of natural dipeptides (K_i = 3–100 μM). Ceftibuten, dicloxacillin, amoxicillin, metampicillin, cloxacillin, ampicillin, cefixime, cefamandole, oxacillin, cefmetazole and 7-aminocephalosporanic acid interacted with PEPT2 with affinity constants (K_i) between 0.1 and 5 mM. For the other β-lactam antibiotics studied—among them the precursor 6-aminopenicillanic acid—the interaction was very low or not measurable (K_i > 5 mM).

At PEPT1 in Caco-2 cells we consider affinity constants for substrates or inhibitors lower than 0.5 mM as high affinity, between 0.5 and 5 mM as medium affinity and above 5 mM as low affinity. Values above 15 mM we consider with great caution [for review see Ref. 22]. Concluding from the present study, we suggest the following classification of PEPT2 substrates and/or inhibitors: (i) affinity constants lower than 0.1 mM as high affinity, (ii) constants between 0.1 and 1 mM as medium affinity and (iii) affinity constants above 1 mM as low

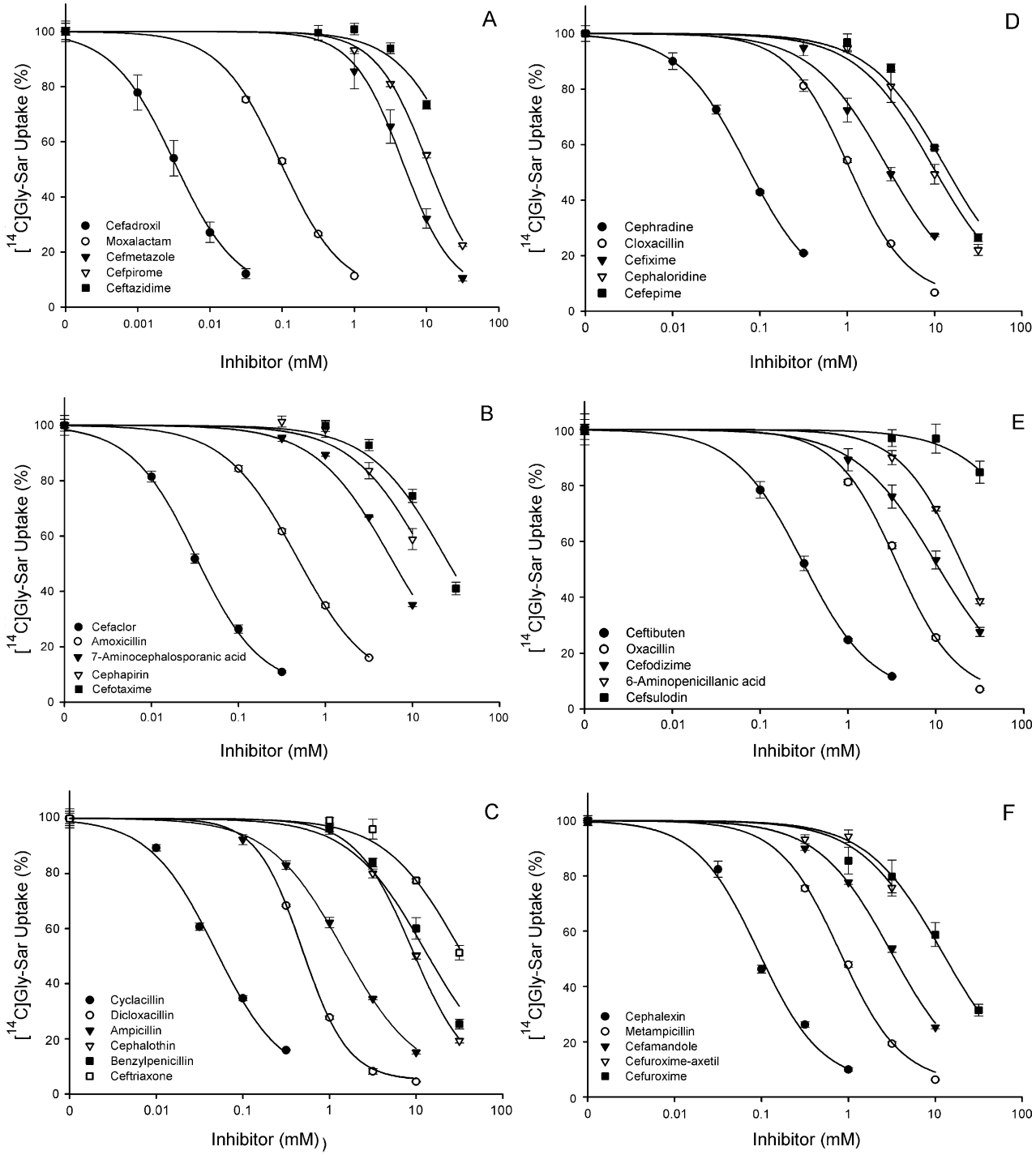


Fig. 1. (A–F) Inhibition of [^{14}C]Gly-Sar uptake by β-lactam antibiotics in SKPT cells. Uptake of 10 μM [^{14}C]Gly-Sar was measured for 10 min in monolayer cultures at pH 6.0 in the absence and presence of increasing concentrations of the substrates (0–31.6 mM or concentration of maximal solubility, respectively). Uptake of [^{14}C]Gly-Sar measured in the absence of the inhibitors was taken as 100%. Data are shown as means ± SEM, $n = 4$.

affinity. Compounds with affinity constants above 5 mM should not be considered as PEPT2 ligands.

In continuation of a previous study [18] the affinity constants of eight new compounds at PEPT1 in Caco-2 cells were measured (Table 1). Cloxacillin, moxalactam, dicloxacillin, metampicillin and oxacillin interacted with PEPT1 with medium to low affinity (3–14 mM). In contrast, for amoxicillin and the precursors 7-aminocephalosporanic acid and 6-aminopenicillanic acid no interaction with PEPT1 was found ($K_i > 14$ mM).

Applying the classification defined above, at first sight PEPT2 has very similar structural requirements as PEPT1. Those β-lactam antibiotics that are recognized by PEPT2 with high or medium affinity are also recognized by PEPT1

(Table 1). An exception is amoxicillin that displays a medium affinity to PEPT2 but in our study no affinity to PEPT1. Vice versa, we found no compound that is a substrate for PEPT1 but not for PEPT2. To a large extent there is a significant correlation of affinity constants. Decisive differences in compound recognition become evident when one compares the rank order and the distribution of affinity constants at SKPT and Caco-2 cells. The rank ordering shown in Table 1 ignores the fact that for the overwhelming majority of compounds PEPT2 displays as expected a higher affinity than PEPT1 but it discloses for particular compounds a differential recognition by the carriers. As stated above, when evaluating such differences it has to be noted that Caco-2 cells express human PEPT1 whereas SKPT cells express rat PEPT2 [9]. But, even for the most striking differences, for example the reversed preference of cefadroxil and cyclacillin by PEPT1 and PEPT2, species differences could be ruled out repeatedly [9,14,15]. To analyze this differential recognition further, the $K_{i\ PEPT1}$:$K_{i\ PEPT2}$ quotients were calculated (Table 1). Most studies of the last 20 years so far described a 3:1–20:1 ratio of affinity constants, i.e. the affinity of substrates is usually 3–20 times higher for PEPT2 than for PEPT1. Indeed, of the 31 compounds studied here, 17 compounds have a $K_{i\ PEPT1}$:$K_{i\ PEPT2}$ ratio between 3 and 20. For five cephalosporins (cefadroxil, cefaclor, cephradine, cephalexin and moxalactam), however, the quotients are >100, for cefadroxil even 2400. These compounds display a disproportionally high affinity for PEPT2. For the penicillin amoxicillin the ratio is 60 conforming an earlier estimate of 72 obtained at heterologously expressed hPEPT1 and hPEPT2 [12]. Looking at the structures, the most striking characteristic is that with the exception of moxalactam they all possess an α-amino group as the classical R_2-residue. This is a structural feature common for many high affinity substrates for both PEPT1 and PEPT2. Of 31 compounds tested, ceftibuten, however, a compound without an α-amino group displays by far the highest affinity for PEPT1 but occupies only rank seven at PEPT2 in the present study. This result supports hypotheses whereby PEPT2 has a disproportionally higher affinity for β-lactam antibiotics carrying an α-amino group than PEPT1 [11,12]. But, in addition to that, it is most interesting that cefadroxil and amoxicillin, the cephalosporin and the penicillin with the highest $K_{i\ PEPT1}$:$K_{i\ PEPT2}$ ratio, both possess not only the α-amino group but also a hydroxyl group at the N-terminal phenyl ring. This moiety increases the affinity for both carriers further, but again disproportionally more for PEPT2. Moxalactam is a high affinity substrate for PEPT2 but a low affinity substrate for PEPT1 and it does not possess an α-amino group but the hydroxyl group at the phenyl ring. In other words, the major difference between the PEPT2 and PEPT1 substrate recognition sites should be manifested in that part of the PEPT2 binding region that interacts with the N-terminal part of the β-lactam. Daniel and Adibi had reported earlier that the marked hydrophobicity of the N-terminal region of aminopenicillins increases the affinity to the renal H^+/peptide cotransporter [7]. Terada et al. [23] suggested that the α-amino group of β-lactam antibiotics interacts with histidine residues of PEPT1 and PEPT2 that may be involved in substrate recognition by peptide transporters. The underlying molecular mechanism could be that the imidazole group of the histidine residue is protonated by the α-amino group. Very recently, in a novel mutagenesis approach Uchiyama and coworkers provided direct evidence that His57 is a principal proton-binding site in hPEPT1 [24]. In hPEPT2, His87 was found to be absolutely essential for the transport activity [25]. β-lactam antibiotics that do not possess an α-amino group but are also transported might interact with the binding site of the transporter other than the α-amino group-histidine interaction. At this point, PEPT2 seems to be much more restrictive than PEPT1.

In summary, we present a large data set of affinity constants of β-lactam antibiotics at PEPT2 that was obtained under identical experimental conditions and covers a broad range of affinity constants and new structures. The inhibition constants reflect the direct single step carrier-compound interaction of β-lactam antibiotics with the binding site of PEPT2 and will therefore be useful for structure–function studies such as molecular modeling and for the development of new β-lactam antibiotics and related peptidomimetics. Since PEPT2 is also expressed in lung, mammary gland and central nervous system the data are also relevant for the accessibility of these tissues by β-lactam antibiotics.

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References

[1] K.-I. Inui, T. Okano, M. Takano, H. Saito, R. Hori, Carrier-mediated transport of cephalexin via the dipeptide transport system in rat renal brush-border membrane vesicles, Biochim. Biophys. Acta 769 (1984) 449–454.
[2] E. Nakashima, A. Tsuji, H. Mizuo, T. Yamana, Kinetics and mechanism of in vitro uptake of amino-β-lactam antibiotics by rat small intestine and relation to the intact-peptide transport system, Biochem. Pharmacol. 33 (1984) 3345–3352.
[3] T. Okano, K.-I. Inui, H. Maegawa, M. Takano, R. Hori, H^+-Coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes, J. Biol. Chem. 261 (1986) 14130–14134.
[4] K.-I. Inui, S. Masuda, H. Saito, Cellular and molecular aspects of drug transport in the kidney, Kidney Int. 58 (2000) 944–958.
[5] I. Rubio-Aliaga, H. Daniel, Mammalian peptide transporters as targets for drug delivery, TIPS 23 (2002) 434–440.

[6] H. Shen, D.E. Smith, T. Yang, Y.G. Huang, J.B. Schnermann, F.C. Brosius III, Localization of PEPT1 and PEPT2 proton-coupled oligopeptide transporter mRNA and protein in rat kidney, Am. J. Physiol. 276 (1999) F658–F665.

[7] H. Daniel, S.A. Adibi, Transport of β-lactam antibiotics in kidney brush border membrane. Determinants of their affinity for the oligopeptide/H^+ symporter, J. Clin. Invest. 92 (1993) 2215–2223.

[8] M. Ries, U. Wenzel, H. Daniel, Transport of cefadroxil in rat kidney brush-border membranes is mediated by two electrogenic H^+-coupled systems, J. Pharmacol. Exp. Ther. 271 (1994) 1327–1333.

[9] M.E. Ganapathy, M. Brandsch, P.D. Prasad, V. Ganapathy, F.H. Leibach, Differential recognition of β-lactam antibiotics by intestinal and renal peptide transporters, PEPT 1 and PEPT 2, J. Biol. Chem. 270 (1995) 25672–25677.

[10] S. Ramamoorthy, W. Liu, Y.Y. Ma, T.L. Yang-Feng, V. Ganapathy, F.H. Leibach, Proton/peptide cotransporter (PEPT 2) from human kidney: functional characterization and chromosomal localization, Biochim. Biophys. Acta 1240 (1995) 1–4.

[11] M. Boll, M. Herget, M. Wagener, W.M. Weber, D. Markovich, J. Biber, W. Clauss, H. Murer, H. Daniel, Expression cloning and functional characterization of the kidney cortex high-affinity proton-coupled peptide transporter, Proc. Natl Acad. Sci. USA 93 (1996) 284–289.

[12] T. Terada, H. Saito, M. Mukai, K.-I. Inui, Recognition of β-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK_1 cells, Am. J. Physiol. 273 (1997) F706–F711.

[13] M. Brandsch, C. Brandsch, M.E. Ganapathy, C.S. Chew, V. Ganapathy, F.H. Leibach, Influence of proton and essential histidyl residues on the transport kinetics of the H^+/peptide cotransport systems in intestine (PEPT 1) and kidney (PEPT 2), Biochim. Biophys. Acta 1324 (1997) 251–262.

[14] K. Takahashi, N. Nakamura, T. Terada, T. Okano, T. Futami, H. Saito, K.-I. Inui, Interaction of β-lactam antibiotics with H^+/peptide cotransporters in rat renal brush-border membranes, J. Pharmacol. Exp. Ther. 286 (1998) 1037–1042.

[15] M.E. Ganapathy, P.D. Prasad, B. Mackenzie, V. Ganapathy, F.H. Leibach, Interaction of anionic cephalosporins with the intestinal and renal peptide transporters PEPT 1 and PEPT 2, Biochim. Biophys. Acta 1324 (1997) 296–308.

[16] M. Brandsch, C. Brandsch, P.D. Prasad, V. Ganapathy, U. Hopfer, F.H. Leibach, Identification of a renal cell line that constitutively expresses the kidney-specific high-affinity H^+/peptide cotransporter, Fed. Am. Soc. Exp. Biol. J. 9 (1995) 1489–1496.

[17] C. Shu, H. Shen, U. Hopfer, D.E. Smith, 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 (2001) 1307–1315.

[18] B. Bretschneider, M. Brandsch, R. Neubert, Intestinal transport of β-lactam antibiotics: analysis of the affinity at the H^+/peptide symporter (PEPT1), the uptake into Caco-2 cell monolayers and the transepithelial flux, Pharm. Res. 16 (1999) 55–61.

[19] K.-I. Inui, T. Terada, S. Masuda, H. Saito, Physiological and pharmacological implications of peptide transporters, PEPT1 and PEPT2, Nephrol. Dial. Transplant. 15 (2000) 11–13.

[20] S. Gebauer, I. Knütter, B. Hartrodt, M. Brandsch, K. Neubert, I. Thondorf, Three-dimensional quantitative structure–activity relationship (3D QSAR) analyses of peptide substrates of the mammalian H^+/peptide cotransporter PEPT1, J. Med. Chem. 46 (2003) 5725–5734.

[21] M. Brandsch, I. Knütter, F. Thunecke, B. Hartrodt, I. Born, V. Börner, F. Hirche, G. Fischer, K. Neubert, Decisive structural determinants for the interaction of proline derivatives with the intestinal H^+/peptide symporter, Eur. J. Biochem. 266 (1999) 502–508.

[22] M. Brandsch, I. Knütter, F.H. Leibach, The intestinal H^+/peptide symporter PEPT1: structure-affinity relationships, Eur. J. Pharm. Sci. 21 (2004) 53–60.

[23] T. Terada, H. Saito, K.-I. Inui, Interaction of β-lactam antibiotics with histidine residue of rat H^+/peptide cotransporters, PEPT1 and PEPT2, J. Biol. Chem. 273 (1998) 5582–5585.

[24] T. Uchiyama, A.A. Kulkarni, D.L. Davies, V.H. Lee, Biophysical evidence for His57 as a proton-binding site in the mammalian intestinal transporter hPepT1, Pharm. Res. 20 (2003) 1911–1916.

[25] Y.J. Fei, W. Liu, P.D. Prasad, R. Kekuda, T.G. Oblak, V. Ganapathy, F.H. Leibach, Identification of the histidyl residue obligatory for the catalytic activity of the human H^+/peptide cotransporters PEPT1 and PEPT2, Biochemistry 36 (1997) 452–460.