JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 543–585
© 2008 The Authors
Received December 4, 2007
Accepted February 15, 2008
DOI 10.1211/jpp.60.5.0002
ISSN 0022-3573

Pharmaceutical and pharmacological importance of peptide transporters

Matthias Brandsch, Ilka Knütter and Eva Bosse-Doenecke

Abstract

Peptide transport is currently a prominent topic in membrane research. The transport proteins involved are under intense investigation because of their physiological importance in protein absorption and also because peptide transporters are possible vehicles for drug delivery. Moreover, in many tissues peptide carriers transduce peptidic signals across membranes that are relevant in information processing. The focus of this review is on the pharmaceutical relevance of the human peptide transporters PEPT1 and PEPT2. In addition to their physiological substrates, both carriers transport many β-lactam antibiotics, valaciclovir and other drugs and prodrugs because of their sterical resemblance to di- and tripeptides. The primary structure, tissue distribution and substrate specificity of PEPT1 and PEPT2 have been well characterized. However, there is a dearth of knowledge on the substrate binding sites and the three-dimensional structure of these proteins. Until this pivotal information becomes available by X-ray crystallography, the development of new drug substrates relies on classical transport studies combined with molecular modelling. In more than thirty years of research, data on the interaction of well over 700 di- and tripeptides, amino acid and peptide derivatives, drugs and prodrugs with peptide transporters have been gathered. The aim of this review is to put the reports on peptide transporter-mediated drug uptake into perspective. We also review the current knowledge on pharmacogenomics and clinical relevance of human peptide transporters. Finally, the reader's attention is drawn to other known or proposed human peptide-transporting proteins.

Introduction

In metazoic organisms, specialized epithelial barriers separate fluid-filled compartments from each other. They restrict and regulate the flux of substances in both directions. In general, the transfer of all substances, from H^+ ions to the largest proteins, across these barriers can occur via paracellular or transcellular routes. The paracellular pathway is very often restricted by tight cell junctions, and the ability of substances to cross epithelia between the cells by simple diffusion depends mainly on their size. The transcellular route requires transport across two morphologically and functionally different cell membranes (e.g. the apical and the basolateral membrane). The extent of simple diffusion of substances across these membranes depends on their size, charge and lipophilicity. Large proteins are translocated across cell layers mainly by specialized transcytotic processes involving membrane invagination and vesicle internalization. For most smaller inorganic and organic solutes, more or less specific transport systems, called carriers or transporters, exist in the membrane of all living cells.

What do we know about the transport of peptides? Historically, the first authors reporting on peptide transport were chemists and physicians working in the field of intestinal protein digestion and absorption. They realized that the end products of intestinal protein digestion are a mixture of free amino acids and small peptides contacting the physical, enzymatic and immunologic barrier of the small intestinal epithelium. At the beginning of the 20th century, O. Cohnheim discovered erepsin (Cohnheim 1901) which, as we know today, is a mixture of brush-border peptidases and soluble peptidases released from the cytosol of the enterocyte. E. Abderhalden discussed the question of whether proteins are absorbed intact or broken down to the level of amino acids (Abderhalden 1911). He did not yet postulate the intestinal absorption of intact small peptides, but he did not rule it out either. He measured, for example, the resistance of various dipeptides against erepsin

Membrane Transport Group, Biozentrum of the Martin-Luther-University Halle-Wittenberg, D-06120 Halle, Germany

Matthias Brandsch, Ilka Knütter

Institute of Biochemistry/ Biotechnology, Faculty of Science I, Martin-Luther-University Halle-Wittenberg, D-06120 Halle, Germany

Eva Bosse-Doenecke

Correspondence: M. Brandsch, Biozentrum of the Martin-Luther-University Halle-Wittenberg, Membrane Transport Group, Weinbergweg 22, D-06120 Halle, Germany. E-mail: matthias.brandsch@biozentrum.uni-halle.de

Funding: This work was funded by the Excellence Initiative of the State Saxony-Anhalt.

hydrolysis and speculated about the maximal size of a peptide that could be absorbed in the gut without potentially triggering an antigenic response (Abderhalden 1911).

Many interesting results and ideas of that early period unfortunately fell by the wayside. New technologies allowing quantification of free amino acids but not peptides in body fluids were introduced. A period of fast discovery of many different amino acid transport systems started in the 1940s and supported the view that all proteins are digested to their constituent amino acids and that only these amino acids are taken up into cells. This dogma prevailed for many more years even though in the late 1950s intestinal peptide transport as a discipline was rediscovered (Newey & Smyth 1959). In the 1960s and 1970s the appearance of hydrolysis-resistant dipeptides at the abluminal side of the intestinal and also the renal epithelium was demonstrated. The uptake of these hydrolysis-resistant peptides into tissue and cell preparations was shown to be saturable (i.e. carrier mediated). It followed the observation that the uptake was uphill (i.e. concentrative). Most importantly, it was observed that the capacity of intestinal amino acid uptake is greater from a solution of dipeptides than from mixtures of the respective free amino acids, a finding that had tremendous nutritional and clinical significance (Steinhardt & Adibi 1986). The pioneers in the field of intestinal and renal peptide transport were D. M. Matthews (Addison et al 1972, 1975; Matthews 1975), A. M. Ugolev (Ugolev 1971), A. N. Radhakrishnan (Hellier et al 1976; Ganapathy & Radhakrishnan 1979), S. A. Adibi (Adibi & Soleimanpour 1974), F. H. Leibach and V. Ganapathy (Ganapathy et al 1980, 1981, 1984; Ganapathy & Leibach 1983) and several others. Readers interested in the history of the field will certainly enjoy the book written by D. M. Matthews (Matthews 1991).

Today we know that, at the intestinal epithelium, di- and tripeptides are transported from the lumen into the enterocytes by the H^+/peptide cotransporter PEPT1 (peptide transporter 1). At the renal epithelium, small peptides are reabsorbed from the glomerular filtrate into the cells by PEPT2 (peptide transporter 2) and by PEPT1. Cytosolic peptidases rapidly hydrolyse most of the di- and tripeptides entering the cells. Peptides resistant to cytosolic peptidases may be transported intact across the basolateral membrane of intestinal and renal cells by a peptide transport system that has been characterized so far only on a functional level.

What is the pharmaceutical relevance of these peptide transporters? In the 1970s and 1980s it was realized that compounds bearing sterical resemblance to the backbone of physiologically occurring di- and tripeptides can be recognized and even transported by peptide transporters. To the best of our knowledge, the first evidence for this phenomenon was reported by J. F. Quay in two abstracts published in *The Physiologist* (Quay & Foster 1970; Quay 1972). The authors showed interaction of Phe-Gly with cephalexin absorption in rat jejunum. Since then it has been well established that it is the activity of PEPT1 at the intestinal epithelium that allows the effective oral bioavailability of such important drugs as cefadroxil, cyclacillin or valaciclovir. Pharmaceutical research in industry and academia now looks on peptide transporters as promising vehicles for drug delivery.

This review will summarize the pharmaceutical and clinical relevance of peptide transporters, recent reports on polymorphisms and data obtained studying knockout animal models. Furthermore, even though the intestinal and renal H^+/peptide cotransporters PEPT1 and PEPT2 are the best characterized and possibly the pharmaceutically most relevant systems, there are several other mammalian peptide transport systems that deserve attention. We will summarize recent results on specific peptide transport at the blood–brain barrier, on the novel Na^+- and Cl^--coupled transport system for opioid peptides and on solute carriers that are known for other prototypical substrates but transport peptides in significant amounts.

Driving forces of intestinal and renal peptide transport

History of peptide transport has shown exemplarily that, for the identification of the driving force of a transport process, the tissue preparation and the uptake technique can be crucial. Today, most reports on mechanism, specificity and regulation of peptide transport are based on cell culture work and on heterologous expression of carriers in *Xenopus laevis* oocytes, mammalian cells (HeLa, LLC-PK_1, CHO, MDCK) and the yeast *Pichia pastoris*. Early studies on peptide transport were performed in feeding or perfusion experiments in-vivo and in-situ. Tissue, cell and membrane preparations, such as the everted sac or ring technique, the Ussing chamber technique and brush-border membrane vesicles, have been used for at least 50 years and are still being used today. It was the use of isolated and purified brush-border membrane vesicles that made it possible to resolve the long-standing argument over the energy source for active peptide transport. This methodology emerged in the late 1960s and was perhaps the most influential technique in membrane transport until the cloning and electrophysiology era. Using such membrane vesicles and radiolabelled Gly-Pro as a substrate, V. Ganapathy and F. H. Leibach discovered that the renal and intestinal dipeptide uptake is driven by an inwardly directed H^+ gradient (Ganapathy et al 1981; Ganapathy & Leibach 1983). The other groups working on peptide transport in the 1970s usually employed an outside pH of 7.5 in their uptake experiments because it was believed at the time that the pH of the intestinal tract would be similar to the pH of plasma (Gray & Dressman 1996). Since such conditions were not associated with transmembrane H^+ gradient, these investigators were unable to observe uphill uptake of peptides.

Ganapathy & Leibach (1983) proposed the model for tertiary-active peptide uptake at intestinal and renal epithelial cells as illustrated in Figure 1. Apical peptide transporters mediate electrogenic uphill transport of their substrates into the cells. The transport is energized by a transmembrane electrochemical H^+ gradient directed from outside to inside. This inwardly directed H^+ gradient is a physiological phenomenon (Said et al 1986). At the polarized epithelia of intestine and kidney there is an acidic microclimate. Values obtained for intervillous pH at the jejunum are in the range of 6.1 (Lucas 1983; McEwan et al 1988) to 6.6 (Daniel et al 1989), whereas the intracellular pH of enterocytes is usually 7.3. The acidic microclimate on the luminal surface of the intestinal epithelium

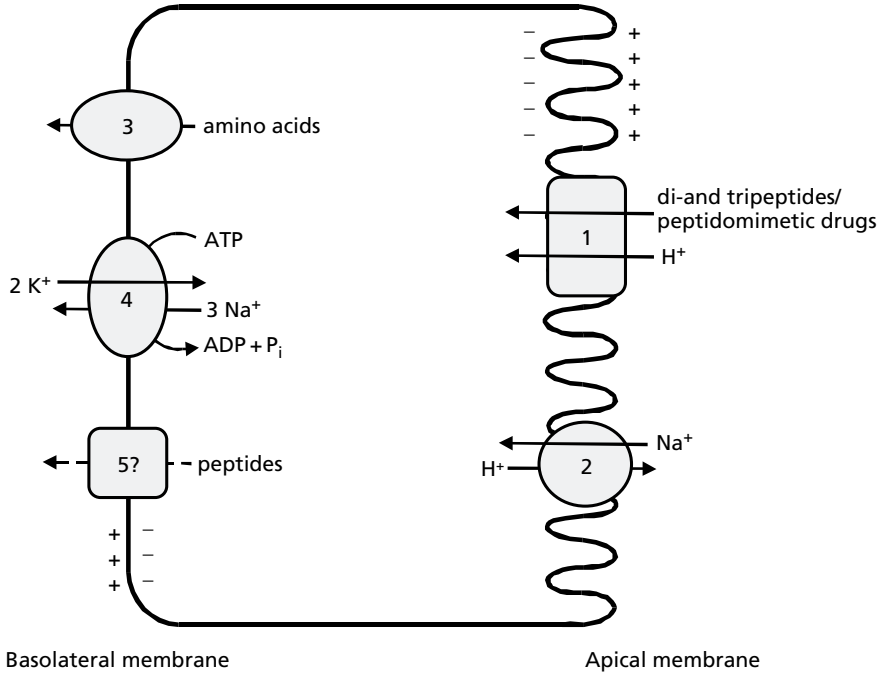


Figure 1 Interplay of translocators involved in intestinal or renal transepithelial absorption of peptides. Apical membrane: 1, PEPT1/2; 2, Na^+/H^+ antiporter. Basolateral membrane: 3, approximately nine different amino acid transporters; 4, Na^+-K^+ ATPase; 5, putative peptide transporter.

is highly relevant not only for all H^+-dependent carriers and channels but also for absorption profiles of non-carrier-mediated dissociable drugs (Lucas 1983; Gray & Dressman 1996). According to the model, this H^+ gradient is established and maintained by the pH-controlled activity of the apical Na^+/H^+ antiporter (secondary-active carrier, Aronson et al 1982). Even though the involvement of the Na^+/H^+ antiporter NHE3 in maintaining the driving force for peptide uptake via PEPT1 and PEPT2 and other transporters has been shown unequivocally (Thwaites et al 1999; Thwaites & Anderson 2007), we cannot rule out a substantial contribution of other H^+ translocators to the acidic microclimate. For example, the relevance of V-type H^+ pumps (Beyenbach & Wieczorek 2006) for establishing the driving force of PEPT1 and PEPT2 has not yet been studied in detail. For the placental H^+/folate cotransport, their decisive role has been well established (Prasad et al 1994). At the colonic surface, bicarbonate plays a major role in maintaining the pH microclimate whereas the K^+-H^+-ATPase has little influence (Genz et al 1999).

The driving force for the Na^+/H^+ antiporter is the inwardly directed Na^+ gradient established by the Na^+-K^+-ATPase located at the basolateral membrane of polarized epithelial cells (primary-active carrier, Figure 1). Assuming that only Na^+/H^+ antiporters contribute to the H^+ gradient, one could speculate that experimental Na^+ depletion of tissues and cells will eventually lead to the breakdown of the transmembrane H^+ gradient. Sometimes such a scenario is used to explain the apparent Na^+ dependency of peptide transport, as has been observed by several authors in the past.

There is no doubt that, in the presence of a H^+ gradient, the H^+/peptide cotransporters work uphill. They accumulate peptides intracellularly against a concentration gradient. It is not yet clear whether, in addition to the concentration gradient, any other driving forces contribute to the basolateral efflux of peptides.

Structure and essential structural elements of peptide transporters

In 1994 the first cDNA encoding the protein responsible for the H^+/di- and tripeptide transport activity was cloned from rabbit intestine (Fei et al 1994) using the *X. laevis* oocyte expression cloning method. Shortly thereafter, cloning of PEPT1 from human (Liang et al 1995) and other species followed (for review see Meredith & Boyd 2000; Daniel 2004). The high-affinity isoform, PEPT2, was isolated by expression cloning and homology screening from a kidney cDNA library (Liu et al 1995; Boll et al 1996). PEPT1 and PEPT2 belong to the POT (proton-oligopeptide transporter) or PTR (peptide transporter) family (Paulsen & Skurray 1994; Steiner et al 1995). Proton-oligopeptide carriers of the POT superfamily have also been identified in bacteria (*Lactococcus lactis*), yeast (*Saccharomyces cerevisiae*), plants (*Arabidopsis thaliana, Hordeum vulgare*), invertebrates (*Caenorhabditis elegans, Homarus americanus*), fish, amphibians and birds (for review see Fei et al 1998a; Herrera-Ruiz & Knipp 2003). According to the Human Genome Organization nomenclature, hPEPT1 and hPEPT2 belong to gene family SLC15 of the human membrane transporters for organic solutes (hPEPT1: SLC15A1, hPEPT2: SLC15A2; Daniel & Kottra 2004; Hediger 2004).

The human PEPT1 consists of 708 amino acid residues with a 50% overall sequence identity and 70% similarity to hPEPT2. Hydropathy analysis of the amino acid sequences of both proteins predicts 12 transmembrane domains. Both

amino and carboxy terminus probably face the cytoplasmic side (Daniel 2004). As for all mammalian SLC members, the three-dimensional protein structure of PEPT1 and PEPT2 is still unknown. Epitope insertion studies suggest that the actual membrane topology of PEPT1 corresponds to that obtained by hydropathy analysis (Covitz et al 1998). Further knowledge on important structural elements has been derived from functional analysis of chimeric proteins, from systematic cysteine-scanning mutagenesis, and from specific site-directed mutagenesis studies: PEPT1–PEPT2 chimeras have provided evidence that the four NH_2-terminal transmembrane regions and domains 7–9 play an important role in determining the substrate affinity (Döring et al 1996, 2002; Fei et al 1998b; Terada et al 2000a). According to Kulkarni et al (2003a), transmembrane segment 5 of hPEPT1 forms part of the substrate translocation pathway. On the basis of scanning cysteine accessibility results, this group also speculated that the extracellular end of transmembrane segment 7 may shift following substrate binding, providing the basis for channel opening and substrate translocation (Kulkarni et al 2003b). Transmembrane segment 3 interacts with other transmembrane domains (Links et al 2007).

In site-directed mutagenesis studies, the obligatory role of histidine residue 57 in PEPT1 has been demonstrated by several investigators (Terada et al 1996; Fei et al 1997; Chen et al 2000). It is assumed that His57 might be involved in binding and translocation of H^+ during the conformational change of the protein when transporting peptides or peptidomimetic drugs in a cotransport mode (Uchiyama et al 2003). For PEPT1 in Caco-2 cells, we have shown that both H^+ and treatment with diethylpyrocarbonate, a compound known to block the H^+ acceptor/donor function of histidine residues of proteins by producing N-carbethoxyhistidine residues, modulate the maximal velocity of peptide transport (Brandsch et al 1997). His121 seems to be involved in substrate binding by PEPT1, whereas His111 and His260 are irrelevant in this regard. Tyr56, Tyr64 and Tyr167 seem to be involved in substrate affinity and substrate translocation (Yeung et al 1998; Chen et al 2000). Arg282 in transmembrane segment 7 of hPEPT1 forms a salt bridge with Asp341 in transmembrane segment 8 (Kulkarni et al 2007). According to Meredith (2004), mutation of Arg282 to Glu uncouples the movement of peptides and protons by the rabbit PEPT1. Other important residues are Trp294 and Glu595 (Bolger et al 1998).

In PEPT2, His87 was found to be absolutely essential for catalytic activity because the corresponding mutants had no detectable peptide transport activity (Fei et al 1997). Klapper and coworkers have shown that a three-amino-acid stretch and tyrosine-based motifs within the COOH tail of PEPT2 are involved in PEPT2's apical membrane localization and membrane steady-state level (Klapper et al 2006). Terada et al (2004) identified a genetic PEPT2 variation Arg57His that abolished transport activity completely.

Tissue distribution and physiological function

The H^+ dependence of intestinal and renal peptide transport is now textbook knowledge and the phenomenon has been confirmed with different tissue preparations. In Table 1 we summarize the expression of both transporters in mammalian tissues. Not in all cases is the evidence based on actual transport data; the experiments sometimes only indicated the presence of mRNA. Convincing evidence was gathered many years ago for the functional expression of PEPT1 in intestine and kidney and for the expression of PEPT2 in the kidney even though the molecular identity of the systems was not elucidated before 1994. We have found PEPT1 expression and function at the extrahepatic biliary duct (Knütter et al 2002). PEPT2 has been detected, in addition to the kidney tubule, in the lung (for review see Groneberg et al 2004), choroid plexus (Teuscher et al 2000), mammary gland and other tissues (for reviews see Ganapathy et al 2001; Meredith & Boyd 2000; Nielsen et al 2002a; Daniel & Kottra 2004; Steffansen et al 2004; Daniel 2004) (Table 1).

The physiological function of peptide transport at the intestinal epithelium and its relation to amino acid transport has been extensively reviewed recently by H. Daniel. Intestinal protein digestion generates a huge variety and quantity of short chain peptides that can be absorbed into intestinal epithelial cells by the PEPT1 transporter in the apical membrane of enterocytes (Daniel 2004). The physiological function of peptide transport in the kidney was initially received with scepticism because of the widely held notion that the concentration of small peptides in the circulation was very low. Yet, the plasma levels of peptide-bound amino acids are many-fold higher than once thought (Seal & Parker 1991). Moreover, due to the hydrolysis of oligopeptides and small-molecular-weight proteins that are present in the glomerular filtrates by the highly active peptidases associated with the renal brush-border membrane, the local concentrations of small peptides in the renal tubular lumen are very likely much higher than in plasma. Even before the cloning of the transporters, it was known that at the renal tubule two peptide transport systems exist, one with high affinity for its substrates and the other with low affinity (Silbernagl et al 1987; Daniel et al 1991). In immunolocalization experiments, Shen et al (1999) detected the low-affinity system PEPT1 in S1 segments of the proximal tubule. PEPT2 immunolocalization was primarily confined to the brush border of S3 segments of the proximal tubule. Hence, PEPT1 is expressed in early regions of the proximal tubule (pars convoluta), whereas PEPT2 is specific for the latter regions of the proximal tubule (pars recta). All other nephron segments in rat were negative for PEPT1 and PEPT2 staining (Shen et al 1999).

We can only speculate about the physiological function of PEPT1 at the biliary epithelium (Knütter et al 2002). Very little is known about the presence of small peptides in bile fluid. Using reverse-phase chromatography, mass spectrometry and Edman degradation several hydrophobic polypeptides have been identified in the bile (Stark et al 1999). It remains to be elucidated whether PEPT1 functions as a recovery system of di- and tripeptides excreted from hepatocytes into the bile. A clinical relevance of biliary duct peptide transport is possible, considering the observation that δ-aminolevulinic acid (δ-ALA), a compound used in photodynamic tumour therapy, is accumulated in biliary epithelial cells by PEPT1 (Neumann & Brandsch 2003).

PEPT2 acts as an efflux transporter in the choroid plexus and might be involved in the regulation of

Table 1 Localization of H^+/peptide symporters PEPT1 and PEPT2 in mammalian tissues

Transporter	Organ/tissue	Localization	Demonstration
PEPT1 (SLC15A1)	Small intestine	Epithelial cells: apical membrane	Function (Ganapathy & Leibach 1983) mRNA (Freeman et al 1995) Protein (Ogihara et al 1996)
	Kidney	S1-segment, epithelial cells: apical membrane	Function (Silbernagl et al 1987) mRNA, protein (Shen et al 1999)
		Lysosomes	Function, protein (Zhou et al 2000)
	Pancreas	Vascular smooth muscle cells: nuclei Schwann cells: nuclei Exocrine pancreas: lysosomes	Protein (Bockman et al 1997)
	Bile duct	Epithelial cells, extrahepatic: apical membrane	Function, mRNA, protein (Knütter et al 2002) mRNA (Fei et al 1994)
	Liver	Lysosomes	Function (Thamotharan et al 1997)
	Blood	Monocytes	Function, mRNA, Protein (Charrier et al 2006)
	Adrenal gland	Neuroendocrine cells	Function, mRNA (Hussain et al 2001)
PEPT2 (SLC15A2)	Kidney	S2-S3 segment, epithelial cells: apical membrane	Function (Silbernagl et al 1987) mRNA, protein (Shen et al 1999)
	Peripheral nervous system	Ganglionic neurons, satellite glial cells	mRNA, protein (Groneberg et al 2001)
	Central nervous system	Choroid plexus, epithelial cells	Function, protein (Novotny et al 2000)
		Choriod plexus, epithelial cells, subependymal & ependymal cells	mRNA (Berger & Hediger 1999)
		Astroglia	Function, mRNA (Dieck et al 1999)
		Cerebral cortex	Function, mRNA, protein (Fujita et al 2004)
	Enteric nervous system	Neuromuscular layers of gastrointestinal tract, enteric glial cells, tissue-resident macrophages	Function, mRNA, protein (Rühl et al 2005)
	Lung	Bronchia, epithelial cells	Function, mRNA, protein (Groneberg et al 2001)
		Alveoli, type II pneumocytes	Function, mRNA, protein (Groneberg et al 2001)
		Small arteries, endothelial cells	mRNA, protein (Groneberg et al 2001)
	Mammary gland	Ducts and glands, epithelial cells	mRNA, protein (Groneberg et al 2002)
	Heart	Cardiomyocytes	Function, mRNA (Lin & King 2007)

neuropeptide levels in the brain (for review see Smith et al 2004). In kidney and brain, the carrier might contribute to glutathione metabolism by providing cysteinylglycine derived from extracellular glutathione for glutathione resynthesis in tubular cells and in astrocytes (Teuscher et al 2001; Frey et al 2007).

Provided that the structural requirements for substrates are fulfilled, peptide carriers translocate peptidic signals across membranes that are relevant in information processing. For example, many peptides derived from dietary proteins, such as Val-Tyr, Ile-Tyr, Ile-Pro-Pro and others, have been shown to reduce blood pressure, which is explained by their angiotensin converting enzyme (ACE)-inhibitory activity (for review see Daniel 2004). Another example is the dipeptide kyotorphin (Tyr-Arg), allegedly the smallest bioactive opioid peptide. As expected for a dipeptide of this type, we observed strong interaction of Tyr-Arg with PEPT2 (Brandsch et al 1995). Its transport by PEPT2 at synaptosomes prepared from rat cerebellum has subsequently been studied in great detail (Fujita et al 1999). Recently, the group of H. Koepsell and coworkers reported that the two tripeptides Gln-Cys-Pro and Gln-Ser-Pro, originating from the human protein RS1, are taken up by PEPT1 into intestinal cells where they inhibit an exocytotic pathway of the Na^+/glucose cotransporter SGLT1 (Vernaleken et al 2007).

Substrate specificity of PEPT1 and PEPT2

Determination and classification of apparent affinity constants

Measuring the interference of compounds with the uptake of standard substrates is the first thing to do when investigating the substrate specificity of a transporter. One of the most commonly used and best known reference substrates of H^+/peptide cotransporters is [^{14}C]glycylsarcosine (Gly-Sar). This substrate is relatively stable against intra- and extracellular enzymatic hydrolysis. Its transport characteristics have been studied in possibly every tissue known to express peptide transport. The apparent affinity constants of Gly-Sar are in the medium range with K_t values of around 0.5–1.5 mM for PEPT1 and 50–150 μM for PEPT2. Other labelled reference substrates used quite often are [^{3}H]D-Phe-L-Ala, [^{3}H]D-Phe-L-Gln and D-Ala-L-Lys-N$^{\varepsilon}$-7-amino-4-methylcoumarin-3-acetic acid. The potency of inhibition of the uptake of labelled reference substrates by test compounds gives a first idea of their affinity. It has to be kept in mind, however, that such an inhibition does not mean that the test compound is indeed a transportable substrate. It could represent a specific or unspecific inhibitor or even a compound that breaks down the H^+ gradient as the driving force of peptide transport thereby inhibiting the uptake of the reference compound indirectly. Moreover, if a compound strongly and

specifically inhibits reference peptide uptake and the inhibition has even been shown to be competitive in kinetic experiments, competition assays only give information about recognition and apparent binding strength but not about actual transport. For that purpose, other techniques such as the two-microelectrode voltage-clamp technique in *X. laevis* oocytes expressing PEPT1 or PEPT2 or HPLC analysis of the compound itself are used.

In over thirty years of research, data on the interaction of several hundred compounds with peptide transporters have been gathered (Addison et al 1975; Matthews 1975; Snyder et al 1997; Börner et al 1998; Brandsch et al 1999; Terada et al 2000b; Nielsen et al 2001, 2002a; Brodin et al 2002; Steffansen et al 2004, 2005; Biegel et al 2006a, b). We estimate that, for the intestinal or renal peptide transporters, the apparent affinity constants of well over 720 compounds are currently known. The list includes ~200 dipeptides, ~50 tripeptides, ~100 backbone modified peptides, ~80 amino acid derivatives, ~150 dipeptide derivatives, ~60 β-lactam antibiotics and ~80 other drugs and prodrugs or potential prodrugs. The available information is most often on IC50 values or K_i values. Only in rare cases do we know the maximal transport velocity, the K_t values of actual transport and the diffusional parameters of the compounds.

Clearly, affinity constants were, and will always be, determined with different assays, and in different laboratories by different people using different protocols. When comparing the affinity data, differences in species, tissues, cell types, the experimental protocols (in particular the outside pH and the reference compound) and data processing (IC50 values or K_i values) cannot be ignored. For cyclacillin, affinity constants between 0.2 mM and > 14 mM have been reported in the literature. For enalapril, reported K_i values attributed to its interaction with peptide transporters differ by a factor of > 300. Furthermore, the affinity constants determined at native cells expressing PEPT1 or PEPT2 constitutively compared with PEPT1 or PEPT2 expressed heterologously can differ by a factor of 10 for certain types of substrates such as charged dipeptides, ω-amino fatty acids or amino acid derivatives (Biegel et al 2006b). These examples illustrate the need for utmost caution in evaluating affinity constants.

Assessments such as high affinity or low affinity need some guiding principles. How do we define these categories for a given substrate at one particular carrier? For PEPT1, we consider affinity constants for substrates or inhibitors lower than 0.5 mM as high affinity, 0.5–5 mM as medium affinity, and above 5 mM as low affinity. We consider values above 15 mM with great caution (for review see Brandsch et al 2004). For PEPT2, we suggested the following: (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 affinity. Compounds with affinity constants above 5 mM should not be considered as PEPT2 ligands (Luckner & Brandsch 2005). In Table 2 we illustrate the classification for substrates of the intestinal-type H^+/peptide cotransporter PEPT1 expressed constitutively in Caco-2 cells. The highest apparent affinity constants of a substrate/inhibitor for PEPT1 reported so far are those of Lys[(Z)NO_2]-Val with 2 μM (Knütter et al 2004) and Val-Lys(Flu) with 5 μM (Abe et al 1999). Most dipeptides made of gene-coded amino acids, the physiological substrates of PEPT1, display affinity constants in the range of 0.07–0.7 mM. The decision to consider a very low-affinity substrate/inhibitor with $K_i > 15$ mM not a substrate/inhibitor at all might be questionable for some researchers. Support for this threshold value comes from our studies on affinity and transepithelial transport of β-lactam antibiotics (Bretschneider et al 1999). Using the Caco-2 cell assay, we determined a threshold value of $K_i \approx 15$ mM for cephalosporins and penicillins with respect to their transport and their oral availability. Compounds with $K_i > 15$ mM are neither transported across

Table 2 Classification of affinity constants at PEPT1

Category (K_i range)	Substrate/inhibitor	K_i (mM)[a]	Reference
High affinity (< 0.5 mM)	Lys[Z(NO_2)]-Val	0.002 ± 0.001[b]	Knütter et al 2004
	Alafosfalin	0.19 ± 0.01	Neumann et al 2004
	Ala-Lys	0.21 ± 0.02	Knütter et al 2004
	Ceftibuten	0.34 ± 0.03	Bretschneider et al 1999
	Valaciclovir	0.49 ± 0.04	Ganapathy et al 1998
Medium affinity (0.5–5 mM)	Gly-Sar	1.1 ± 0.1	Brandsch et al 1994
	Pro-Pro	1.2 ± 0.1	Brandsch et al 1999
	δ-Aminolevulinic acid	1.5 ± 0.1	Irie et al 2001
	Cloxacillin	3.0 ± 1.0	Luckner & Brandsch 2005
	Lys-Lys	3.4 ± 0.7	Eddy et al 1995
Low affinity (5–15 mM)	D-Ala-Lys	7.0 ± 0.6	Hartrodt et al 2001
	Cefadroxil	7.2 ± 0.8	Bretschneider et al 1999
	Pro-Ala	9.5 ± 0.4	Brandsch et al 1999
	4-Aminophenylacetic acid	14 ± 1	Börner et al 1998
	Cephalexin	14 ± 2	Bretschneider et al 1999

[a]K_i values were obtained in Caco-2 cell (PEPT1) uptake competition assays. [b]Highest affinity measured for a PEPT1 ligand reported so far. Compounds with K_i or $K_t > 15$ mM were not considered PEPT1 substrates or inhibitors, respectively.

Caco-2 cell monolayers in significant amounts nor are they orally available in man.

It is generally assumed that PEPT2 displays a 10- to 15-times higher affinity for its substrates than PEPT1. For most di- and tripeptides this is certainly the case and it is the reason why PEPT2 is called the high-affinity peptide transporter as opposed to PEPT1, the low-affinity isoform. In Table 3 we illustrate the classification of apparent affinity constants in SKPT cells, a rat proximal tubular cell line. These cells express PEPT2 constitutively (Brandsch et al 1995). A human cell line expressing PEPT2 with sufficient activity has not yet been identified.

Di- and tripeptides and derivatives

D. M. Matthews' laboratory showed in extensive studies in the late 1960s and early 1970s that only di- and tripeptides could cross the intestinal brush-border membrane (Matthews 1975). Larger peptides are not transported. Numerous studies have been carried out since these early reports and there is now general consensus that only di- and tripeptides are substrates for PEPT1 and PEPT2. The number of physiologically occurring substrates is therefore sometimes calculated as 400 dipeptides (20^2) and 8000 tripeptides (20^3). Today we know that PEPT1 and PEPT2 accept most but not all proteinogenic di- and tripeptides as substrates. We recently found that Trp-Trp-Trp, even though it displays a very high affinity to PEPT1 ($K_i = 0.15$ mM), is not transported. Whether the size (i.e. the molecular weight of the peptide) is a limiting factor for translocation has not yet been studied in detail. Moreover, reviewing the available literature reveals that peptides with proline in N-terminal position are not particularly good substrates for peptide carriers. Pro-Ala, Pro-Asp, Pro-Ser, Pro-Glu and Pro-Gly display very low affinity or no affinity at all for PEPT1 in Caco-2 cells (Brandsch et al 1999; Vig et al 2006).

Cationic and anionic dipeptides are transported by the same systems utilized by zwitterionic dipeptides. However, they appear to have a lower affinity compared with structurally similar dipeptides (Eddy et al 1995; Terada et al 2000b). In a systematic approach, we confirmed this for Xaa-Ala dipeptides (Brandsch et al 1999). Studies by Amasheh et al (1997) and Kottra et al (2002) for PEPT1 and by Fei et al (1999) for PEPT2 have shown that H^+/peptide transporters preferentially recognize zwitterionic dipeptides or the zwitterionic form of charged dipeptides in aqueous solution.

Modifying the N-terminal amino group by methylation, acetylation or other substitutions weakens the affinity for the transporters (Hartrodt et al 1998; Meredith et al 2000; Terada et al 2000b). N-Terminal incorporation of β-amino acids into dipeptides such as in β-Ala-His (L-carnosine) lowers the affinity (Nielsen et al 2002b). Modification of the C-terminal carboxy group (e.g. converting it to an amide) also decreases the affinity but to a lower extent than in the case of the modification of the N-terminus (Meredith et al 2000; for review see Brandsch et al 2004; Biegel et al 2006b). Removal of the amino terminus, the carboxy terminus, or both, leads to total loss of affinity. Very interesting data have been obtained in experiments where the distance between the two charged terminal groups as related to peptide linkage was increased. Increasing the distance between the peptide bond and the C-terminal carboxy group is much better tolerated than doing the same at the amino terminus (Biegel et al 2006b).

A peptide bond in the narrower sense is not an essential structural requirement for a substrate. It can be replaced by a ketomethylene bond without diminishing the affinity (arphamenine A, Daniel & Adibi 1994; Enjoh et al 1996). δ-AA, which also possesses a ketomethylene group instead of a peptide bond, is transported by PEPT1 and PEPT2 with affinity constants in the range of 0.2–2.2 mM (Döring et al 1998a; Terada et al 2000b; Irie et al 2001). Ala-ψ[CS-N]-Pro, a compound where the peptide bond is replaced by the isosteric thioxo peptide bond, is recognized by PEPT1 with high affinity ($K_i = 0.3$ mM) and also transported

Table 3 Classification of affinity constants at PEPT2

Category (K_i range)	Substrate/inhibitor	K_i[a]	Reference
High affinity (< 0.1 mM)	Lys[Z(NO_2)]-Lys[Z(NO_2)]	0.010 ± 0.001 μM[b]	Theis et al 2002b
	Cefadroxil	3.0 ± 1.0 μM	Luckner & Brandsch 2005
	Ala-Ala	6.3 ± 0.3 μM	Brandsch et al 1995
	Fosinopril	29.5 ± 2.4 μM	Shu et al 2001
	Valganciclovir	43 ± 5 μM	Sugawara et al 2000
Medium affinity (0.1–1 mM)	Gly-Sar	0.11 ± 0.02 mM	Theis et al 2002b
	δ-Aminolevulinic acid	0.23 ± 0.09 mM	Bravo et al 2005
	Ceftibuten	0.28 ± 0.01 mM	Luckner & Brandsch 2005
	Amoxicillin	0.43 ± 0.02 mM	Luckner & Brandsch 2005
	D-Leu-Gly-Gly	0.59 ± 0.02 mM	Biegel et al 2006b
Low affinity (1–5 mM)	β-Ala-Ala	2.1 ± 0.2 mM	Biegel et al 2006b
	Pro-Glu	2.6 ± 0.3 mM	Biegel et al 2006b
	Cefamandole	2.8 ± 1.1 mM	Luckner & Brandsch 2005
	Oxacillin	3.3 ± 0.9 mM	Luckner & Brandsch 2005
	Ala-D-Ala-Ala	4.2 ± 0.2 mM	Biegel et al 2006b

[a]K_i values were obtained in SKPT cell (PEPT2) uptake competition assays. [b]Highest affinity measured for a PEPT2 ligand reported so far. Compounds with K_i or $K_t > 5$ mM were not considered PEPT2 substrates or inhibitors, respectively.

(Brandsch et al 1998). 4-Aminomethylbenzoic acid has been reported as an inhibitor for PEPT1 expressed in *X. laevis* oocytes with an apparent affinity constant of 3.1 mM (Meredith et al 1998). These results on the relevance of the functional groups and the peptide bond raise the question: what are the minimal molecular determinants of substrates for recognition by peptide transporters? For PEPT1 it has been shown that ω-amino fatty acids serve as substrates though with widely differing affinities (Döring et al 1998b; Terada et al 2000b; Irie et al 2001; Biegel et al 2006b). The optimal distance between the two charged centres is 500–630 pm (Döring et al 1998b). For PEPT1 in Caco-2 cells, the affinity of ω-amino fatty acids is low and can be increased drastically by introduction of a carbonyl function. ω-Amino fatty acids do not serve as substrates for PEPT2 (Theis et al 2002a). The compounds with an isopeptide bond (isopeptides) tested so far, such as γ-Glu-Ala, γ-Glu-N^{ε}-Lys and Ala-N^{ε}-Lys, are neither bound nor transported by peptide transporters.

Much effort has been invested in the synthesis of side-chain modified peptides. The idea is to design a prodrug by conjugating the drug to an anchor moiety that fulfills the minimal structural requirements for a substrate (Taub et al 1998; Nielsen et al 2001, 2002a; Knütter et al 2004). The side-chain modified dipeptides D-Asp(OBzl)-Ala, D-Glu(OBzl)-Ala and D-Ser(Bzl)-Ala have been reported as possible substrates for PEPT1 with high to medium affinity (Taub et al 1998; Nielsen et al 2001) but actual transport of such compounds has not been established unequivocally. Among side-chain modified dipeptides, very interesting inhibitors of PEPT1 have been found (e.g. Val-Lys(Flu) (Abe et al 1999) or Lys[Z(NO_2)]-Pro (Knütter et al 2001, see below)).

A subject of very thorough investigations was the stereospecificity of peptide transporters. PEPT1 is stereospecific in the sense that L-L-dipeptides and L-L-L-tripeptides display a much higher affinity than dipeptides or tripeptides containing D-amino acids (Li et al 1998; Meredith et al 2000). D-Leu-D-Leu and D-Ala-D-Ala are not able to inhibit Gly-Sar transport in Caco-2 cells (Thwaites et al 1994). The affinities of dipeptides consisting of the D-isomers of natural amino acids are in most cases > 30 mM or not measurable. In contrast, the D-D-isomers of Ala-Lys(Z), Lys(Z)-Ala and Ala-Asp(OBzl) displayed appreciable affinity constants between 1 and 10 mM (Hartrodt et al 2001). Even though the affinity of these compounds is low compared with L-L-dipeptides, it might be high enough to consider hydrolytically stable prodrugs of this type (Brandsch et al 2004). Actual transport, however, of these D-D-isomers across the cellular membrane has not yet been shown.

Besides stereospecificity, there is the question of conformational specificity of peptide transporters. Having observed 4- to 12-fold lower affinities of Gly-Pro and Gly-Sar compared with Gly-Gly and Gly-Ala at the renal peptide transporter, Daniel et al (1992) were first to discuss that isomerization at the peptide bond might be responsible for these differences. Gly-Gly and Gly-Ala exist in aqueous solution only in *trans* conformation whereas Xaa-Pro dipeptides exist as *cis*/*trans* conformer mixtures. To measure *cis*/*trans* conformational effects, ideally, conformers of one and the same substrate should be used. We used the thioxodipeptide Ala-ψ[CS-N]-Pro, which is characterized by a low *cis*/*trans* isomerization rate as a model substrate. We could show that only the *trans* conformer interacted with the transporter and was taken up into the cell (Brandsch et al 1998). In a further study, we examined the correlation of the content of *trans* conformer (%) and affinity constant (mM) of Xaa-Pro dipeptides at PEPT1 and compared the affinity constants with the values of the respective Xaa-Ala dipeptides (Brandsch et al 1999). From both studies we concluded that PEPT1 accepts almost exclusively the *trans* conformers of Xaa-Pro dipeptides. Hence, when measuring concentration-related constants such as IC50, K_i and K_t values of Xaa-Pro dipeptides, the *cis*/*trans* ratio should not be ignored (Brandsch et al 1999; Payne et al 2001; Brandsch 2006). Bailey and coworkers later confirmed our results using pH control of *cis*/*trans* ratios of a set of substrates (Bailey et al 2005) and filed a patent on the use of 'thiopeptide conjugates for drug delivery' (Bailey 2005, WO/2005/067978).

It should be noted that *cis* conformers can be detected not only in aqueous solutions of all Xaa-Pro dipeptides but also for derivatives such as Xaa-pipecolic acids, Xaa-thiazolidides, Sar-Pro, Xaa-Sar dipeptides and several peptidic ACE-inhibitors. Therefore, conformational specificity also concerns the prototypic peptide transporter substrate Gly-Sar itself, which was found to exist in buffer at 61% in *trans* conformation (Brandsch et al 1999; Payne et al 2001). The specificity of PEPT1 for tripeptide *cis*/*trans* conformes and the conformational specificity of PEPT2 have not yet been investigated.

Amino acid derivatives

When working on peptide transport in *Saccharomyces cerevisiae*, Jeffrey M. Becker observed that leucyl-*p*-nitroanilide is a competitive inhibitor of peptide transport and used it as a photoaffinity label for the carrier protein (Becker et al 1982). Daniel & Adibi (1994) found that amino acid 4-nitroanilides are recognized by mammalian renal peptide transporters with high affinity. The actual translocation of amino acid derivatives such as Ala-anilide, Ala-7-amido-4-methylcoumarin, Ala-4-nitroanilide and Ala-4-methylanilide could be shown by electrophysiological methods (Börner et al 1998). Inui's group studied the recognition of amino acid ester compounds by rPEPT1 and rPEPT2 (Sawada et al 1999b). Several esters of L-valine were able to inhibit [^{14}C]Gly-Sar uptake in LLC-PK_1 cells expressing PEPT1. Interestingly, the affinity of alkyl esters of other amino acids such as tyrosine, phenylalanine, leucine, glycine and alanine was much weaker or not measurable. Valine has been shown independently by several investigators to be the favourable N-terminal amino acid when designing prodrugs (see below).

Xaa-pyrrolidides show no affinity towards PEPT1. In contrast, amino acid thiazolidides, such as Ala-thiazolidide and Ile-thiazolidide, are potential substrates with low or very low affinity (Brandsch et al 1999). This finding is of pharmaceutical relevance: based on the known oral availability and the in-vivo efficacy of the dipeptidyl peptidase IV inhibitor Ile-thiazolidide and its peptide-like structure, Foltz et al (2004) examined whether the derivatives were not only recognized but actually transported by peptide transporters. They identified various compounds that serve as substrates for PEPT1 but not for PEPT2. Acceptance of the compounds by PEPT1 might explain their oral availability in man.

As a short summary of the substrate specificity of intestinal and renal peptide transporter, it can be concluded that for a high-affinity substrate the following structural features are essential (Figure 2): (i) a free N-terminal α-amino group in L-configuration; (ii) a peptide bond that can be replaced by a ketomethylene group but not by a -CH_2-NH-group and that should be separated by one or two methine groups or methylene carbon atoms from the N-terminal nitrogen; (iii) in case of dipeptides, *trans* conformation of the peptide bond; (iv) an acidic C-terminal group (e.g. a carboxy group)—the distance between the carbonyl group of the first peptide bond and the carboxylic group is less relevant; (v) high hydrophobicity; and (vi) for tripeptides, an uncharged amino acid residue in position 3 (for review see Biegel et al 2006b). These general requirements are valid both for substrates and inhibitors of peptide transporters and both for PEPT1 and PEPT2. This raises two additional questions: first, which are the structural modifications that convert a substrate into an inhibitor and, second, are there differences between PEPT1 and PEPT2 with regard to substrate recognition?

High-affinity inhibitors

In competition studies with radiolabelled dipeptides and in electrophysiological measurements we have shown that Lys[Z(NO_2)]-Pro binds with high affinity to PEPT1 (K_i = 5–10 μM). The compound competes with various dipeptides for uptake into cells but, however, is not transported itself (Knütter et al 2001). It is the Z-group added to the ε-amino group of the Lys-residue that turns Lys-Pro from a normal transported substrate into a non-transportable derivative with significantly higher affinity. Addition of the NO_2-function to the hydrophobic ring moiety (Lys[Z(NO_2)]-Pro) enhances the affinity for binding to PEPT1 further while maintaining its inability to be transported (Knütter et al 2001). In another study, synthesis and functional analysis of Lys-Lys derivatives containing benzyloxycarbonyl (Z) or 4-nitrobenzyloxycarbonyl (Z(NO_2)) side-chain protection groups provided a set of inhibitors that reversibly inhibited the uptake of dipeptides by PEPT2 with K_i values as low as 10 nM (Theis et al 2002b). Based on structure–function relationship analyses, it was concluded that, firstly, the spatial location of the side-chain amino-protecting group in a dipeptide containing a diaminocarbonic acid and, secondly, its intramolecular distance from the ω-C-atom are key factors for the transformation of a substrate into an inhibitor of PEPT2. Figure 3 illustrates the structural modifications identified for PEPT1 inhibitors. We had synthesized a series of Xaa(R)-Ala and Ala-Xaa(R) dipeptides with the functional groups of the side chains modified by structurally different blocking groups R. Both the affinity constants in Caco-2 cells and transgenic *Pichia pastoris* cells, as well as inward-directed currents in *X. laevis* oocytes expressing PEPT1, were measured (Knütter et al 2004). Transport of side-chain modified dipeptides into enterocytes depends firstly on the position of the modified

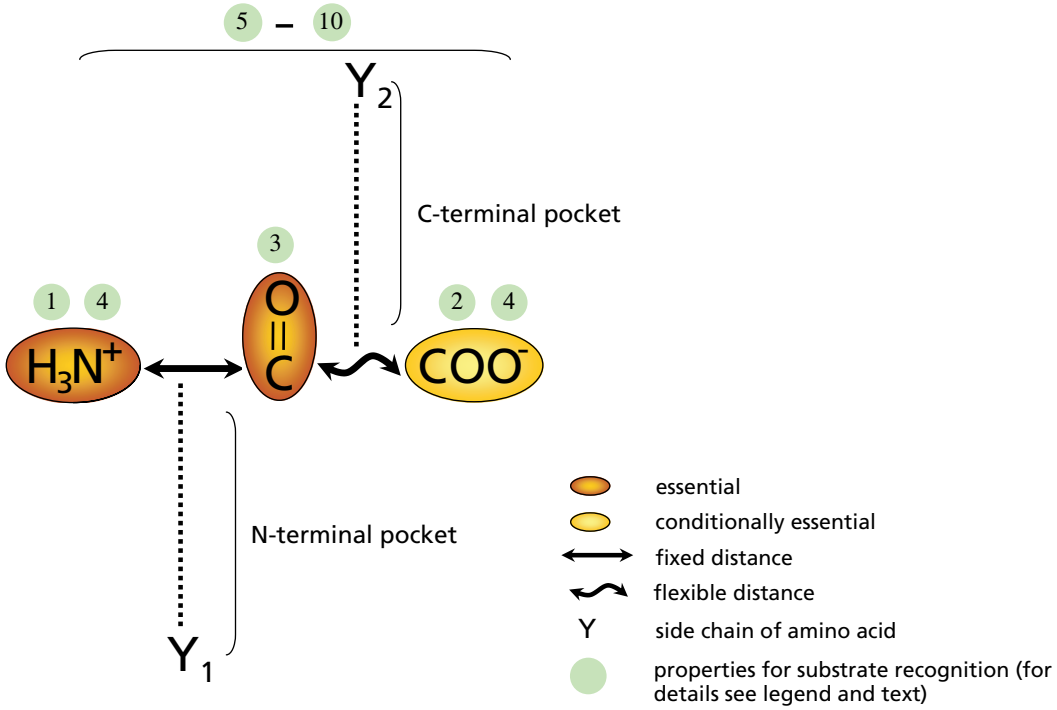


Figure 2 Structural determinants for high-affinity substrates of H^+/peptide cotransporters PEPT1 and PEPT2. 1, free NH_3^+-group in α-position, cannot be replaced by other basic groups; 2, COO^- group conditionally essential, can be replaced with phosphoric acid group or arylamides; 3, carbonyl group of the peptide bond essential for high affinity, can be replaced by a thiocarbonyl group; the peptide bond must be in *trans*-conformation; 4, backbone cyclization abolishes affinity; 5, N-terminal Pro is disadvantageous; 6, bulky side chain is advantageous; 7, C-terminal cationic side chain decreases affinity; 8, hydrophobic side chain blocking groups increase affinity; 9, L-L-stereoselective (but: D-D-dipeptides with protected side chain of a trifunctional amino acid display medium affinity); 10, overall hydrophobicity increases affinity.

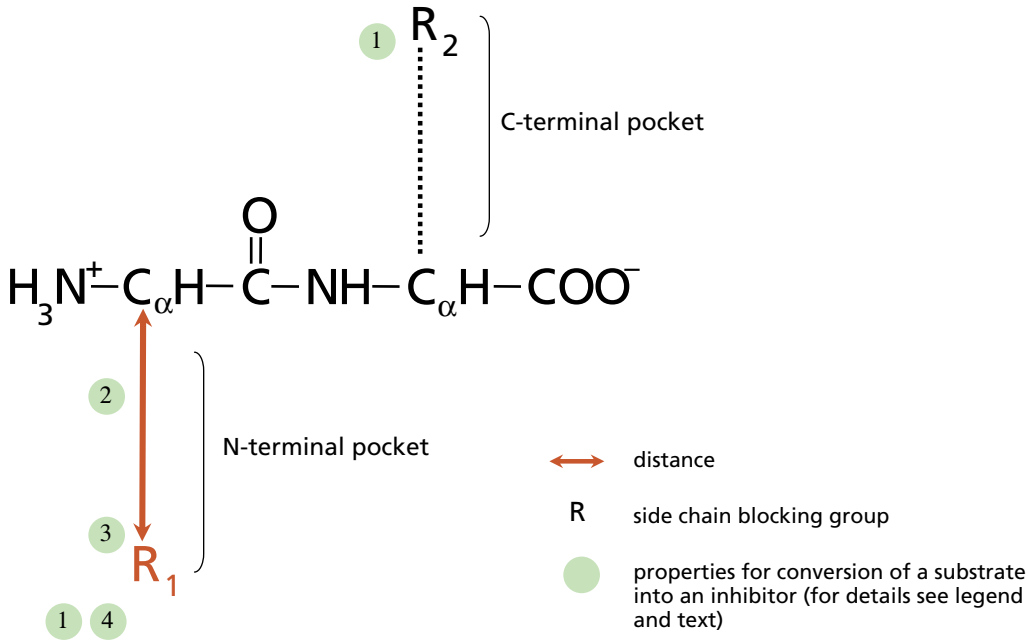


Figure 3 Structural determinants for high-affinity inhibitors of H^+/peptide cotransporters PEPT1 and PEPT2. 1, hydrophobic side chain blocking group at the N- but not C-terminal amino acid; 2, distance of the blocking group to the C_α-atom is decisive; 3, insertion of a blocking group of the urethane type; 4, NO_2-group at the N-terminal side chain protection group increases affinity.

trifunctional amino acid in the dipeptide, secondly on the distance between its α-carbon and the side-chain blocking group and thirdly on the hydrophobic character of the side-chain modification.

Such high-affinity inhibitors should be very useful for probing the protein structure of peptide transporters as well as for identifying their biological role in the various cell types and tissues where the transporters are expressed (Theis et al 2002b).

Comparison of PEPT1 and PEPT2 substrate specificity

A number of studies concerning the similarities and differences in substrate recognition between PEPT1 and PEPT2 have been published (Daniel et al 1992; Daniel & Adibi 1993; Ganapathy et al 1995, 1997; Terada et al 1997b, 2000b; Döring et al 1998a; Sugawara et al 2000; Theis et al 2002b; Knütter et al 2004; Vabeno et al 2004; Luckner & Brandsch 2005). It has been well established that there are several significant differences. First of all, certain amino acid derivatives and ω-amino fatty acids are recognized by PEPT1 but not PEPT2. Second, the affinity constants of most substances to the transporter isoforms differ significantly, PEPT2 being the transporter with higher binding affinity in most cases (for review see Biegel et al 2006b). We usually calculate the quotients $K_{i\ PEPT1}/K_{i\ PEPT2}$ to highlight the differences in affinity. It is often stated in reviews that PEPT2 transports its substrates with 10-to 15-fold higher affinity than PEPT1. Hence, assuming that PEPT2 binds the same substrates with the same rank order, a $K_{i\ PEPT1}/K_{i\ PEPT2}$ ratio of 10–15 would be expected. When we compiled the data measured in Caco-2 and SKPT cells, however, it became apparent that these ratios vary from 0.16 for Lys[Z(NO2)]-Lys to 31 000 for Lys(Z)-Lys(Z) (Biegel et al 2006b). We are aware of the fact that these cell lines originate from different species, man and rat, respectively, but differences between hPEPT1 and rPEPT2 could so far be confirmed using hPEPT1 and hPEPT2 expression systems (Ganapathy et al 1995, 1997; Brandsch et al 1997; Shu et al 2001). The main differences in substrate recognition between PEPT1 and PEPT2 are the following (Biegel et al 2006b):

In general, PEPT2 accepts the same substrates as PEPT1 but in case of natural dipeptides with higher affinity and lower maximal uptake rates.

The more hydrophobic a substrate the higher is the binding affinity to PEPT2. This phenomenon was not observed for PEPT1.

PEPT2 has disproportionally higher affinities for those β-lactam antibiotics that contain an α-amino group than PEPT1 (see below).

Tripeptides containing a charged amino acid in position 3 are medium to low affinity substrates for PEPT2, whereas they show high binding affinities to PEPT1.

Very generally it can be said that PEPT2 is more selective than PEPT1, and that PEPT2 has more specific, confined requirements for substrate recognition.

Molecular modelling of PEPT1 and PEPT2 substrates

The results discussed so far were all obtained experimentally. Until the exact structural features of the substrate-binding sites of PEPT1 and PEPT2 become available, this classic approach of determining affinities followed by proof of actual membrane translocation is the only way to identify new

physiological substrates, drugs and prodrugs. In the last five years, however, much progress has been made in the field of computational molecular modelling studies of substrates (Gebauer et al 2003; Biegel et al 2005, 2006a; Andersen et al 2006; Bailey et al 2006). In several earlier molecular modelling studies, inconsistent or very limited data sets from the literature, or parameters rather than actual affinity data, were collected and used. In cooperation with the group of I. Thondorf, we investigated a high number of substrates with a very broad range of K_i values that had been obtained under conditions as highly standardized as possible. In the first study, a comparative molecular field analysis (CoMFA) and a comparative molecular similarity indices analysis (CoMSIA) were performed on a series of 79 dipeptide-type substrates of PEPT1 (Gebauer et al 2003). The rigid dipeptide analogue Ala-ψ[CS-N]-Pro (see above) was used as template for the identification of pharmacophore features of substrates. The models were validated by an external test set of 19 dipeptides and dipeptide derivatives. By combining five CoMSIA contour maps (i.e. steric, electrostatic, hydrophobic/hydrophilic, hydrogen-bond-donor and hydrogen-bond-acceptor), six recognition elements that are favourable for binding to PEPT1 were identified: (i) the presence of bulky side chains; (ii) a positively charged N-terminus and a region of high electron density at the C-terminus, which are commensurate with (iii) two hydrophilic regions; (iv) a preferred hydrophobic region at the C-terminal part; (v) a hydrogen-bond-donor region at the N-terminus; and (vi) a hydrogen-bond-acceptor region crucial for differentiation between L-L-, D-L-, L-D-, and D-D-isomers. The 3D-QSAR models allow the prediction of K_i values of new compounds (Gebauer et al 2003). The investigation was then extended by including tripeptides and β-lactam antibiotics, thereby increasing the predictive power of the model, particularly for larger peptidomimetics (Biegel et al 2005). Andersen et al (2006) followed a similar strategy to develop a 3D-QSAR model for binding of tripeptides and tripeptidomimetics to hPEPT1 based on 25 diverse tripeptides with K_i values in the range 0.15–25 mM. The structural diversity of the compounds was described by VolSurf descriptors and structural information on tripeptide properties influencing the binding to hPEPT1 was extracted from the 3D-QSAR model (Andersen et al 2006). Our third study focused on PEPT2. A comprehensive 3D-QSAR model based on 83 compounds was developed. Again, a statistically reliable model of high predictive power was obtained. Comparing the CoMSIA contour plots with those of the PEPT1 plots, differences in the selectivity between the intestinal and the renal type peptide carriers became evident (Biegel et al 2006a).

The idea behind these efforts is that it might become possible to predict the interaction of any structure with both peptide transporters with sufficient precision of K_i. It should be noted that the computational model cannot predict K_i values for classes of compounds it was not trained for. It should also be noted that the models discussed so far are based on affinity data, not transport data. Hence, the model can only predict affinity, not transport. Such a prediction of transport rates has been attempted by Wanchana et al (2004). These authors performed two- and three-dimensional QSAR of uptake rates of 20 β-lactam antibiotics into Caco-2 cells we had published in 1999 (Bretschneider et al 1999). The authors concluded that the simple 2D-QSAR approach gives a sufficient predictability of uptake (Wanchana et al 2004).

There were even attempts to predict total transepithelial net flux of peptide transporter substrates. In our view, the process of transepithelial flux is much too complex. It is the sum of binding to the carrier, uptake into the cell, intracellular transport, intracellular metabolism, possibly apical efflux and, finally, basolateral efflux. For most steps of this process we do not have any experimental data, and whether binding of substrates to PEPT1 is a good indicator of transport via PEPT1 is still a matter of discussion (Brandsch et al 2004; Bailey et al 2006; Vig et al 2006).

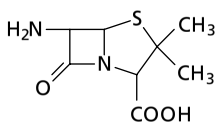
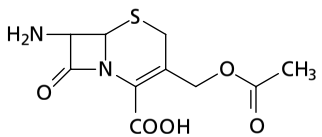
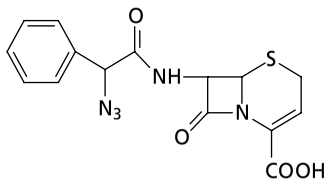
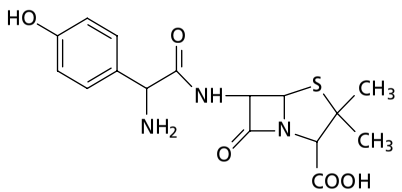
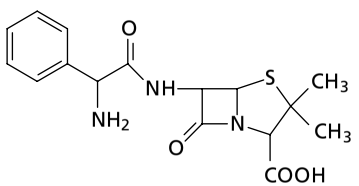
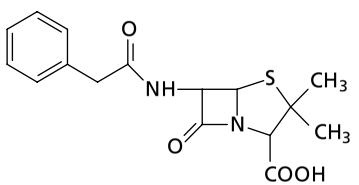
Drug and prodrug transport

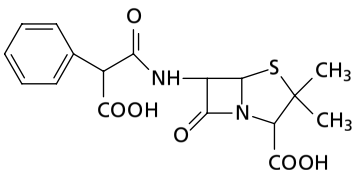
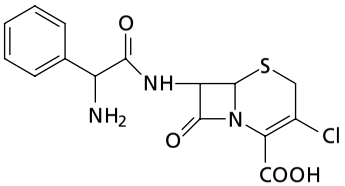
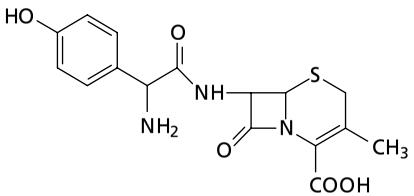
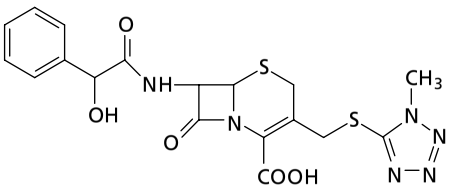
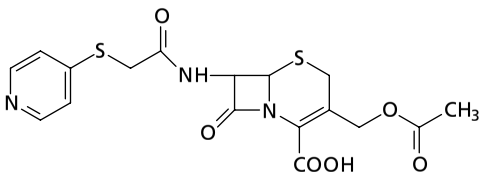
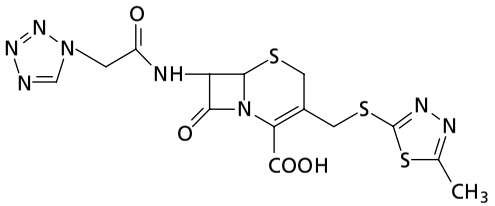
The subject of drug and prodrug transport is one of the focal points in the field of membrane transport in general. Well-known examples for transporters involved in drug delivery are the organic anion transporters (OATs), the organic cation transporters (OCTs), the organic anion transporting polypeptides (OATPs) and primary active pumps such as P-glycoprotein. In all cases, compounds bearing structural resemblance to physiologically occurring substrates are potential substrates. For peptide transporters, first evidence for drug transport came from the observation that Phe-Gly and cephalexin interact during absorption in rat jejunum (Quay & Foster 1970; Quay 1972). In the last three to four decades, several hundred reports on the subject have been published and there are many reviews and books available on the topic of drug/prodrug transport via the peptide transporters (for review see Leibach & Ganapathy 1996; Brodin et al 2002; Daniel & Rubio-Aliaga 2003; Nielsen & Brodin 2003; Brandsch et al 2004; Terada & Inui 2004; Biegel et al 2006b). Several authors consider transporters as drug targets when talking about transporter-mediated drug delivery across membranes (Amidon & Sadée 1999). But, as Nielsen et al (2002a) point out, peptide transporters are not drug targets per-se. Peptide transporters are routes or mediators for drug delivery, the actual drug targets being located in distal compartments behind the epithelial barriers.

β-Lactam antibiotics

It has been well established that it is the activity of PEPT1 at the intestinal epithelium that allows the effective oral availability of several β-lactam antibiotics and many other drugs or prodrugs. β-Lactam antibiotics bear sterical similarities to the backbone of physiologically occurring tripeptides. In an early study by Addison et al (1975), cephalexin at a concentration of 20 mM inhibited Gly-Sar-Sar uptake in hamster jejunum in-vitro by 56%. In the mid-1980s, after the identification of the driving force of renal and intestinal peptide transport, it was demonstrated conclusively that several aminocephalosporins and penicillins are substrates for PEPT1 and that the energy source for their active transport is the electrochemical proton gradient (Nakashima et al 1984; Okano et al 1986; Tsuji et al 1987; Iseki et al 1989). In Table 4 we compiled the structures of 45 penicillins and cephalosporins together with general statements regarding their affinity for PEPT1 and PEPT2 and the available evidence for their actual membrane translocation mediated by these carriers.

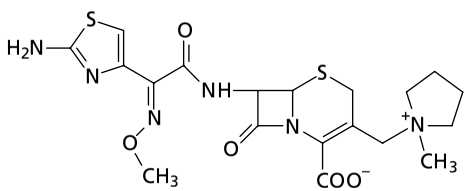
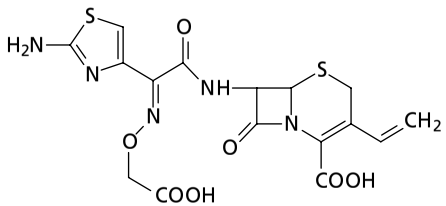
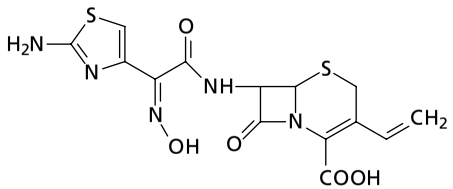
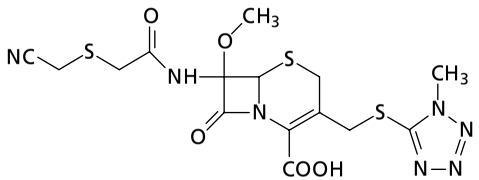
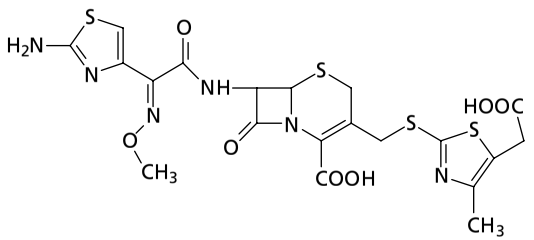
Table 4 Interaction of β-lactam antibiotics with the H^+/peptide symporters PEPT1 and PEPT2

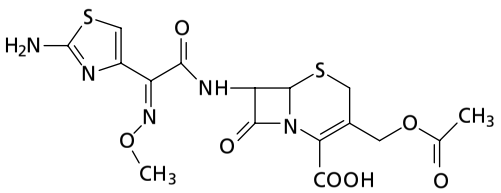
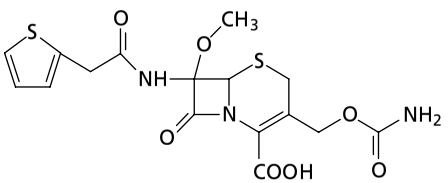
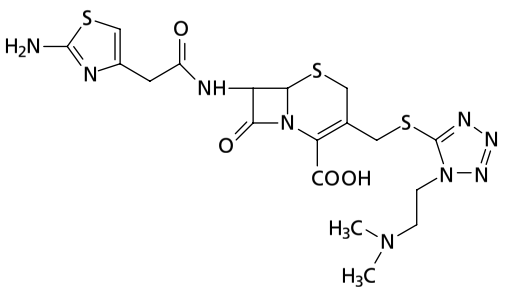
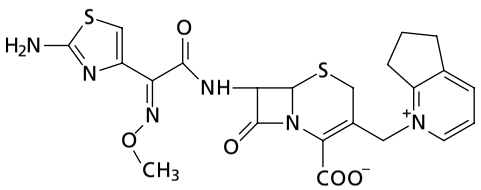
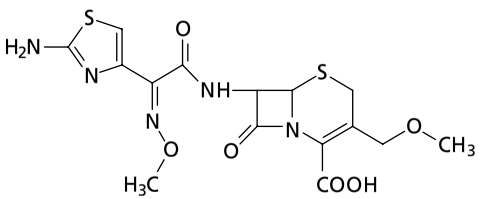
Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
6-Aminopenicillanic acid		no[a]		no[a]	
7-Aminocephalosporanic acid		low[a]		low[a]	
Acidocillin		low[b]		medium[c]	
Amoxicillin		no[a, d, e] low[f, g]	no[d] yes[e]	medium[a, e, f, h]	yes[e]
Ampicillin		no[d, f, i, j] low[k]	no[d]	low[a, h] medium[f]	yes[h]
Benzylpenicillin		no[j, k] low[g]		no[a, h]	

Carbenicillin				no[h]	
Cefaclor		low[d, i, k, l] medium[e, j]	yes[d, e, i, l, m]	high[a, e, h]	yes[e]
Cefadroxil		low[g, k] medium[d, f, j, n]	yes[d, g, j, m]	high[a, f, h, n, o]	yes[o]
Cefamandole		no[j] low[k]	no[m]	low[a]	
Cefapirin		no[k]	no[m]	no[a, h]	
Cefazolin		no[b, k]	no[m]	no[c]	

(Continued)

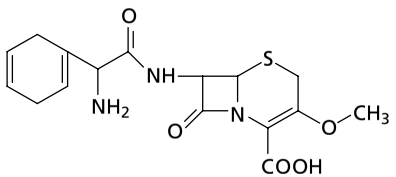
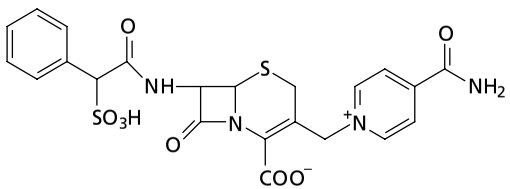
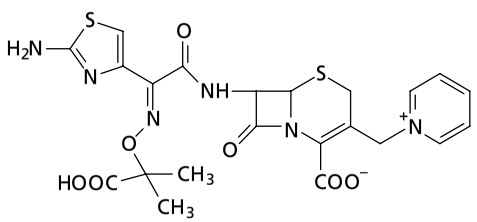
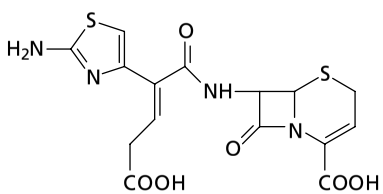
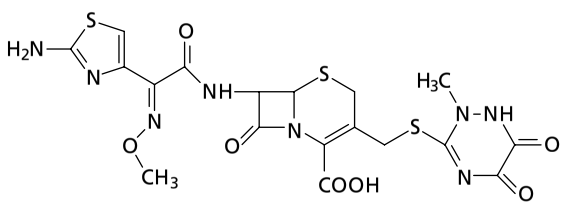
Table 4 *(Continued)*

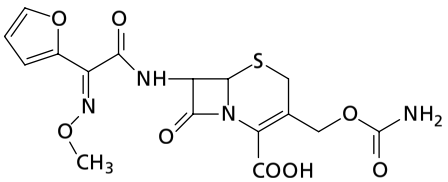
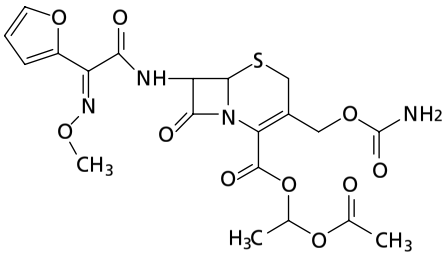
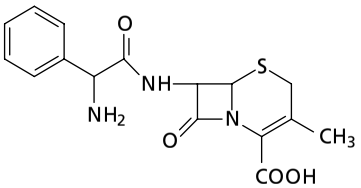
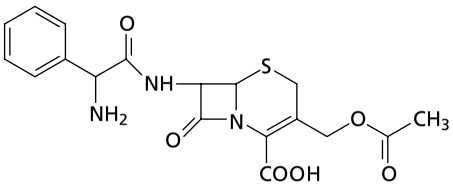
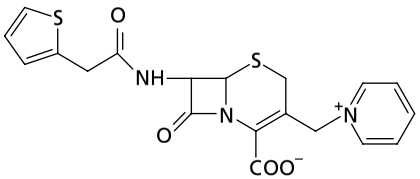
Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Cefepime		no[k]		no[a]	
Cefixime		no[l] low[f, g, k, p, q]	yes[g, l]	no[f] low[a, p]	
Cefdinir		no[p] low[f]		no[f, p]	
Cefmetazole		no[k]		low[a]	
Cefodizime		no[k]		no[a]	

Cefotaxime		no[k]	no[m]	no[a]
Cefoxitin		low[b]	no[m]	no[c]
Cefotiam		no[r]	no[q]	no[r]
Cefpirome		no[k]		no[a]
Cefpodoxime		no[b]		no[c]

(Continued)

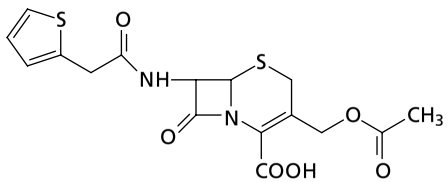
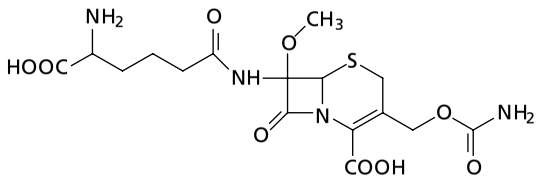
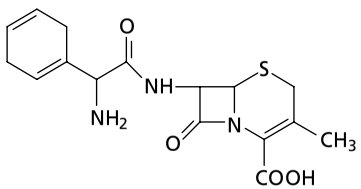
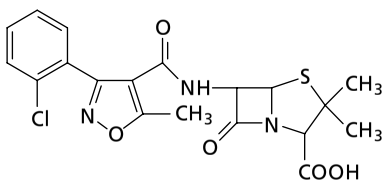
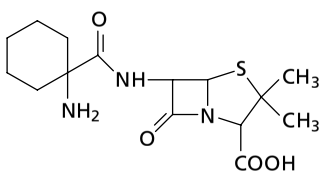
Table 4 *(Continued)*

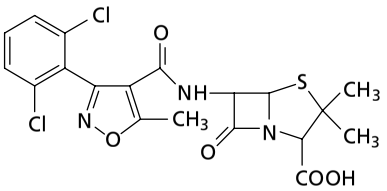
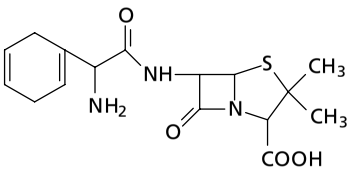
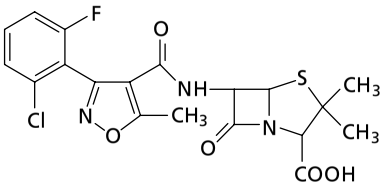
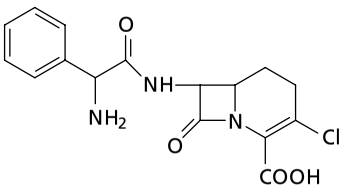
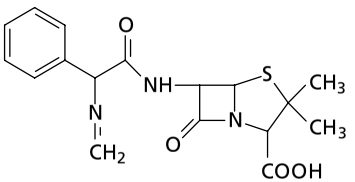
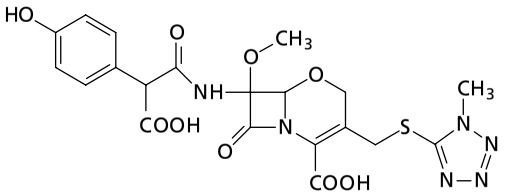
Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Cefroxadine				medium[h]	
Cefsulodine		no[k]	no[m]	no[a]	
Ceftazidime		no[k]	no[m]	no[a]	
Ceftibuten		medium[f, p, q] high[k]	yes[p, q]	low[f] medium[a, p]	
Ceftriaxone		no[k]		no[a]	

Cefuroxime		no[d, k]	no[d, m]	no[a]	
Cefuroxime axetil		low[k]		no[a]	
Cephalexin		low[g, i, k, l] medium[d, f]	yes[d, i, l, m]	high[a, f, h, n]	yes[h, n]
Cephaloglycine		no[j]	yes[m]	low[h]	
Cephaloridine		no[k]	no[m]	no[a, h]	

(Continued)

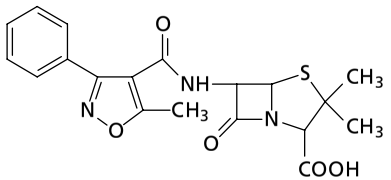
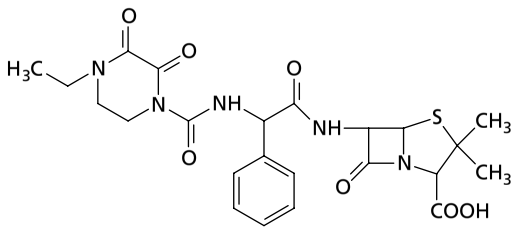
Table 4 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Cephalothin		no[d, j] low[g, k]	no[d, m]	no[a, h]	
Cephamycin C				no[h]	
Cephradine		low[d, f, i, k, q]	yes[d, i, m, q]	medium[h] high[a, f]	
Cloxacillin		medium[a]		medium[a]	
Cyclacillin		medium[n] high[f, k, n]		high[a, f, n]	

Dicloxacillin		low[a]		medium[a]
Epicillin				low[h]
Flucloxacillin		low[b]		low[c]
Loracarbef		low[g, l]	yes[l]	high[h]
Metampicillin		low[a]		low[h] medium[a]
Moxalactam		low[a]		high[a]

(Continued)

Table 4 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Oxacillin		low[a]		low[a]	
Piperacillin		no[b]		no[r]	

Assessment of apparent affinity was done according to our classification for PEPT1 in Caco-2 cells (Table 2) and PEPT2 in SKPT cells (Table 3): PEPT1: affinity constants < 0.5 mM = high affinity, 0.5–5 mM = medium affinity, > 5 mM = low affinity, > 15 mM = no interaction. PEPT2: affinity constants < 0.1 mM = high affinity, 0.1–1 mM = medium affinity, > 1 mM = low affinity, > 5 mM = no interaction. [a]Luckner & Brandsch 2005, [b]Biegel et al 2005, [c]Biegel et al 2006b, [d]Faria et al 2004, [e]Li et al 2006b, [f]Terada et al 1997b, [g]Wenzel et al 1996, [h]Daniel & Adibi 1993, [i]Han et al 1999, [j]Boll et al 1994, [k]Bretschneider et al 1999, [l]Dantzig et al 1994a, [m]Raeissi et al 1999, [n]Ganapathy et al 1995, [o]Boll et al 1996, [p]Ganapathy et al 1997, [q]Terada et al 1997a, [r]own unpublished data.

In an extensive study on structure–activity relationships, Snyder et al (1997) evaluated 47 analogues of the carbacephalosporin loracarbef and the cephalosporin cephalexin with regard to structural features necessary for interaction with PEPT1 and for antimicrobial activity. It was concluded that: (i) the free amino group may not be necessary for interaction; (ii) the carrier shows preference for L-stereoisomers; (iii) the less polar compounds competed best for drug uptake; and (iv) the phenylglycine side chain is not absolutely required for binding (Snyder et al 1997). The presence of an α-amino group on cephalosporins increases recognition by PEPT1 but is not an absolute requirement for substrate interaction (Raeissi et al 1999). In a systematic approach we studied the structure–transport relationship of 23 β-lactam antibiotics by measuring their recognition by PEPT1, their cellular uptake and their total transepithelial flux across Caco-2 cell monolayers (Bretschneider et al 1999). Ceftibuten and cyclacillin were recognized by PEPT1 with high affinity ($K_i = 0.3$ and 0.5 mM, respectively, Table 4). Cefadroxil, cefaclor, cephalexin, ampicillin and many others interacted with PEPT1 with low affinity ($K_i = 7–14$ mM). Cefapirin, cefuroxime, benzylpenicillin, ceftriaxone, cefsulodin and others displayed no affinity to the transport system ($K_i > 20$ mM). The following structural features seemed relevant to us for β-lactam antibiotic recognition: (i) sterical resemblance to the tripeptide backbone; (ii) N-terminal peptide bond with an α-amino group; (iii) carboxy group at the dihydrothiazine ring of the cephalosporins or the thiazolidine of the penicillins; and (iv) substituents on and saturation of the N-terminal ring systems. These conclusions were later supported by molecular modelling studies (Biegel et al 2005). The amino group of those β-lactam antibiotics containing an aminothiazole ring (type I, see Biegel et al 2005) has donor properties just like the N-terminal ammonium group of high affinity substrates, which may also explain the high affinity of ceftibuten.

The total transepithelial flux of the β-lactam antibiotics through the Caco-2 cell monolayers differed by a factor of 70 and was highest for cefadroxil (Bretschneider et al 1999). Uptake into the cells and transepithelial flux were highest for those β-lactam antibiotics that showed the highest affinity to PEPT1. We concluded that the overall variation of transepithelial flux is mainly determined by the variation of the affinity to PEPT1 ($r^2 = 0.61$). When the route of medical application and the oral availability (%) were taken into account it became clear that the affinity constants measured in this study are very well correlated with the bioavailability data of the compounds. Only those β-lactam antibiotics with $K_i < 14$ mM are orally available. From all this evidence, we finally concluded that the route of application for β-lactam antibiotics is mainly determined by their affinity to PEPT1.

With regard to actual membrane translocation, Tamai et al (1997) using the *X. laevis* oocytes expression system demonstrated the predominant role of PEPT1 in the carrier-mediated intestinal absorption of β-lactam antibiotics. Wenzel et al (1995, 1996) conclude from their studies that PEPT1 is capable of stereoselective transport of neutral as well as anionic/dianionic β-lactams in their zwitterionic form.

It has been known for 20 years that the renal type peptide transporter PEPT2 also accepts β-lactam antibiotics as substrates. The first systematic study focused on determinants of affinity was performed by Daniel & Adibi (1993) in kidney brush-border membrane vesicles. Ganapathy et al (1995) found differences in the recognition of β-lactam antibiotics between PEPT1 and PEPT2. The penicillin cyclacillin was 9-fold more potent than the cephalosporin cefadroxil in competing with Gly-Sar for uptake via PEPT1. In contrast, cefadroxil was 13-fold more potent than cyclacillin in competing with the dipeptide for uptake via PEPT2. To compare the substrate recognition pattern between PEPT1 and PEPT2 systematically, we examined the interaction of 31 cephalosporins and penicillins with PEPT2 expressed in renal SKPT cells (Luckner & Brandsch 2005). Cefadroxil, cefaclor, cyclacillin, cephradine, cephalexin and moxalactam were recognized by PEPT2 with a very high affinity, comparable with that of natural dipeptides (Table 4). Ceftibuten, dicloxacillin, amoxicillin, metampicillin, cloxacillin, ampicillin, cefixime, cefamandole, oxacillin and cefmetazole interacted with PEPT2 with medium affinity. For the other β-lactam antibiotics, interaction was very low or not measurable. The affinity constants of β-lactam antibiotics at rPEPT2 and hPEPT1 were significantly correlated. We found no compound that is a ligand for PEPT1 but not for PEPT2. Decisive differences in compound recognition became evident when, as described above, $K_{i\ PEPT1}/K_{i\ PEPT2}$ quotients were calculated. Of the 31 β-lactam antibiotics studied, 17 compounds displayed a ratio between 3 and 20. For five cephalosporins (cefadroxil, cefaclor, cephradine, cephalexin and moxalactam), however, the quotients were > 100 (for cefadroxil even 2400). This result supports hypotheses according to which PEPT2 has a disproportionally higher affinity for β-lactam antibiotics carrying an α-amino group than PEPT1 (Boll et al 1996; Terada et al 1997b; Raeissi et al 1999; Luckner & Brandsch 2005). A hydroxyl group at the N-terminal phenyl ring increases the affinity for PEPT1 and PEPT2 further, but again disproportionally more for PEPT2. In general, 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 & Adibi (1993) had already reported that the marked hydrophobicity of the N-terminal region of aminopenicillins increases the affinity to the renal H^+/peptide cotransporter. Terada et al (1998) 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.

Compared with PEPT1, there are not as many reports on actual transport of β-lactam antibiotics by PEPT2. Most measurements were done by the group of H. Daniel (Boll et al 1996) on PEPT2 expressing *X. laevis* oocytes. Several other groups directly confirmed the uptake of radiolabelled or unlabelled amoxicillin, cefaclor, ceftibuten or cephalexin in native renal cells or PEPT2-transfected cells (Ganapathy et al 1995, 1997; Li et al 2006b).

PEPT2 is also expressed in the choroid plexus. Ocheltree et al (2004b) studied the uptake of [^{3}H]cefadroxil in PEPT2 wild-type and null mice plexus as a function of temperature, transport inhibitors, pH and saturability. Gly-Sar or cefadroxil coadministration could inhibit the uptake of cefadroxil in

PEPT2$^{+/+}$ mice but not PEPT2$^{-/-}$ mice. Except for tissue-specific modifications and modulations, the PEPT2 substrate specificity is presumably the same no matter whether a renal or a choroid plexus cell is studied.

It should be noted that peptide transporters are not the only membrane carriers for β-lactam antibiotics (Tsuji et al 1993; Ganapathy et al 2000). Moreover, β-lactam antibiotics taken up into the cell can be subject to enzymatic degradation or to efflux processes at the apical membrane back into the intestinal or tubule lumen (Saitoh et al 1996). Hence, it is not exclusively the activity of peptide transporters that affects the absorption of β-lactam antibiotics.

Angiotensin-converting enzyme inhibitors

In almost every review on H^+/peptide transporters and in the introduction of many original papers, it is stated that angiotensin-converting enzyme (ACE) inhibitors are peptide transporter substrates. Just as for β-lactam antibiotics, the oral availability of ACE inhibitors is explained by uptake via PEPT1 at the intestinal epithelium. In the beginning, this view was based on publications from the group of G. L. Amidon, reporting that the intestinal H^+/peptide transporter takes up ACE inhibitors such as captopril, enalapril, lisinopril, quinapril, benazepril and ceronapril (Table 5, for review see Bai & Amidon 1992; Amidon & Sadée 1999). However, the transport of these compounds is still a matter of controversy. For instance, the affinity constants for enalapril transport attributed to the intestinal peptide transporter reported in the literature range from 0.07 mM to > 20 mM (Bai & Amidon 1992; Moore et al 2000). Several authors consider the orally active ACE inhibitors captopril and enalapril as PEPT1 substrates and itemize them as such in their reviews. Swaan et al (1995) evaluated the affinity of enalapril, enalaprilat and lisinopril for the intestinal peptide carrier based on their ability to inhibit the transport of cephalexin at pH 7.4 in an Ussing chamber and tried to explain their affinity with three-dimensional structural data. However, the K_i values of enalapril, enalaprilat and lisinopril reported were 0.15, 0.28 and 0.39 mM, respectively, suggesting a surprisingly high affinity. Moore et al (2000) and most others found no affinity of captopril, enalapril, enalaprilat and lisinopril for PEPT1 ($K_i > 20$ mM). Thwaites et al (1995) showed weak interaction of captopril and enalapril with PEPT1 but suggested their actual translocation. So far, in our hands these compounds did not show any convincing substrate or inhibitor properties at PEPT1: the affinity constants for captopril and enalapril measured in our laboratory were > 30 mM and 12 mM, respectively (Brandsch et al 2004). For enalapril, K_i values of 6.2 and 4.3 mM have been determined for PEPT2 (Lin et al 1999; Zhu et al 2000).

In contrast, fosinopril undoubtedly interacts with PEPT1 and PEPT2 with very high affinity. For PEPT1, a K_i value of 110 μM has been reported (Moore et al 2000). Lin et al (1999) have reported K_i values of 55 μM and 81 μM for fosinopril and zofenopril, respectively, for PEPT2. Moreover, the high affinity of fosinopril was confirmed in studies using Caco-2 cells expressing PEPT1 ($K_i = 35.5$ μM) and in SKPT cells expressing PEPT2 ($K_i = 29.6$ μM; Shu et al 2001). These authors showed in subsequent experiments that fosinopril is transported intact by a proton-coupled, saturable process. Intracellular accumulation of fosinopril was 3-to 4-times higher from the apical side than from the basolateral side. From these results it could be concluded that fosinopril is a high-affinity substrate that is indeed transported by peptide transporters, even though this might seem unlikely when looking at the structure of the molecule (Table 5).

Another matter of controversy concerns quinapril. In some reports this compound is considered a noncompetitive, non-transported inhibitor because quinapril inhibited reference peptide transport with affinity constants of 0.81 mM for PEPT1 and 0.41 mM for PEPT2 (Zhu et al 2000) but could not elicit any current in *X. laevis* oocytes expressing PEPT1 or PEPT2 (Akarawut et al 1998; Chen et al 1999; Zhu et al 2000). Kitagawa et al (1997) demonstrated in intestinal brush-border membrane vesicles a competitive nature of peptide transport inhibition by quinapril ($K_i = 0.46$ mM). Other reports specifically suggest that quinapril is a peptide transporter substrate (Bai & Amidon 1992; Hu et al 1995). It should be noted that quinapril interacts also with several other organic solute transporters such as organic anion transporter 3 (Chu et al 2007).

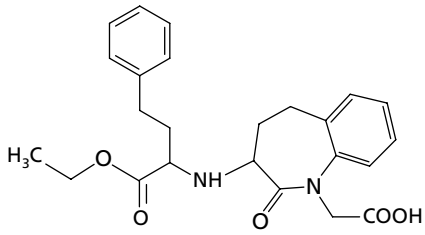
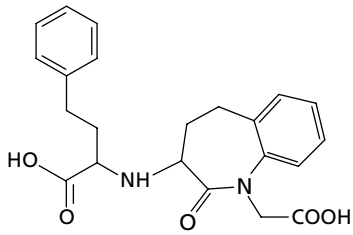
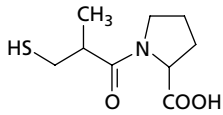
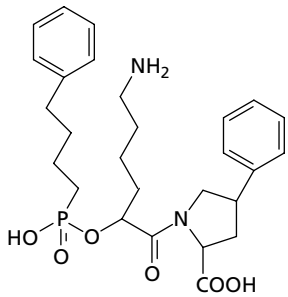
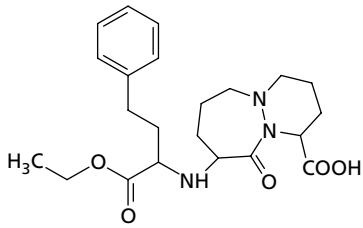
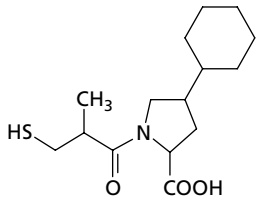
In our view, the topic of ACE inhibitor transport by peptide carriers should be revisited. Systematic studies on the translocation of ACE inhibitors using today's advanced experimental techniques are warranted. In electrophysiological experiments of PEPT1 and PEPT2 performed so far, low signals elicited by cotransport of H^+ with enalapril or captopril were reported (Boll et al 1994; Zhu et al 2000; Faria et al 2004). For the conflicting results, experimental differences such as buffer composition and the cell and tissue preparations used might be responsible. In particular for academic groups and companies working on the development of new ACE inhibitors, the current situation is unsatisfactory.

Other drugs and prodrugs

In the early 1990s, Beauchamp and coworkers synthesized and tested 18 amino acid esters of the antiviral agent aciclovir. They observed that, compared with orally administered aciclovir, ten of the esters produced greater amounts of aciclovir in rat urine than the parent compound (Beauchamp et al 1992). The L-valyl ester was the best prodrug: 63% of its administered dose was excreted as aciclovir in the urine compared with administered aciclovir for which this value was 19%. After clinical evaluation, the prodrug was approved and is now marketed under the name Valtrex or Zelitrex, being used for the oral treatment of several viral infections. To the best of our knowledge, Lee et al (1996) were first to suggest hPEPT1-mediated uptake of valaciclovir into intestinal cells as the underlying mechanism for elevated absorption. In an extensive study, Ganapathy et al (1998) characterized valaciclovir as a hPEPT1 substrate of high affinity ($K_i = 0.49$ mM). The phenomenon has been confirmed by Balimane et al (1998) and other groups (Table 6). Similarly, valganciclovir, the valine ester of ganciclovir, has been reported to be a substrate of PEPT1 (Sugawara et al 2000). Both prodrugs are also substrates for PEPT2, interacting with the carrier protein with medium or high affinity, respectively (Ganapathy et al 1998; Sugawara et al 2000).

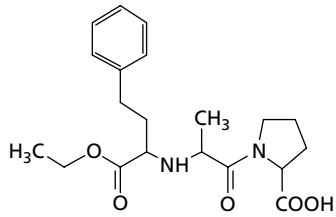
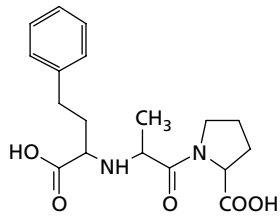
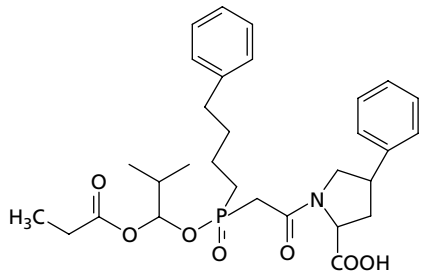
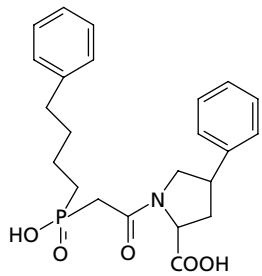
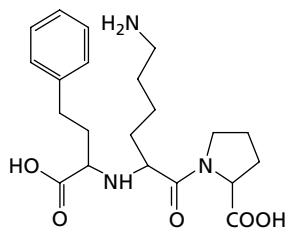
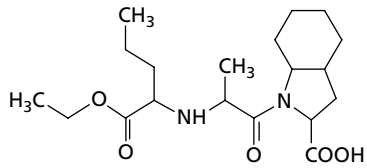
Other L-valyl prodrugs are Val-2-bromo-5, 6-dichloro-1-(β-D-ribofuranosyl) benzimidazole, Val-cytarabine, Val-floxuridine, Val-gemcitabine, Val-levovirin (R1518) and

Table 5 Interaction of ACE inhibitors with the H^+/peptide symporters PEPT1 and PEPT2

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Benazepril		medium[a]			
Benazeprilat		low[a]			
Captopril		no[a, b] low[c]	yes[d, c]	low[e, f]	yes[d]
Ceronapril		medium[g, a, h]	yes[g, h]		
Cilazapril		medium[a]			
4-Cyclohexylcaptopril		medium[a]			

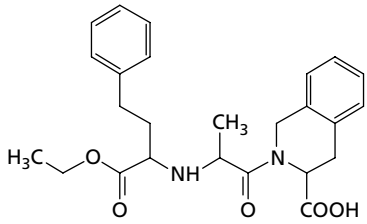
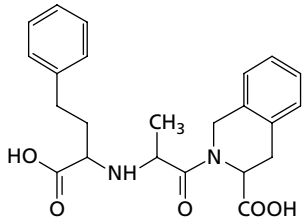
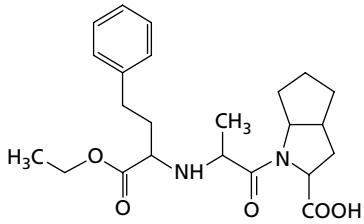
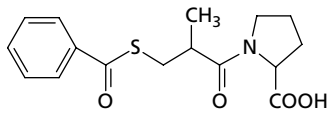
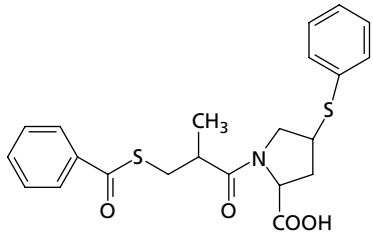
(Continued)

Table 5 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Enalapril		no[a, b] low[d, c, h] medium[i, j]	yes[d, h]	no[e, f] low[d]	yes[d]
Enalaprilat		no[a] low[j]		low[e]	
Fosinopril		high[a, k]	yes[k]	high[e, k]	yes[k]
Fosinoprilat		medium[a]			
Lisinopril		low[a, b, c, h]	no[b, h]	low[e]	
Perindopril		low[a]			

(Continued)

Table 5 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Quinapril		medium[a, d]	no[d, l]	medium[e, s]	no[d]
Quinaprilat				low[e]	
Ramipril				medium[e]	
S-Benzylcaptopril		medium[a]			
Zofenopril				high[e]	

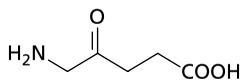
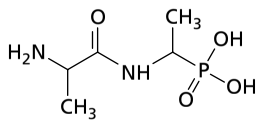
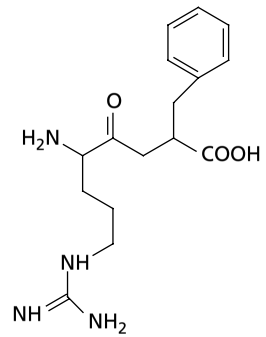
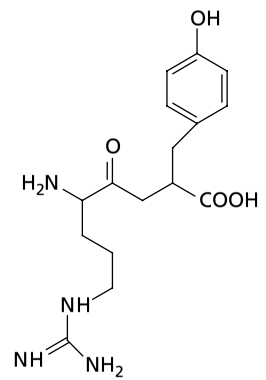
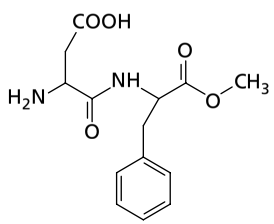
Assessment of apparent affinity was done according to our classification for PEPT1 in Caco-2 cells (Table 2) and PEPT2 in SKPT cells (Table 3): PEPT1: affinity constants < 0.5 mM = high affinity, 0.5–5 mM = medium affinity, > 5 mM = low affinity, > 15 mM = no interaction. PEPT2: affinity constants < 0.1 mM = high affinity, 0.1–1 mM = medium affinity, > 1 mM = low affinity, > 5 mM = no interaction. [a]Moore et al 2000, [b]Thwaites et al 1995, [c]Boll et al 1994, [d]Zhu et al 2000, [e]Lin et al 1999, [f]Boll et al 1996, [g]Nicklin et al 1996, [h]Faria et al 2004, [i]Han et al 1999, [j]Yuasa et al 1994, [k]Shu et al 2001, [l]Chen et al 1999.

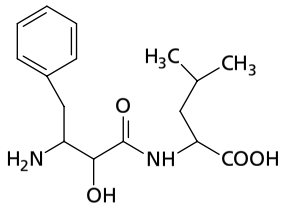
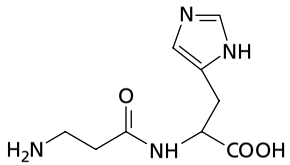
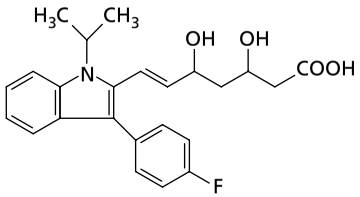
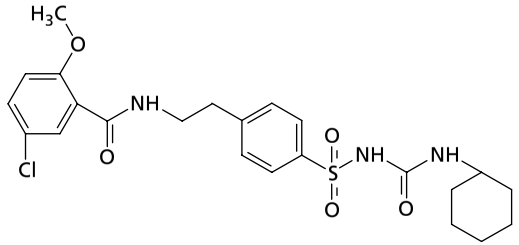
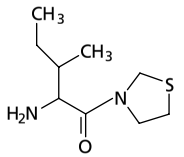
Val-zidovudine (Table 6). For most of them, not only interaction but also actual transport by peptide transporters have been shown. For other prodrugs, glycine has been used as N-terminal amino acid (e.g. for midodrine) (Tsuda et al 2006, Table 6).

The prodrug strategy has also been applied to L-α-methyldopa (Table 6). Intestinal permeability of five dipeptidyl derivatives of the drug was studied by an in-situ intestinal perfusion method (Hu at al 1989). The dipeptides displayed higher permeabilities than the parent drug. The prodrugs are hydrolysed to the active drug within the intestinal cells. Involvement of PEPT1 in the translocation of L-dopa-L-Phe has been shown (Hu et al 1989; Tsuji et al 1990; Tamai et al 1998; Amidon & Sadée 1999).

A noteworthy example for drug delivery by peptide transporters is δ-ALA (Table 2, 6). This compound has gained interest as an endogenous photosensitizer for fluorescence diagnosis and photodynamic tumour therapy. When administered

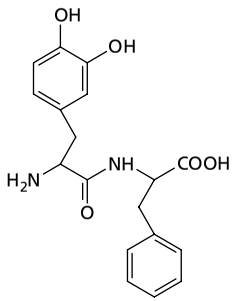
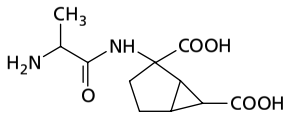
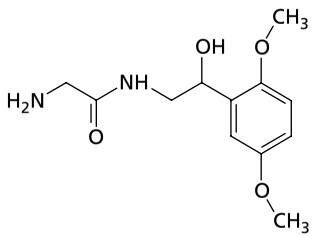
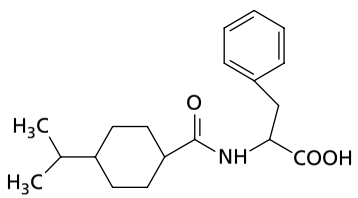
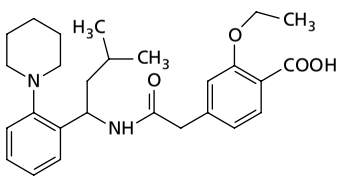
Table 6 Interaction of other drugs with the H^+/peptide symporters PEPT1 and PEPT2

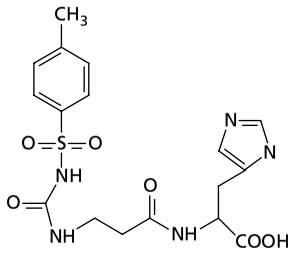
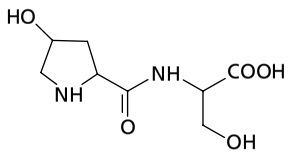
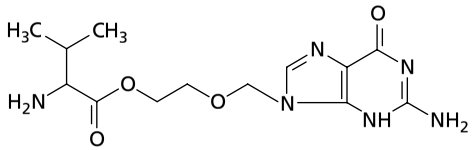
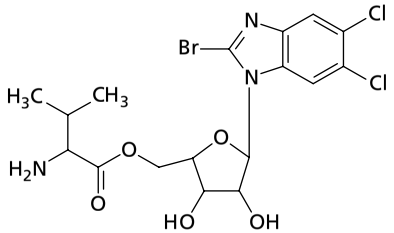
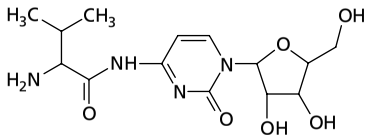
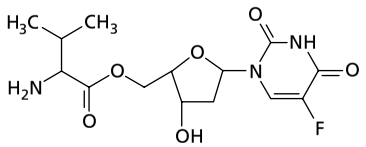
Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
δ-Aminolevulinic acid		medium[a, b, c, d] high[e]	yes[b, d, e]	medium[a, e, f]	yes[e]
Alafosfalin		high[g]	yes[g]	high[g]	
Arphamenine A		high[h]	yes[h]	high[i]	
Arphamenine B				high[i]	
Aspartame		low[j]			

Bestatin		low[k] medium[a, l]	yes[k, l, m]	high[a, i]	yes[m]
Carnosine		low[a] medium[n]	yes[n, o]	high[a, p]	yes[p]
Fluvastatin		high[j]			
Glibenclamide		high[q]	no[q]	high[q]	no[q]
Ile-thiazolidide		low[r] medium[s]	yes[s]	medium[s]	no[s]

(Continued)

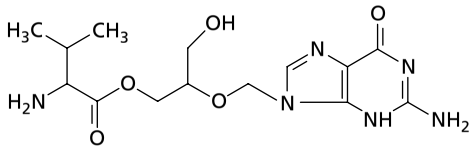
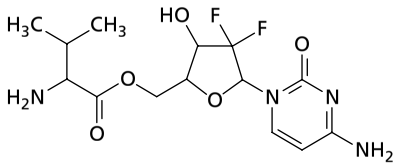
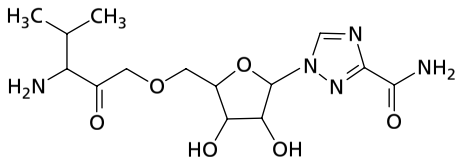
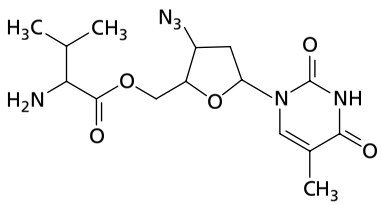
Table 6 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
L-Dopa-Phe		medium[t] high[u]	yes[t, u]		
LY544344		high[v]			
Midodrine		medium[w]	yes[w]		
Nateglinide		high[x]	no[x]	high[x]	no[x]
Repaglinide		high[j]			

4-Toluenesulfonyl-ureido-carnosine		medium[n]		
trans-4-L-Hydroxyprolyl-L-serine (JBP923)		low[y]		
Val-aciclovir		medium[a, b, l, z] high[d]	yes[b, d, l]	medium[a, z]
Val-2-bromo-5, 6-dichloro-1-(β-D-ribofuranosyl) benzimidazole		high[aa]		
Val-cytarabine			yes[bb]	
Val-floxuridine		medium[cc]	yes[cc]	

(Continued)

Table 6 *(Continued)*

Drug	Structure	PEPT1		PEPT2	
		Affinity	Transport	Affinity	Transport
Val-ganciclovir		medium[dd]	yes[dd]	high[dd]	
Val-gemcitabine		high[ee]	yes[ee]		
Val-levovirin (R1518)		low[ff]	yes[ff]		
Val-zidovudine		high[gg]			

Assessment of apparent affinity was done according to our classification for PEPT1 in Caco-2 cells (Table 2) and PEPT2 in SKPT cells (Table 3): PEPT1: affinity constants < 0.5 mM = high affinity, 0.5–5 mM = medium affinity, > 5 mM = low affinity, > 15 mM = no interaction. PEPT2: affinity constants < 0.1 mM = high affinity, 0.1–1 mM = medium affinity, > 1 mM = low affinity, > 5 mM = no interaction. [a]Terada et al 2000b, [b]Bhardwaj et al 2005, [c]Brandsch et al 2004, [d]Irie et al 2001, [e]Döring et al 1998a, [f]Bravo et al 2005, [g]Neumann et al 2004, [h]Enjoh et al 1996, [i]Daniel & Adibi 1994, [j]Ekins et al 2005, [k]Terada et al 1997a, [l]Faria et al 2004, [m]Saito et al 1996, [n]Nielsen et al 2002b, [o]Vabeno et al 2004, [p]Teuscher et al 2001, [q]Sawada et al 1999a, [r]Brandsch et al 1999, [s]Foltz et al 2004, [t]Tsuji et al 1990, [u]Tamai et al 1998, [v]Bueno et al 2005, [w]Tsuda et al 2006, [x]Terada et al 2000d, [y]Liu et al 2000, [z]Ganapathy et al 1998, [aa]Song et al 2005b, [bb]Cheon et al 2006, [cc]Landowski et al 2005, [dd]Sugawara et al 2000, [ee]Song et al 2005a, [ff]Li et al 2006a, [gg]Han et al 1998.

orally, intact δ-ALA is very well absorbed in the gastrointestinal tract (Dalton et al 1999). In the kidney, δ-ALA is efficiently reabsorbed. This observation and the interesting feature that δ-ALA contains a ketomethylene group instead of a peptide bond prompted Döring et al (1998a) to perform a thorough investigation of δ-ALA transport focused on peptide transporters. They reported that δ-ALA represents a high affinity substrate for the H^+/peptide cotransporters PEPT1 and PEPT2. Irie et al (2001) confirmed these results and showed, with Caco-2 cells grown on filters, greater transport activity from the apical-to-basolateral than in the opposite direction. In the choroid plexus epithelium, δ-ALA is also transported by PEPT2 (Novotny et al 2000; Ocheltree et al 2004a). More recent studies have established the neuroprotective role for PEPT2 in modulating the toxicity of δ-ALA (Hu et al 2007).

Photodynamic tumour therapy can also be employed for treatment of tumours of the extrahepatic biliary duct (Whitaker et al 2000). Having shown the expression of the low-affinity H^+/peptide cotransporter PEPT1 at the epithelium of the extrahepatic biliary duct (Knütter et al 2002), we investigated the transport characteristics of [^{3}H]δ-ALA in bile duct tumour cells (Neumann & Brandsch 2003). Uptake of [^{3}H]δ-ALA into human cholangiocarcinoma SK-ChA-1 cells was pH dependent and carrier mediated with an apparent affinity (K_t) of 2.1 mM. Further experiments revealed that Gly-Sar and δ-ALA are transported via one and the same system, PEPT1. We concluded that δ-ALA could be accumulated in bile duct tumour cells via PEPT1 before photodynamic therapy (Neumann & Brandsch 2003). It has been shown several times that bestatin, an inhibitor of aminopeptidases, is transported by peptide transporters in intestinal (Tomita et al 1990) and renal (Hori et al 1993) epithelial cells. The evidence that PEPT1 and PEPT2 recognize and actually translocate bestatin is unequivocal (Daniel & Adibi 1994; Terada et al 1997a, 2000b; Faria et al 2004). Similarly, transport of β-Ala-His (carnosine) by peptide transporters was studied more than 30 years ago (Matthews et al 1974). The dipeptide is a low to medium affinity substrate for PEPT1 (Terada et al 2000b; Nielsen et al 2002b; Vabeno et al 2004) and a high-affinity substrate for PEPT2 (Terada et al 2000b; Teuscher et al 2001). To investigate transport characteristics of its anticancer derivative 4-toluenesulfonylureido-carnosine, Nielsen et al (2002b) performed uptake and flux experiments with Caco-2 cells. L-Carnosine was transported across the apical membrane with an apparent K_m of 2.5 mM. 4-Toluenesulfonylureido-carnosine had a similar affinity ($K_i = 2.3$ mM), but transepithelial transport was low. The authors concluded that L-carnosine is not a suitable dipeptide moiety for hPEPT1-mediated absorption of sulfonamide-type anti-cancer compounds (Nielsen et al 2002b).

The interaction of the antibacterial dipeptide derivative alafosfalin with mammalian H^+/peptide cotransporters has been demonstrated in Caco-2 and in SKPT cells. Alafosfalin displays high affinity to both H^+/peptide symporters and is accumulated in intestinal and renal epithelial cells via a H^+-symport mechanism, thus explaining its oral availability (Neumann et al 2004). The observation of H^+/alafosfalin cotransport also helped change the definition of structural requirements for peptide transporter substrates. It demonstrates that dipeptides where the C-terminal carboxyl group is substituted by a phosphonic function represent high affinity substrates for mammalian H^+/peptide cotransporters.

Other prodrugs are the orally active hydroxyprolylserine derivatives such as trans-4-L-hydroxyprolyl-L-serine (JBP923, Liu et al 2000). JBP923 is almost completely absorbed from the gastrointestinal lumen. The compound was able to inhibit the H^+-dependent transport of Gly-Sar in brush-border membrane vesicles, suggesting the involvement of peptide transporters (Liu et al 2000). For the anti-diabetic drugs nateglinide and glibenclamide, high interaction with peptide transporters but no actual transport has been found (Sawada et al 1999a; Terada et al 2000d, Table 6).

For further reading on drug transport by peptide transporters, we would like to draw the reader's attention to reviews by Amidon & Sadée (1999), Inui & Terada (1999), Lee et al (1999), Tsuji (1999), Meredith & Boyd (2000), Nielsen et al (2002a), Brodin et al (2002), Rubio-Aliaga & Daniel (2002), Herrera-Ruiz & Knipp (2003), Nielsen & Brodin (2003); Steffansen et al (2004), Sai & Tsuji (2004), Steffansen et al (2005) and others.

We would like to emphasize that even though most results were obtained with rabbit, mouse and human peptide transporters, the results discussed here are certainly also relevant for the veterinary care of domestic animals.

As stated above, PEPT2 is also expressed in the respiratory tract (Meredith & Boyd 1995). The expression of transporter mRNA and protein was localized to the airway epithelium and alveolar type II pneumocytes. Therefore, we would also like to emphasize that the results on substrate specificity, drug and prodrug transport obtained with other tissues or isolated carriers might also be relevant for treatment of lung diseases or for pulmonary drug delivery (for review see Groneberg et al 2006).

Third, it should be noted that there are additional strategies to increase drug absorption by peptide transporter other than modifying substrate structure (e.g. it is possible to increase the transmembrane pH gradient as the driving force for peptide and drug uptake). Nozawa et al (2003) accomplished this in-situ and in-vivo by administration of a proton-releasing polymer. Eudragit L100-55 decreased the pH in rat intestinal loops and increased the disappearance of both cefadroxil and cefixime from the loops. After oral coadministration of cephalosporin and a proton-releasing polymer, the drug plasma concentrations were increased significantly (Nozawa et al 2003).

Clinical relevance of peptide transport

The intestinal peptide transport has not only pharmaceutical importance for oral drug delivery but also clinical relevance for enteral nutritional support in hospitalized patients. Mixtures of free amino acids are nutritionally inferior to mixtures of small peptides of comparable amino acid composition (Matthews 1975; Ganapathy et al 1994; Adibi 1997; Daniel 2004). Ganapathy et al (1994) summarized the physiological evidence for this conclusion as follows: (i) faster absorption of amino acids when given in the form of peptides than in the form of free amino acids; (ii) more even appearance of amino acids in blood after absorption from peptide mixtures than

from amino acid mixtures; (iii) avoidance of competition during transport between amino acids when absorbed as peptides instead of free amino acids; (iv) conservation of metabolic energy in transporting amino acids as oligomeric peptides rather than in the monomeric form; and (v) relative resistance of peptide transport compared to amino acid transport to numerous adverse conditions such as starvation, protein–calorie malnutrition, vitamin deficiency and intestinal diseases.

Peptide-based artificial enteral diets have additional important advantages: several amino acid-based enteral solutions lack tyrosine, glutamine, and cysteine because tyrosine is insoluble, and glutamine and cysteine are unstable. These amino acids can be conveniently included in the form of dipeptides in peptide-based solutions. Inclusion of the crucial amino acid glutamine in the form of dipeptides has been recommended not only in preparations meant for enteral nutrition but also in those for parenteral nutrition and for cell culture media. Such peptides are well tolerated and efficiently utilized by the body (Fürst et al 1990).

Furthermore, enteral diets based on free amino acids are hyperosmolar, which may be at least one of the contributing factors in the commonly encountered diarrhoeal complications of enteral nutrition. The tonicity of these solutions can be considerably decreased by providing the amino acids in the peptide form (Ganapathy et al 1994).

Since free amino acids and peptides do not share the same transporters, PEPT1 and PEPT2 can compensate for the lost ability to absorb and utilize specific amino acids in diseases related to amino acid transport defects. There are numerous genetic disorders affecting amino acid transport in the small intestine, the kidney or both (e.g. Hartnup disease, cystinuria, lysinuric protein intolerance and iminoglycinuria). Iminoglycinuria is characterized by a reduced reabsorption of proline, glycine and hydroxyproline in the kidney (Goodman et al 1967; Law & Sardharwalla 1978; Bröer et al 2006). Hartnup disease is a genetic disorder in which intestinal and renal transport of dipolar amino acids is defective, caused by mutations in the neutral amino acid transporter B^0AT1 (SLC6A19) expressed predominantly in kidney and intestine (Seow et al 2004). In spite of the defect, most patients with Hartnup disease do not exhibit obvious symptoms of protein malabsorption. These patients obtain adequate amounts of the affected amino acids via peptide transporters (Asatoor et al 1970; for review see Ganapathy et al 1994). In cystinuria, the defect is in the intestinal and renal absorption of cationic amino acids and cystine caused by mutations in either of the two genes coding for the two subunits of the heteromeric amino acid transporter $b^{0,+}$ (Palacin et al 2005). Again, just as in Hartnup disease, cystinuria patients also do not show any evidence of malnutrition because the amino acids are absorbed adequately in the form of small peptides (Hellier et al 1972; Daniel 2004). No genetic disorder has been reported in which the primary defect is in peptide transport systems.

Pharmacogenomics, polymorphisms and knockout models

Using genetic information, the field of pharmacy is now moving in the direction of more individual treatment and personalized drug dosage. Genetic variability of peptide transporters could have not only nutritional implications but also account for inter-individual differences in the disposition of peptidomimetic drugs. A recent study by Gerloff (2004) gives an overview about the possible impact of single nucleotide polymorphisms (SNPs) of transmembrane transporters on the pharmacokinetics of therapeutic substances. By virtue of high-throughput sequencing methods, screening of many samples for SNPs has become feasible in recent years. Several reports describe the identification of polymorphisms for hPEPT1 and hPEPT2 in different genomic DNA sample collections.

Some genetic variations of hPEPT1 without corresponding functional data were published by Leabman et al (2003). Zhang et al (2004) found nine non-synonymous hPEPT1 SNPs out of a panel of 44 ethnically diverse individuals. Characterization of the variants, when expressed in HeLa cells, revealed no significant differences in substrate transport with exception of the P586L variant. Its greatly reduced uptake rate could be explained by a post-translational reduction in plasma membrane expression. The two common SNPs S117N and G419A were also found to retain the wild type transport features in *X. laevis* oocytes (Sala-Rabanal et al 2006). In a study by Anderle et al (2006), besides several SNPs in non-coding regions, nine nonsynonymous coding SNPs were discovered. Eight were tested for function in COS-7 or CHO cells. Typical transport activity was verified for all of them except for the low frequency PEPT1-F28Y. This variant showed increased K_m values for dipeptides and cephalexin and a change in pH dependency. Furthermore, the authors found differences among ethnic populations and small effects of *cis* acting elements on transporter expression. All in all, the limited variability of the PEPT1 gene suggests a high evolutionary pressure on this protein preventing the survival of mutations which result in severe loss of function.

The situation seems to be different for hPEPT2. The group of Inui (Terada et al 2004) characterized two out of five previously identified SNPs selected based on high conservation of the respective positions across the species using HEK293 cells and *X. laevis* oocytes. The mutants P409S and R57H were both expressed in the plasma membrane, but in contrast to P409S, which exhibited wild-type like transport function, R57H had no detectable transport function. Pinsonneault et al (2004) selected two major variants out of 27 SNPs found in 247 DNA samples for their study. The variants are present at high frequency in all ethnic groups. CHO cells transfected with hPEPT2*1 and hPEPT2*2 displayed similar capacity but different affinity and different pH sensitivity for Gly-Sar transport. Moreover, variable mRNA expression, probably caused by *cis* acting polymorphisms, was observed. These results indicate a considerable extent of variability in the hPEPT2 gene with possible influence on pharmacokinetic profiles of peptide-like drugs.

The conclusions drawn from studies on the functional consequences of these naturally occurring variations match well the findings from site-directed mutagenesis studies. In general, changes within the putative transmembrane regions (TM) tend to be fatal whereas, for example, the large extracellular loop between TM9 and TM10 can be changed without any phenotypic implications in terms of transport function. Of course, drastic mutations at highly conserved

positions might not be found in nature because such mutations would result in structurally or functionally dead protein.

The insights gained from studies on polymorphisms of PEPT1 and PEPT2 are supported by results obtained using knockout animals. The predominant role of PEPT1 for the delivery of sufficient amounts of amino acids is undermined by deletion of the homologue PEP-2 in *Caenorhabditis elegans* (Meissner et al 2004). The animals are severely retarded in development and growth and produce fewer offspring than the wild type animals. Very recently, a PEPT1 knockout mouse line became commercially available but studies with this animal have not yet been published. Two groups succeeded in generating PEPT2 null mouse strains (Rubio-Aliaga et al 2003; Shen et al 2003). Animals were viable, healthy and fertile and showed no obvious abnormalities although no compensatory upregulation of related genes could be detected. The study by Rubio-Aliaga et al (2003) focused on the nutritional consequences of the PEPT2 deficiency. It confirmed the zonal expression of the transporter in the kidney and demonstrated an impaired renal reabsorption of a fluorophore- and radiolabelled dipeptide after in-vivo administration. Recently, further physiological parameters were determined in these mice without revealing any major conspicuous phenotype compared with wild type animals (e.g., in blood pressure, plasma and urine composition) (Frey et al 2006). Profiling by a combination of DNA microarray, proteome and metabolite analyses revealed that PEPT2 might play an important role in the reabsorption of Cys-Gly and consequently in the resynthesis and other related metabolic pathways connected to the supply of glutathione (Frey et al 2007). The relevance of PEPT2 in brain was shown by the group of Smith. They used $PEPT2^{+/+}$ and $PEPT2^{-/-}$ mice choroid plexus tissue preparations to measure uptake of various substrates of the carrier. Impaired accumulation of Gly-Sar (Shen et al 2003; Ocheltree et al 2005), carnosine (Teuscher et al 2004), δ-ALA (Ocheltree et al 2004a) and cefadroxil (Ocheltree et al 2004b) was detected in choroid plexus of PEPT2 deficient mice. More recent studies by the same group deal with the analysis of the disposition of Gly-Sar (Ocheltree et al 2005), cefadroxil (Shen et al 2007) and δ-ALA (Hu et al 2007) in whole animals. PEPT2 null mice clearly exhibited elevated clearance of Gly-Sar, cefadroxil and δ-ALA resulting in lower systemic concentrations. The levels of these compounds in cerebrospinal fluid on the other hand were elevated. Although some peptidomimetics are also substrates for other transport systems like organic anion transporters to some extent, PEPT2 is obviously the predominant system regulating the homeostasis of these compounds in kidney and in brain. It can reduce not only the exposure and potential toxicity of drugs or endogenous substrates like δ-ALA but also their efficacy in certain tissues.

Modulation of PEPT1 and PEPT2

Epithelial peptide transporters are under regulatory control by extra- and intracellular signals of exogenous or endogenous origin. The signals can elicit their effect from the luminal or from the abluminal side. Regulation of peptide transport has been described as a result of development, disease, intestinal resection, inflammation, nutritional status and food composition, hormones and drugs (for review see Ganapathy et al 1994; Meredith & Boyd 2000; Brodin et al 2002; Adibi 2003; Herrera-Ruiz & Knipp 2003; Nielsen & Brodin 2003; Daniel 2004; Terada & Inui 2004). The underlying mechanisms may be nonspecific (e.g. changes in the absorptive surface area, changes in the physical state of the membrane across which absorption occurs) or specific for a particular carrier. Such specific regulation can occur on the transcriptional level, the translational level, by insertion of carrier proteins into or retrieval out of the membrane (trafficking) and by direct modifications of the protein. Virtually all possible regulatory mechanisms have been described for PEPT1 and PEPT2. The capacity to absorb peptides at the intestinal epithelium is maximal at birth and then decreases with age to reach adult levels. Intestinal transport of peptides is upregulated by the presence of high levels of protein in the intestinal lumen. Similarly, short-term restriction of diet increases peptide transport. The intestinal peptide transport is upregulated on the protein expression level at diabetes, after intestinal resection and during inflammation. Insulin, EGF, thyroid hormone, leptin, phorbol esters, cholera toxin, forskolin, the vasoactive intestinal peptide, flavonoids, Ca^{2+}-channel blockers, ciclosporin, 5-fluorouracil, the sigma receptor ligand pentazocine, lipopolysaccharides, clonidine, progesterone and many other compounds have been shown to modulate peptide uptake (Ganapathy et al 1994; Meredith & Boyd 2000; Adibi 2003; Nielsen & Brodin 2003). Some of these biologically and pharmaceutically active compounds affect peptide transport indirectly (e.g. via modulation of the H^+ gradient as the driving force (Thwaites et al 2002; Thwaites & Anderson 2007)). For other signals, direct effects at the carrier, such as phosphorylation and dephosphorylation of the carrier protein (e.g. by protein kinase C), have been described (Brandsch et al 1994; Beattie & Boyd 2001).

Other peptide transporters

Di- and tripeptides

The H^+/peptide cotransporters PEPT1 and PEPT2 are the best characterized and possibly the pharmaceutically most relevant peptide transport systems. In addition to PEPT1 and PEPT2, the proton oligopeptide cotransporter family SLC15 also consists of the peptide/histidine transporters, PHT1 and PHT2 (Herrera-Ruiz & Knipp 2003; Daniel 2004; Daniel & Kottra 2004). PHT1 cDNA had been cloned from rat brain (Yamashita et al 1997). The protein sequence reveals very weak similarity to PEPT1 and PEPT2 (32% and 27%, respectively). When expressed in *X. laevis* oocytes, PHT1 mediated H^+-dependent high-affinity uptake of histidine and di- and tripeptides. The system is also expressed in retina and placenta. Recently the human PHT1 was cloned and functionally characterized in COS-7 cells (Bhardwaj et al 2006). Unexpectedly, uptake of Gly-Sar by this transporter is almost negligible and is unaffected by pH. Gly-Leu and Gly-Gly-Leu at a concentration of 1 mM were able to inhibit hPHT1-mediated uptake of histidine by less than 50%. The study did not assess the affinity of the peptides. Rat PHT2 encodes a protein of 582 amino acid residues showing 49% identity with rat PHT1 (Sakata et al 2001) and is expressed mainly in spleen, thymus and lung. PHT1 and PHT2 have not been analysed

systematically with respect to their substrate specificity (Daniel & Kottra 2004). Nothing is known about their specific pharmaceutical or pharmacological relevance.

In 1994, Dantzig and coworkers used a monoclonal antibody that blocked uptake of cephalexin to identify and clone a gene that encodes a protein that was associated with the acquisition of peptide transport activity by transport-deficient cells (HPT-1, human peptide transporter 1; Dantzig et al 1994b). The amino acid sequence indicated a protein related to the cadherin superfamily of calcium-dependent, cell–cell adhesion proteins. The function of this protein as a physiologically relevant peptide transporter has not yet been convincingly established.

An interesting transcript related to PEPT1 was cloned by Saito et al (1997). Amino acid residues 18–195 of the protein are identical to residues 8–185 in hPEPTl, whereas residues 1–17 and 196–208 are unique (Saito et al 1997; Daniel 2004). The protein has been termed PEPT1-RF for hPEPTl-regulating factor: hPEPTI-RF alone does not transport peptides but coexpression with hPEPTl in *X. laevis* oocytes leads to a shift in the pH sensitivity profile for Gly-Sar uptake.

The mechanisms involved in the transfer of peptides across the intestinal basolateral membrane to the blood side are still under debate. The investigation by Dyer et al (1990) using rabbit enterocyte basolateral membrane vesicles was the first to study basolateral peptide transport. They described a system relatively specific for small peptides that, just as PEPT1 in the apical membrane, is stimulated by an inwardly directed H^+ gradient. Yet, the H^+ gradient across the basolateral membrane is expected to be very small. This might provide the basis for transcellular movement of small peptides across the enterocyte despite the fact that the peptide transport systems in both poles of the cell are H^+ dependent (Ganapathy et al 1994). Using basolateral membrane vesicles prepared from rat kidney, Sugawara et al (2003) demonstrated basolateral uptake of Gly-Sar and β-lactam antibiotics at the renal epithelium.

Most evidence for basolateral peptide transporters, however, was gathered by the group of Inui in a long series of investigations using intestinal and renal epithelial cells cultured on permeable filter membranes (Inui et al 1992; Saito & Inui 1993; Matsumoto et al 1994; Terada et al 1999, 2000c; Irie et al 2001, 2004). Terada et al (1999) reported that uptake of [^{14}C]Gly-Sar across the basolateral membrane into Caco-2 cells cultured on filters was less sensitive to extracellular pH than uptake across the apical membrane by PEPT1. Importantly, the uptake did not proceed against a concentration gradient. This result led to the conclusion that the basolateral system is a facilitative peptide transporter whereas PEPT1 is an active transporter (Terada et al 1999). Characteristics of Gly-Sar uptake across the basolateral membrane of renal MCDK cells cultured on filters differ significantly from those at Caco-2 cells with respect to the affinity towards dipeptides and the pH profile. The authors suggested that intestinal and renal basolateral peptide transport is mediated by different proteins (Terada et al 2000c). Shepherd et al (2002) claimed to have identified a candidate protein for the basolateral peptide transporter of rat jejunum. According to the authors, photoaffinity labelling of basolateral membrane vesicles with [4-azido-3,5-^{3}H-D-Phe]-L-Ala revealed that the majority of label was incorporated into a single, novel 112 kDa protein with no obvious similarity to PEPT1. None of these putative basolateral peptide transport proteins have yet been identified on a molecular level.

Larger peptides

There are several transporters for larger peptides known in mammalian tissues. Some are specific peptide transporters while some are carriers that are better known for other prototypic substrates but do transport also peptides (e.g. the organic anion transporting polypeptides (OATPs)).

A classic field of peptide transport research is transport at the blood–brain barrier. As far as we know, at the endothelial cells of brain capillaries separating blood from brain tissue, H^+/peptide cotransporters are not expressed. However, much functional evidence for the transfer of peptides up to 10 amino acids in size by specific systems has been gathered. Four different peptide transport systems have been described (PTS-1 to PTS-4). It has been reported that peptides such as Leu- and Met-enkephalins, corticotrophin-releasing hormone, small tyrosinated peptides, vasopressin-related peptides, luteinizing hormone-releasing hormone, interleukin 1α, somatostatin, neurotensin and glutathione cross the barrier by saturable and nonsaturable mechanisms in the direction of brain to blood or blood to brain, or both (for review see Pardridge et al 1981; Pardridge 1992; Ermisch et al 1993; Zlokovic 1995; Banks & Kastin 1996; Begley 1996; Rochat & Audus 1999; Bickel et al 2001; Kastin & Pan 2003; Pan & Kastin 2004; Ganapathy & Miyauchi 2005; Su & Sinko 2006; Banks 2006). There are several lines of evidence for the pharmaceutical and pharmacological importance of these transport processes. First, blood–brain barrier passage of peptides is modulated by pharmacological agents. Second, pharmaceutically active peptide derivatives might be delivered as therapeutic agents for disorders of the central nervous system. Third, as for H^+/peptide cotransporters, a prodrug approach (i.e. the attachment of a nontransportable drug to a transportable peptide) has been investigated. The potential of peptide transporters to serve as selective carriers for therapeutic agents is currently under intense investigation but many of the transport processes lack sufficient characterization to propose their precise roles for drug delivery (Kastin & Pan 2003; Pan & Kastin 2004; Su & Sinko 2006). Most importantly, the blood–brain barrier peptide transporters have not yet been identified on a molecular level.

Some of these peptide transporters might actually be organic anion-transporting polypeptides (OATPs), a growing gene family of polyspecific membrane transporters (Hagenbuch & Meier 2004; König et al 2006). The expression of OATPs is widespread, detectable in the brain, liver, intestine, kidney, placenta, and eye. At the human blood–brain barrier, OATP-A (SLC21A3) can mediate the transport of the opioid peptides [D-penicillamine(2,5)]enkephalin and deltorphin II (Gao et al 2000). In several tissues OATPs have also been shown to transport cholecystokinin octapeptide (Ismair et al 2001). The peptide-based thrombin inhibitor CRC 220 is a substrate of the basolateral rat liver OATP (Eckhardt et al 1996). Certain larger peptides and derivatives thereof (Bertrams & Ziegler 1991) and peptide–bile acid conjugates might be substrates for bile acid transporters (Petzinger et al

1999). Fujiya et al (2007) recently reported that the competence and sporulation factor (CSF) of *Bacillus subtilis* activates key survival pathways in intestinal epithelial cells. The effects of CSF, which is a pentapeptide, depend on its uptake by the apical membrane organic cation transporter-2 (OCTN2). The ATP-dependent efflux pump P-glycoprotein encoded by the multidrug resistance gene MDR1 also transports peptides. The brain-to-blood transport of opioid peptides is impaired when P-glycoprotein is inhibited, down regulated or knocked out (for review see Ganapathy & Miyauchi 2005).

In 2003, a completely novel peptide transporter was discovered by the group of Ganapathy—a transport system for opioid peptides (Hu et al 2003; Ganapathy & Miyauchi 2005). The system has been found in human retinal pigment epithelial cells. It is energized by transmembrane Na^+ and Cl^- gradients and is distinct from any of the previously identified transport systems for opioid peptides in mammalian cells. Free amino acids, dipeptides, tripeptides and non-peptide opiate receptor antagonists, such as naloxone or naltrexone, are excluded by the system. The affinity constants of endogenous opioid peptides, such as several dynorphins, enkephalins and endorphins, consisting of 4–13 amino acid residues are in the range of 0.4–40 μM (Hu et al 2003). The molecular identity of the system is not yet known. The carrier is very likely involved in the physiology and biopharmacokinetics of opioid peptides. It is not only expressed in the retinal pigment epithelium: very recently the same group described Na^+,Cl^--dependent transport of deltorphin II in the human neuronal cell line SK-N-SH (Miyauchi et al 2007).

One of the most thoroughly investigated carriers for larger peptides is the transporter referred to as Transporter associated with Antigen Processing, TAP (Lankat-Buttgereit & Tampe 2002; Herget & Tampe 2007). This ATP-dependent system is not expressed at epithelial cell plasma membranes but at the membrane of the endoplasmatic reticulum (ER). The peptides, which constitute TAP substrates, are generated from endogenous proteins in the proteosomal pathway. Human TAP preferentially recognizes peptides 8–16 residues in length (Uebel et al 1997). They are translocated by TAP into the ER lumen and assembled with major histocompatibility complex class I molecules.

Conclusions and future direction

Protein digestion products are absorbed into intestinal cells predominantly in the form of di- and tripeptides. Inside the cells, most peptides are hydrolysed by peptidases to their constituent amino acids. The individual amino acids are transported out of the cell via basolateral amino acid transporters. In addition to their physiological substrates, peptide transporters accept many drugs and prodrugs as substrates because of their structural resemblance to di- and tripeptides. They transport many β-lactam antibiotics and prodrugs such as valaciclovir, thereby allowing oral administration of these compounds. In other tissues, peptide transporters mediate the transfer of peptides, drugs and prodrugs of various origins between intra- and extracellular fluids and between compartments separated by epithelial barriers.

Efforts to clone peptide transporters and to obtain detailed insight into the substrate specificity of these transporters have met with considerable success in recent years. Similar success has also been realized with investigations of their tissue distribution, and their regulation by extra- and intracellular signals in health and disease. The use of knockout animals largely expanded our knowledge of the physiological role of peptide transporters. As demonstrated by the amazing reports on the Na^+,Cl^--dependent opioid peptide transporters, the time of discovering new peptide transporters is far from over.

Despite the recent gains made in the field, much remains to be understood in critical areas, especially in those dealing with protein structure and the operational mechanism of translocation. Current hypotheses on binding sites, conformational changes during translocation and substrate release are largely speculative. Weitz and coworkers recently cloned, overexpressed and purified a prokaryotic peptide transporter with features similar to mammalian PEPT1 (Weitz et al 2007). Hopefully, a similar approach will pave the way to produce sufficient amounts of purified mammalian peptide transporter proteins allowing X-ray crystallography and NMR measurements to elucidate their three-dimensional structure.

Many years ago, Ganapathy and coworkers, when reviewing intestinal amino acid and peptide transport, stated that 'an intriguing question for which there is no answer at this time is how these transport systems are differentially sorted in the enterocyte to be inserted into the brush border and/or the basolateral membrane' (Ganapathy et al 1994). Not much has been done in the last 10 years to answer this question. Another serious lack of knowledge exists regarding the number of carriers per cell and the substrate turnover rates. Triggered, for example, by new imaging techniques and by knockout models now available, we currently observe a shift in direction from pure molecular biology to more cell and system biology. There are still many open questions regarding the intracellular fate of peptides. The identity of the postulated basolateral peptide transporters remains to be elucidated. The intestinal absorption of ACE inhibitors needs to be re-evaluated. Furthermore, inter-individual differences in peptide transport should be one of the priorities of future research in this area. From a pharmaceutical point of view, one final aim of these efforts is to build better drugs for new therapeutic approaches.

References

Abderhalden, E. (1911) *Die Bedeutung der Verdauung für den Zellstoffwechsel im Lichte neuer Forschungen auf dem Gebiete der physiologischen Chemie*. Urban & Schwarzenberg, Berlin-Wien

Abe, H., Satoh, M., Miyauchi, S., Shuto, S., Matsuda, A., Kamo, N. (1999) Conjugation of dipeptide to fluorescent dyes enhances its affinity for a dipeptide transporter (PEPT1) in human intestinal Caco-2 cells. *Bioconjug. Chem.* **10**: 24–31

Addison, J. M., Burston, D., Matthews, D. M. (1972) Evidence for active transport of the dipeptide glycylsarcosine by hamster jejunum *in vitro*. *Clin. Sci.* **43**: 907–911

Addison, J. M., Burston, D., Dalrymple, J. A., Matthews, D. M., Payne, J. W., Sleisenger, M. H., Wilkinson, S. (1975) A common mechanism for transport of di- and tri-peptides by hamster jejunum *in vitro*. *Clin. Sci. Mol. Med.* **49**: 313–322

Adibi, S. A. (1997) The oligopeptide transporter (Pept-1) in human intestine: biology and function. *Gastroenterology* **113**: 332–340

Adibi, S. A. (2003) Regulation of expression of the intestinal oligopeptide transporter (Pept-1) in health and disease. *Am. J. Physiol*. **285**: G779–G788

Adibi, S. A., Soleimanpour, M. R. (1974) Functional characterization of dipeptide transport system in human jejunum. *J. Clin. Invest*. **53**: 1368–1374

Akarawut, W., Lin, C. J., Smith, D. E. (1998) Noncompetitive inhibition of glycylsarcosine transport by quinapril in rabbit renal brush border membrane vesicles: effect on high-affinity peptide transporter. *J. Pharmacol. Exp. Ther*. **287**: 684–690

Amasheh, S., Wenzel, U., Boll, M., Dorn, D., Weber, W., Clauss, W., Daniel, H. (1997) Transport of charged dipeptides by the intestinal H^+/peptide symporter PepT1 expressed in *Xenopus laevis* oocytes. *J. Membr. Biol*. **155**: 247–256

Amidon, G. L., Sadée, W. (eds) (1999) *Membrane transporters as drug targets. Pharmaceutical biotechnology volume 12*. Kluwer Academic/Plenum Publishers. New York

Anderle, P., Nielsen, C. U., Pinsonneault, J., Krog, P. L., Brodin, B., Sadee, W. (2006) Genetic variants of the human dipeptide transporter PEPT1. *J. Pharmacol. Exp. Ther*. **316**: 636–646

Andersen, R., Jorgensen, F. S., Olsen, L., Vabeno, J., Thorn, K., Nielsen, C. U., Steffansen, B. (2006) Development of a QSAR model for binding of tripeptides and tripeptidomimetics to the human intestinal di-/tripeptide transporter hPEPT1. *Pharm. Res*. **23**: 483–492

Aronson, P. S., Nee, J., Suhm, M. A. (1982) Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Nature* **299**: 161–163

Asatoor, A. M., Cheng, B., Edwards, K. D., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F., Richards, A. J. (1970) Intestinal absorption of two dipeptides in Hartnup disease. *Gut* **11**: 380–387

Bai, J. P., Amidon, G. L. (1992) Structural specificity of mucosal-cell transport and metabolism of peptide drugs: implication for oral peptide drug delivery. *Pharm. Res*. **9**: 969–978

Bailey, P. D. (2005) Thiopeptide conjugates for drug delivery. Patent WO/2005/067978

Bailey, P. D., Boyd, C. A., Collier, I. D., Kellett, G. L., Meredith, D., Morgan, K. M., Pettecrew, R., Price, R. A. (2005) Probing dipeptide *trans/cis* stereochemistry using pH control of thiopeptide analogues, and application to the PepT1 transporter. *Org. Biomol. Chem*. **3**: 4038–4039

Bailey, P. D., Boyd, C. A., Collier, I. D., George, J. P., Kellett, G. L., Meredith, D., Morgan, K. M., Pettecrew, R., Price, R. A. (2006) Affinity prediction for substrates of the peptide transporter PepT1. *Chem. Commun. (Camb.)* **2006**: 323–325

Balimane, P. V., Tamai, I., Guo, A., Nakanishi, T., Kitada, H., Leibach, F. H., Tsuji, A., Sinko, P. J. (1998) Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. *Biochem. Biophys. Res. Commun*. **250**: 246–251

Banks, W. A. (2006) The CNS as a target for peptides and peptide-based drugs. *Expert Opin. Drug Deliv*. **3**: 707–712

Banks, W. A., Kastin, A. J. (1996) Passage of peptides across the blood-brain barrier: pathophysiological perspectives. *Life Sci*. **59**: 1923–1943

Beattie, L. A., Boyd, C. A. R. (2001) Protein kinase C activation directly alters the kinetics of PepT1-mediated peptide transport. *J. Physiol*. 535P

Beauchamp, L. M., Orr, G. F., de Miranda, P., Burnette, T., Krenitsky, T. A. (1992) Amino acid ester prodrugs of acyclovir. *Antiv. Chem. Chemother*. **3**: 157–164

Becker, J. M., Dunsmore, K. P., Steinfeld, A. S., Naider, F. (1982) Photoinactivation of peptide transport in Saccharomyces cerevisiae. *Biochemistry* **21**: 5967–5971

Begley, D. J. (1996) The blood-brain barrier: principles for targeting peptides and drugs to the central nervous system. *J. Pharm. Pharmacol*. **48**: 136–146

Berger, U. V., Hediger, M. A. (1999) Distribution of peptide transporter PEPT2 mRNA in the rat nervous system. *Anat. Embryol. (Berl.)* **199**: 439–449

Bertrams, A., Ziegler, K. (1991) Hepatocellular uptake of peptides by bile acid transporters: relationship of carrier-mediated transport of linear peptides with renin-inhibiting activity to multispecific bile acid carriers. *Biochim. Biophys. Acta* **1091**: 337–348

Beyenbach, K. W., Wieczorek, H. (2006) The V-type H^+ ATPase: molecular structure and function, physiological roles and regulation. *J. Exp. Biol*. **209**: 577–589

Bhardwaj, R. K., Herrera-Ruiz, D., Sinko, P. J., Gudmundsson, O. S., Knipp, G. (2005) Delineation of human peptide transporter 1 (hPepT1)-mediated uptake and transport of substrates with varying transporter affinities utilizing stably transfected hPepT1/Madin-Darby Canine Kidney clones and Caco-2 cells. *J. Pharmacol. Exp. Ther*. **314**: 1093–1100

Bhardwaj, R. K., Herrera-Ruiz, D., Eltoukhy, N., Saad, M., Knipp, G. T. (2006) The functional evaluation of human peptide/histidine transporter 1 (hPHT1) in transiently transfected COS-7 cells. *Eur. J. Pharm. Sci*. **27**: 533–542

Bickel, U., Yoshikawa, T., Pardridge, W. M. (2001) Delivery of peptides and proteins through the blood-brain barrier. *Adv. Drug Deliv. Rev*. **46**: 247–279

Biegel, A., Gebauer, S., Hartrodt, B., Brandsch, M., Neubert, K., Thondorf, I. (2005) Three-dimensional quantitative structure-activity relationship analyses of β-lactam antibiotics and tripeptides as substrates of the mammalian H^+/peptide cotransporter PEPT1. *J. Med. Chem*. **48**: 4410–4419

Biegel, A., Gebauer, S., Brandsch, M., Neubert, K., Thondorf, I. (2006a) Structural requirements for the substrates of the H+/peptide cotransporter PEPT2 determined by three-dimensional quantitative structure-activity relationship analysis. J. Med. Chem. 49: 4286–4296

Biegel, A., Knütter, I., Hartrodt, B., Gebauer, S., Theis, S., Luckner, P., Kottra, G., Rastetter, M., Zebisch, K., Thondorf, I., Daniel, H., Neubert, K., Brandsch, M. (2006b) The renal type H^+/peptide symporter PEPT2: structure-affinity relationships. *Amino Acids* **31**: 137–156

Bockman, D. E., Ganapathy, V., Oblak, T. G., Leibach, F. H. (1997) Localization of peptide transporter in nuclei and lysosomes of the pancreas. *Int. J. Pancreatol*. **22**: 221–225

Bolger, M. B., Haworth, I. S., Yeung, A. K., Ann, D., von Grafenstein, H., Hamm-Alvarez, S., Okamoto, C. T., Kim, K. J., Basu, S. K., Wu, S., Lee, V. H. (1998) Structure, function, and molecular modeling approaches to the study of the intestinal dipeptide transporter PepT1. *J. Pharm. Sci*. **87**: 1286–1291

Boll, M., Markovich, D., Weber, W. M., Korte, H., Daniel, H., Murer, H. (1994) Expression cloning of a cDNA from rabbit small intestine related to proton-coupled transport of peptides, ß-lactam antibiotics and ACE-inhibitors. *Pflügers Arch*. **429**: 146–149

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

Börner, V., Fei, Y. J., Hartrodt, B., Ganapathy, V., Leibach, F. H., Neubert, K., Brandsch, M. (1998) Transport of amino acid aryl amides by the intestinal H^+/peptide cotransport system, PEPT1. *Eur. J. Biochem*. **255**: 698–702

Brandsch, M. (2006) Transport of L-proline, L-proline-containing peptides and related drugs at mammalian epithelial cell membranes. *Amino Acids* **31**: 119–136

Brandsch, M., Miyamoto, Y., Ganapathy, V., Leibach, F. H. (1994) Expression and protein kinase C-dependent regulation of peptide/H^+ cotransport system in the Caco-2 human colon carcinoma cell line. *Biochem. J*. **299**: 253–260

Brandsch, M., Brandsch, C., Prasad, P. D., Ganapathy, V., Hopfer, U., Leibach, F. H. (1995) Identification of a renal cell line that constitutively expresses the kidney-specific high-affinity H^+/peptide cotransporter. *FASEB J*. **9**: 1489–1496

Brandsch, M., Brandsch, C., Ganapathy, M. E., Chew, C. S., Ganapathy, V., Leibach, F. H. (1997) 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**: 251–262

Brandsch, M., Thunecke, F., Küllertz, G., Schutkowski, M., Fischer, G., Neubert, K. (1998) Evidence for the absolute conformational specificity of the intestinal H^+/peptide symporter, PEPT1. *J. Biol. Chem*. **273**: 3861–3864

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

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

Bravo, S. A., Nielsen, C. U., Frokjaer, S., Brodin, B. (2005) Characterization of rPEPT2-mediated Gly-Sar transport parameters in the rat kidney proximal tubule cell line SKPT-0193 cl.2 cultured in basic growth media. *Mol. Pharm*. **2**: 98–108

Bretschneider, B., Brandsch, M., Neubert, R. (1999) Intestinal transport of ß-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**: 55–61

Brodin, B., Nielsen, C. U., Steffansen, B., Frokjaer, S. (2002) Transport of peptidomimetic drugs by the intestinal di/tri-peptide transporter, PepT1. *Pharmacol. Toxicol*. **90**: 285–296

Bröer, A., Cavanaugh, J. A., Rasko, J. E., Bröer. S. (2006) The molecular basis of neutral aminoacidurias. *Pflügers Arch*. **451**: 511–517

Bueno, A. B., Collado, I., de Dios, A., Dominguez, C., Martin, J. A., Martin, L. M., Martinez-Grau, M. A., Montero, C., Pedregal, C., Catlow, J., Coffey, D. S., Clay, M. P., Dantzig, A. H., Lindstrom, T., Monn, J. A., Jiang, H., Schoepp, D. D., Stratford, R. E., Tabas, L. B., Tizzano, J. P., Wright, R. A., Herin, M. F. (2005) Dipeptides as effective prodrugs of the unnatural amino acid (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740), a selective group II metabotropic glutamate receptor agonist. *J. Med. Chem*. **48**: 5305–5320

Charrier, L., Driss, A., Yan, Y., Nduati, V., Klapproth, J. M., Sitaraman, S. V., Merlin, D. (2006) hPepT1 mediates bacterial tripeptide fMLP uptake in human monocytes. *Lab. Invest*. **86**: 490–503

Chen, X. Z., Zhu, T., Smith, D. E., Hediger, M. A. (1999) Stoichiometry and kinetics of the high-affinity H^+-coupled peptide transporter PepT2. *J. Biol. Chem*. **274**: 2773–2779

Chen, X. Z., Steel, A., Hediger, M. A. (2000) Functional roles of histidine and tyrosine residues in the H^+-peptide transporter PepT1. *Biochem. Biophys. Res. Commun*. **272**: 726–730

Cheon, E. P., Hong, J. H., Han, H. K. (2006) Enhanced cellular uptake of Ara-C via a peptidomimetic prodrug, L-valyl-ara-C in Caco-2 cells. *J. Pharm. Pharmacol*. **58**: 927–932

Chu, X. Y., Bleasby, K., Yabut, J., Cai, X., Chan, G. H., Hafey, M. J., Xu, S., Bergman, A. J., Braun, M. P., Dean, D. C., Evers, R. (2007) Transport of the dipeptidyl peptidase-4 inhibitor sitagliptin by human organic anion transporter 3, organic anion transporting polypeptide 4C1, and multidrug resistance P-glycoprotein. *J. Pharmacol. Exp. Ther*. **321**: 673–683

Cohnheim, O. (1901) Die Umwandlung des Eiweiss durch die Darmwand. *Z. Physiol. Chemie* **33**: 451–465

Covitz, K. M., Amidon, G. L., Sadee, W. (1998) Membrane topology of the human dipeptide transporter, hPEPT1, determined by epitope insertions. *Biochemistry* **37**: 15214–15221

Dalton, J. T., Meyer, M. C., Golub, A. L. (1999) Pharmacokinetics of aminolevulinic acid after oral and intravenous administration in dogs. *Drug Metab. Dispos*. **27**: 432–435

Daniel, H. (2004) Molecular and integrative physiology of intestinal peptide transport. *Annu. Rev. Physiol*. **66**: 361–384

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

Daniel, H., Adibi, S. A. (1994) Functional separation of dipeptide transport and hydrolysis in kidney brush border membrane vesicles. *FASEB J*. **8**: 753–759

Daniel, H., Kottra, G. (2004) The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. *Pflügers Arch*. **447**: 610–618

Daniel, H., Rubio-Aliaga, I. (2003) An update on renal peptide transporters. *Am. J. Physiol*. **284**: F885–F892

Daniel, H., Fett, C., Kratz, A. (1989) Demonstration and modification of intervillous pH profiles in rat small intestine *in vitro*. *Am. J. Physiol*. **257**: G489–G495

Daniel, H., Morse, E. L., Adibi, S. A. (1991) The high and low affinity transport systems for dipeptides in kidney brush border membrane respond differently to alterations in pH gradient and membrane potential *J. Biol. Chem*. **266**: 19917–19924

Daniel, H., Morse, E. L., Adibi, S. A. (1992) Determinants of substrate affinity for the oligopeptide/H^+ symporter in the renal brush border membrane. *J. Biol. Chem*. **267**: 9565–9573

Dantzig, A. H., Duckworth, D. C., Tabas, L. B. (1994a) Transport mechanisms responsible for the absorption of loracarbef, cefixime, and cefuroxime axetil into human intestinal Caco-2 cells. *Biochim. Biophys. Acta* **1191**: 7–13

Dantzig, A. H., Hoskins, J. A., Tabas, L. B., Bright, S., Shepard, R. L., Jenkins, I. L., Duckworth, D. C., Sportsman, J. R., Mackensen, D., Rosteck, P. R., Jr. (1994b) Association of intestinal peptide transport with a protein related to the cadherin superfamily. *Science* **264**: 430–433

Dieck, S. T., Heuer, H., Ehrchen, J., Otto, C., Bauer, K. (1999) The peptide transporter PepT2 is expressed in rat brain and mediates the accumulation of the fluorescent dipeptide derivative β-Ala-Lys-N-epsilon-AMCA in astrocytes. *Glia* **25**: 10–20

Döring, F., Dorn, D., Bachfischer, U., Amasheh, S., Herget, M., Daniel, H. (1996) Functional analysis of a chimeric mammalian peptide transporter derived from the intestinal and renal isoforms. *J. Physiol*. **497**: 773–779

Döring, F., Walter, J., Will, J., Focking, M., Boll, M., Amasheh, S., Clauss, W., Daniel, H. (1998a) δ-aminolevulinic acid transport by intestinal and renal peptide transporters and its physiological and clinical implications. *J. Clin. Invest*. **101**: 2761–2767

Döring, F., Will, J., Amasheh, S., Clauss, W., Ahlbrecht, H., Daniel, H. (1998b) Minimal molecular determinants of substrates for recognition by the intestinal peptide transporter. *J. Biol. Chem*. **273**: 23211–23218

Döring, F., Martini, C., Walter, J., Daniel, H. (2002) Importance of a small N-terminal region in mammalian peptide transporters for substrate affinity and function. *J. Membr. Biol*. **186**: 55–62

Dyer, J., Beechey, R. B., Gorvel, J. P., Smith, R. T., Wootton, R., Shirazi-Beechey, S. P. (1990) Glycyl-L-proline transport in rabbit enterocyte basolateral-membrane vesicles. *Biochem. J*. **269**: 565–571

Eckhardt, U., Horz, J. A., Petzinger, E., Stuber, W., Reers, M., Dickneite, G., Daniel, H., Wagener, M., Hagenbuch, B., Stieger, B., Meier, P. J. (1996) The peptide-based thrombin inhibitor CRC

220 is a new substrate of the basolateral rat liver organic anion-transporting polypeptide. *Hepatology* **24**: 380–384

Eddy, E. P., Wood, C., Miller, J., Wilson, G., Hidalgo, I. J. (1995) A comparison of the affinities of dipeptides and antibiotics for the di-/tripeptide transporter in Caco-2 cells. *Int. J. Pharm*. **115**: 79–86

Ekins, S., Johnston, J. S., Bahadduri, P., D'Souza, V. M., Ray, A., Chang, C., Swaan, P. W. (2005) *In vitro* and pharmacophore-based discovery of novel hPEPT1 inhibitors. *Pharm. Res*. **22**: 512–517

Enjoh, M., Hashimoto, K., Arai, S., Shimizu, M. (1996) Inhibitory effect of arphamenine A on intestinal dipeptide transport. *Biosci. Biotechnol. Biochem*. **60**: 1893–1895

Ermisch, A., Brust, P., Kretzschmar, R., Rühle, H.-J. (1993) Peptides and blood-brain barrier transport. *Physiol. Rev*. **73**: 489–527

Faria, T. N., Timoszyk, J. K., Stouch, T. R., Vig, B. S., Landowski, C. P., Amidon, G. L., Weaver, C. D., Wall, D. A., Smith, R. L. (2004) A novel high-throughput PepT1 transporter assay differentiates between substrates and antagonists. *Mol. Pharm*. **1**: 67–76

Fei, Y. J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F. H., Romero, M. F., Singh, S. K., Boron, W. F., Hediger, M. A. (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368**: 563–566

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

Fei, Y. J., Ganapathy, V., Leibach, F. H. (1998a) Molecular and structural features of the proton-coupled oligopeptide transporter superfamily. *Prog. Nucleic Acid Res. Mol. Biol*. **58**: 239–261

Fei, Y. J., Liu, J. C., Fujita, T., Liang, R., Ganapathy, V., Leibach, F. H. (1998b) Identification of a potential substrate binding domain in the mammalian peptide transporters PEPT1 and PEPT2 using PEPT1-PEPT2 and PEPT2-PEPT1 chimeras. *Biochem. Biophys. Res. Commun*. **246**: 39–44

Fei, Y. J., Nara, E., Liu, J. C., Boyd, C. A., Ganapathy, V., Leibach, F. H. (1999) Preferential recognition of zwitterionic dipeptides as transportable substrates by the high-affinity peptide transporter PEPT2. *Biochim. Biophys. Acta* **1418**: 344–351

Foltz, M., Meyer, A., Theis, S., Demuth, H. U., Daniel, H. (2004) A rapid *in vitro* screening for delivery of peptide-derived peptidase inhibitors as potential drug candidates via epithelial peptide transporters. *J. Pharmacol. Exp. Ther*. **310**: 695–702

Freeman, T. C., Bentsen, B. S., Thwaites, D. T., Simmons, N. L. (1995) H^+/di-tripeptide transporter (PepT1) expression in the rabbit intestine. *Pflügers Arch*. **430**: 394–400

Frey, I. M., Rubio-Aliaga, I., Klempt, M., Wolf, E., Daniel, H. (2006) Phenotype analysis of mice deficient in the peptide transporter PEPT2 in response to alterations in dietary protein intake. *Pflügers Arch*. **452**: 300–306

Frey, I. M., Rubio-Aliaga, I., Siewert, A., Sailer, D., Drobyshev, A., Beckers, J., de Angelis, M. H., Aubert, J., Bar, H. A., Fiehn, O., Eichinger, H. M., Daniel, H. (2007) Profiling at mRNA, protein, and metabolite levels reveals alterations in renal amino acid handling and glutathione metabolism in kidney tissue of $Pept2^{-/-}$ mice. *Physiol. Genomics* **28**: 301–310

Fürst, P., Albers, S., Stehle, P. (1990) Dipeptides in clinical nutrition. *Proc. Nutr. Soc. (UK)* **49**: 343–359

Fujita, T., Kishida, T., Okada, N., Ganapathy, V., Leibach, F. H., Yamamoto, A. (1999) Interaction of kyotorphin and brain peptide transporter in synaptosomes prepared from rat cerebellum: implication of high affinity type H^+/peptide transporter PEPT2 mediated transport system. *Neurosci. Lett*. **271**: 117–120

Fujita, T., Kishida, T., Wada, M., Okada, N., Yamamoto, A., Leibach, F. H., Ganapathy, V. (2004) Functional characterization of brain peptide transporter in rat cerebral cortex: identification of the high-affinity type H^+/peptide transporter PEPT2. *Brain Res*. **997**: 52–61

Fujiya, M., Musch M. W., Nakagawa, Y., Hu, S., Alverdy, J., Kohgo, Y., Schneewind, O., Jabri, B., Chang, E. B. (2007) The *Bacillus subtilis* quorum-sensing molecule CSF contributes to intestinal homeostasis via OCTN2, a host cell membrane transporter. *Cell Host Microbe* **1**: 299–308

Ganapathy, V., Leibach, F. H. (1983) Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-border membrane vesicles from the rabbit. Studies with L-carnosine and glycyl-L-proline. *J. Biol. Chem*. **258**: 14189–14192

Ganapathy, V., Miyauchi, S. (2005) Transport systems for opioid peptides in mammalian tissues. *AAPS. J*. **7**: E852–E856

Ganapathy, V., Radhakrishnan, A. N. (1979) Interaction of amino acids with glycl-L-leucine hydrolysis and transport in monkey small intestine. *Clin. Sci. (Lond.)* **57**: 521–527

Ganapathy, V., Mendicino, J., Pashley, D. H., Leibach, F. H. (1980) Carrier-mediated transport of glycyl-L-proline in renal brush border vesicles. *Biochem. Biophys. Res. Commun*. **97**: 1133–1139

Ganapathy, V., Mendicino, J. F., Leibach, F. H. (1981) Transport of glycyl-L-proline into intestinal and renal brush border vesicles from rabbit. *J. Biol. Chem*. **256**: 118–124

Ganapathy, V., Burckhardt, G., Leibach, F. H. (1984) Characteristics of glycylsarcosine transport in rabbit intestinal brush-border membrane vesicles. *J. Biol. Chem*. **259**: 8954–895

Ganapathy, V., Brandsch, M., Leibach, F. H. (1994) Intestinal transport of amino acids and peptides. In: Johnson, L. R. (ed.) *Physiology of the gastrointestinal tract*. 3rd edn, Raven Press Ltd, New York, pp 1773–1794

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

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

Ganapathy, M. E., Huang, W., Wang, H., Ganapathy, V., Leibach, F. H. (1998) Valacyclovir: a substrate for the intestinal and renal peptide transporters PEPT1 and PEPT2. *Biochem. Biophys. Res. Commun*. **246**: 470–475

Ganapathy, M. E., Huang, W., Rajan, D. P., Carter, A. L., Sugawara, M., Iseki, K., Leibach, F. H., Ganapathy, V. (2000) β-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J. Biol. Chem*. **275**: 1699–1707

Ganapathy, V., Ganapathy, M. E., Leibach, F. H. (2001) Intestinal transport of peptides and amino acids. In: Barrett, K. E., Donowitz, M. (ed.) *Gastrointestinal transport. Molecular physiology*. Vol. 50, Academic Press, San Diego, pp 379–412

Gao, B., Hagenbuch, B., Kullak-Ublick, G. A., Benke, D., Aguzzi, A., Meier, P. J. (2000) Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J. Pharmacol. Exp. Ther*. **294**: 73–79

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

Genz, A. K., von Engelhardt, W., Busche, R. (1999) Maintenance and regulation of the pH microclimate at the luminal surface of the distal colon of guinea-pig. *J. Physiol*. **517**: 507–519

Gerloff, T. (2004) Impact of genetic polymorphisms in transmembrane carrier-systems on drug and xenobiotic distribution. *Naunyn Schmiedebergs Arch. Pharmacol*. **369**: 69–77

Goodman, S. I., McIntyre, C. A., O'Brien, D. (1967) Impaired intestinal transport of proline in a patient with familial iminoaciduria. *J. Pediatr*. **71**: 246–249

Gray, V. A., Dressman, J. B. (1996) Change of pH requirements for simulated intestinal fluid TS. *Pharmacopeial Forum* **22**: 1943–1945

Groneberg, D. A., Nickolaus, M., Springer, J., Döring, F., Daniel, H., Fischer, A. (2001) Localization of the peptide transporter PEPT2 in the lung: implications for pulmonary oligopeptide uptake. *Am. J. Pathol*. **158**: 707–714

Groneberg, D. A., Döring, F., Theis, S., Nickolaus, M., Fischer, A., Daniel, H. (2002) Peptide transport in the mammary gland: expression and distribution of PEPT2 mRNA and protein. *Am. J. Physiol*. **282**: E1172–E1179

Groneberg, D. A., Fischer, A., Chung, K. F., Daniel, H. (2004) Molecular mechanisms of pulmonary peptidomimetic drug and peptide transport. *Am. J. Respir. Cell Mol. Biol*. **30**: 251–260

Groneberg, D. A., Paul, H., Welte, T. (2006) Novel strategies of aerosolic pharmacotherapy. *Exp. Toxicol. Pathol*. **57**: 49–53

Hagenbuch, B., Meier, P. J. (2004) Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflügers Arch*. **447**: 653–665

Han, H., de Vrueh, R. L., Rhie, J. K., Covitz, K. M., Smith, P. L., Lee, C. P., Oh, D. M., Sadee, W., Amidon, G. L. (1998) 5'-Amino acid esters of antiviral nucleosides, acyclovir, and AZT are absorbed by the intestinal PEPT1 peptide transporter. *Pharm. Res*. **15**: 1154–1159

Han, H. K., Rhie, J. K., Oh, D. M., Saito, G., Hsu, C. P., Stewart, B. H., Amidon, G. L. (1999) CHO/hPEPT1 cells overexpressing the human peptide transporter (hPEPT1) as an alternative in vitro model for peptidomimetic drugs. *J. Pharm. Sci*. **88**: 347–350

Hartrodt, B., Börner, V., Knütter, I., Born, I., Brandsch, M., Neubert, K. (1998) Intestinal H^+/peptide cotransport: Investigation of transporter-substrate interaction. In: Bajusz, S., Hudecz, F. (eds) *Peptides 1998*. Akademiai Kiado, Budapest, pp 586–587

Hartrodt, B., Theis, S., Knütter, I., Börner, V., Born, I., Brandsch, M., Daniel, H., Neubert, K. (2001) New insight into the stereospecificity of the intestinal H^+/peptide symporter. In: Martinez, J., Fehrentz, J.-A. (eds) *Peptides 2000*. Editions EDK, Paris, pp 983–984

Hediger, M. A. (special editor) (2004) The ABC of solute transporters. *Pflügers Arch*. **447**: 5

Hellier, M. D., Holdsworth, C. D., Perrett, D., Thirumalai, C. (1972) Intestinal dipeptide transport in normal and cystinuric subjects. *Clin. Sci*. **43**: 659–668

Hellier, M. D., Radhakrishnan, A. N., Ganapathy, V., Mathan, V. I., Baker, S. J. (1976) Intestinal perfusion studies in tropical sprue. 1. Amino acid and dipeptide absorption. *Gut* **17**: 511–516

Herget, M., Tampe, R. (2007) Intracellular peptide transporters in human-compartmentalization of the peptidome. *Pflügers Arch*. **453**: 591–600

Herrera-Ruiz, D., Knipp, G. T. (2003) Current perspectives on established and putative mammalian oligopeptide transporters. *J. Pharm. Sci*. **92**: 691–714

Hori, R., Tomita, Y., Katsura, T., Yasuhara, M., Inui, K., Takano, M. (1993) Transport of bestatin in rat renal brush-border membrane vesicles. *Biochem. Pharmacol*. **45**: 1763–1768

Hu, M., Subramanian, P., Mosberg, H. I., Amidon, G. L. (1989) Use of the peptide carrier system to improve the intestinal absorption of L-alpha-methyldopa: carrier kinetics, intestinal permeabilities, and *in vitro* hydrolysis of dipeptidyl derivatives of L-α-methyldopa. *Pharm. Res*. **6**: 66–70

Hu, M., Zheng, L., Chen, J., Liu, L., Zhu, Y., Dantzig, A. H., Stratford, R. E. (1995) Mechanisms of transport of quinapril in Caco-2 cell monolayers: comparison with cephalexin. *Pharm. Res*. **12**: 1120–1125

Hu, H., Miyauchi, S., Bridges, C. C., Smith, S. B., Ganapathy, V. (2003) Identification of a novel Na^+- and Cl^--coupled transport system for endogenous opioid peptides in retinal pigment epithelium and induction of the transport system by HIV-1 Tat. *Biochem. J*. **375**: 17–22

Hu, Y., Shen, H., Keep, R. F., Smith, D. E. (2007) Peptide transporter 2 (PEPT2) expression in brain protects against 5-aminolevulinic acid neurotoxicity. *J. Neurochem*. **103**: 2058–2065

Hussain, I., Zanic-Grubisic, T., Kudo, Y., Boyd, C. A. (2001) Functional and molecular characterization of a peptide transporter in the rat PC12 neuroendocrine cell line. *FEBS Lett*. **508**: 350–354

Inui, K., Terada, T. (1999) Dipeptide transporters. *Pharm. Biotechnol*. **12**: 269–288

Inui, K., Yamamoto, M., Saito, H. (1992) Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: specific transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther*. **261**: 195–201

Irie, M., Terada, T., Sawada, K., Saito, H., Inui, K. (2001) Recognition and transport characteristics of nonpeptidic compounds by basolateral peptide transporter in Caco-2 cells. *J. Pharmacol. Exp. Ther*. **298**: 711–717

Irie, M., Terada, T., Okuda, M., Inui, K. (2004) Efflux properties of basolateral peptide transporter in human intestinal cell line Caco-2. *Pflügers Arch*. **449**: 186–194

Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K., Arita, T. (1989) Comparison of transport characteristics of amino ß-lactam antibiotics and dipeptides across rat intestinal brush border membrane. *J. Pharm. Pharmacol*. **41**: 628–632

Ismair, M. G., Stieger, B., Cattori, V., Hagenbuch, B., Fried, M., Meier, P. J., Kullak-Ublick, G. A. (2001) Hepatic uptake of cholecystokinin octapeptide by organic anion-transporting polypeptides oatp4 and oatp8 of rat and human liver. *Gastroenterology* **121**: 1185–1190

Kastin, A. J., Pan, W. (2003) Peptide transport across the blood-brain barrier. *Prog. Drug Res*. **61**: 79–100

Kitagawa, S., Takeda, J., Kaseda, Y., Sato, S. (1997) Inhibitory effects of angiotensin-converting enzyme inhibitor on cefroxadine uptake by rabbit small intestinal brush border membrane vesicles. *Biol. Pharm. Bull*. **20**: 449–451

Klapper, M., Daniel, H., Döring, F. (2006) Cytosolic COOH terminus of the peptide transporter PEPT2 is involved in apical membrane localization of the protein. *Am. J. Physiol*. **290**: C472–C483

Knütter, I., Theis, S., Hartrodt, B., Born, I., Brandsch, M., Daniel, H., Neubert, K. (2001) A novel inhibitor of the mammalian peptide transporter PEPT1. *Biochemistry* **40**: 4454–4458

Knütter, I., Rubio-Aliaga, I., Boll, M., Hause, G., Daniel, H., Neubert, K., Brandsch, M. (2002) H^+-peptide cotransport in the human bile duct epithelium cell line SK-ChA-1. *Am. J. Physiol*. **283**: G222–G229

Knütter, I., Hartrodt, B., Theis, S., Foltz, M., Rastetter, M., Daniel, H., Neubert, K., Brandsch, M. (2004) Analysis of the transport properties of side chain modified dipeptides at the mammalian peptide transporter PEPT1. *Eur. J. Pharm. Sci*. **21**: 61–67

König, J., Seithel, A., Gradhand, U., Fromm, M. F. (2006) Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch. Pharmacol*. **372**: 432–443

Kottra, G., Stamfort, A., Daniel, H. (2002) PEPT1 as a paradigm for membrane carriers that mediate electrogenic bidirectional transport of anionic, cationic, and neutral substrates. *J. Biol. Chem*. **277**: 32683–32691

Kulkarni, A. A., Haworth, I. S., Lee, V. H. (2003a) Transmembrane segment 5 of the dipeptide transporter hPepT1 forms a part of the substrate translocation pathway. *Biochem. Biophys. Res. Commun*. **306**: 177–185

Kulkarni, A. A., Haworth, I. S., Uchiyama, T., Lee, V. H. (2003b) Analysis of transmembrane segment 7 of the dipeptide transporter

hPepT1 by cysteine-scanning mutagenesis. *J. Biol. Chem*. **278**: 51833–51840

Kulkarni, A. A., Davies, D. L., Links, J. S., Patel, L. N., Lee, V. H., Haworth, I. S. (2007) A charge pair interaction between Arg282 in transmembrane segment 7 and Asp341 in transmembrane segment 8 of hPepT1. *Pharm. Res*. **24**: 66–72

Landowski, C. P., Vig, B. S., Song, X., Amidon, G. L. (2005) Targeted delivery to PEPT1-overexpressing cells: acidic, basic, and secondary floxuridine amino acid ester prodrugs. *Mol. Cancer Ther*. **4**: 659–667

Lankat-Buttgereit, B., Tampe, R. (2002) The transporter associated with antigen processing: function and implications in human diseases. *Physiol. Rev*. **82**: 187–204

Law, E. A., Sardharwalla, I. B. (1978) A new type of heterozygote of familial renal iminoglycinuria. *Monogr. Hum. Genet*. **9**: 152–154

Leabman, M. K., Huang, C. C., DeYoung, J., Carlson, E. J., Taylor, T. R., De La, C. M., Johns, S. J., Stryke, D., Kawamoto, M., Urban, T. J., Kroetz, D. L., Ferrin, T. E., Clark, A. G., Risch, N., Herskowitz, I., Giacomini, K. M. Pharmacogenetics of membrane transporters investigators 2003. Natural variation in human membrane transporter genes reveals evolutionary and functional constraints. *Proc. Natl Acad. Sci. USA* **100**: 5896–5901

Lee, C. P., de Vrueh, R. L., Smith, P. L. (1996) Transport of a prodrug of acyclovir, L-valacyclovir, via the oligopeptide transporter. *Proc. Int. Symp. Controlled Release Bioact. Mater*. **23**: 47–48

Lee, V. H., Chu, C., Mahlin, E. D., Basu, S. K., Ann, D. K., Bolger, M. B., Haworth, I. S., Yeung, A. K., Wu, S. K., Hamm-Alvarez, S., Okamoto, C. T. (1999) Biopharmaceutics of transmucosal peptide and protein drug administration: role of transport mechanisms with a focus on the involvement of PepT1. *J. Control. Release* **62**: 129–140

Leibach, F. H., Ganapathy, V. (1996) Peptide transporters in the intestine and the kidney. *Annu. Rev. Nutr*. **16**: 99–119

Li, J., Tamura, K., Lee, C. P., Smith, P. L., Borchardt, R. T., Hidalgo, I. J. (1998) Structure-affinity relationships of Val-Val and Val-Val-Val stereoisomers with the apical oligopeptide transporter in human intestinal Caco-2 cells. *J. Drug Target*. **5**: 317–327

Li, F., Hong, L., Mau, C. I., Chan, R., Hendricks, T., Dvorak, C., Yee, C., Harris, J., Alfredson, T. (2006a) Transport of levovirin prodrugs in the human intestinal Caco-2 cell line. *J. Pharm. Sci*. **95**: 1318–1325

Li, M., Anderson, G. D., Phillips, B. R., Kong, W., Shen, D. D., Wang, J. (2006b) Interactions of amoxicillin and cefaclor with human renal organic anion and peptide transporters. *Drug Metab. Dispos*. **34**: 547–555

Liang, R., Fei, Y. J., Prasad, P. D., Ramamoorthy, S., Han, H., Yang-Feng, T. L., Hediger, M. A., Ganapathy, V., Leibach, F. H. (1995) Human intestinal H^+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. *J. Biol. Chem*. **270**: 6456–6463

Lin, H., King, N. (2007) Demonstration of functional dipeptide transport with expression of PEPT2 in guinea pig cardiomyocytes. *Pflügers Arch*. **453**: 915–922

Lin, C. J., Akarawut, W., Smith, D. E. (1999) Competitive inhibition of glycylsarcosine transport by enalapril in rabbit renal brush border membrane vesicles: interaction of ACE inhibitors with high-affinity H^+/peptide symporter. *Pharm. Res*. **16**: 609–615

Links, J. L., Kulkarni, A. A., Davies, D. L., Lee, V. H., Haworth, I. S. (2007) Cysteine scanning of transmembrane domain three of the human dipeptide transporter: implications for substrate transport. *J. Drug Target*. **15**: 218–225

Liu, W., Liang, R., Ramamoorthy, S., Fei, Y. J., Ganapathy, M. E., Hediger, M. A., Ganapathy, V., Leibach, F. H. (1995) Molecular cloning of PEPT 2, a new member of the H^+/peptide cotransporter family, from human kidney. *Biochim. Biophys. Acta* **1235**: 461–466

Liu, K. X., Kato, Y., Kaku, T. I., Santa, T., Imai, K., Yagi, A., Ishizu, T., Sugiyama, Y. (2000) Hydroxyprolylserine derivatives JBP923 and JBP485 exhibit the antihepatitis activities after gastrointestinal absorption in rats. *J. Pharmacol. Exp. Ther*. **294**: 510–515

Lucas, M. (1983) Determination of acid surface pH *in vivo* in rat proximal jejunum. *Gut* **24**: 734–739

Luckner, P., Brandsch, M. (2005) Interaction of 31 ß-lactam antibiotics with the H^+/peptide symporter PEPT2: analysis of affinity constants and comparison with PEPT1. *Eur. J. Pharm. Biopharm*. **59**: 17–24

Matsumoto, S., Saito, H., Inui, K. (1994) Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: interaction with dipeptide transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther*. **270**: 498–504

Matthews, D. M. (1975) Intestinal absorption of peptides. *Physiol. Rev*. **55**: 537–608

Matthews, D. M. (1991) *Protein absorption: development and present state of the subject*. Wiley-Liss, New York

Matthews, D. M., Addison, J. M., Burston, D. (1974) Evidence for active transport of the dipeptide carnosine (*β*-alanyl-L-histidine) by hamster jejunum *in vitro. Clin. Sci. Mol. Med*. **46**: 693–705

McEwan, G. T., Daniel, H., Fett, C., Burgess, M. N., Lucas, M. L. (1988) The effect of *Escherichia coli* ST_a enterotoxin and other secretagogues on mucosal surface pH of rat small intestine *in vivo. Proc. R. Soc. Lond. B Biol. Sci*. **234**: 219–237

Meissner, B., Boll, M., Daniel, H., Baumeister, R. (2004) Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans. J. Biol. Chem*. **279**: 36739–36745

Meredith, D. (2004) Site-directed mutation of arginine 282 to glutamate uncouples the movement of peptides and protons by the rabbit proton-peptide cotransporter PepT1. *J. Biol. Chem*. **279**: 15795–15798

Meredith, D., Boyd, C. A. (1995) Dipeptide transport characteristics of the apical membrane of rat lung type II pneumocytes. *Am. J. Physiol*. **269**: L137–L143

Meredith, D., Boyd, C. A. (2000) Structure and function of eukaryotic peptide transporters. *Cell. Mol. Life Sci*. **57**: 754–778

Meredith, D., Boyd, C. A., Bronk, J. R., Bailey, P. D., Morgan, K. M., Collier, I. D., Temple, C. S. (1998) 4-Aminomethylbenzoic acid is a non-translocated competitive inhibitor of the epithelial peptide transporter PepT1. *J. Physiol*. **512**: 629–634

Meredith, D., Temple, C. S., Guha, N., Sword, C. J., Boyd, C. A., Collier, I. D., Morgan, K. M., Bailey, P. D. (2000) Modified amino acids and peptides as substrates for the intestinal peptide transporter PepT1. *Eur. J. Biochem*. **267**: 3723–3728

Miyauchi, S., Gopal, E., Thakkar, S. V., Ichikawa, S., Prasad, P. D., Ganapathy, V. (2007) Differential modulation of sodium- and chloride-dependent opioid peptide transport system by small non-opioid peptides and free amino acids. *J. Pharmacol. Exp. Ther*. **321**: 257–264

Moore, V. A., Irwin, W. J., Timmins, P., Lambert, P. A., Chong, S., Dando, S. A., Morrison, R. A. (2000) A rapid screening system to determine drug affinities for the intestinal dipeptide transporter 2: affinities of ACE inhibitors. *Int. J. Pharm*. **210**: 29–44

Nakashima, E., Tsuji, A., Mizuo, H., Yamana, T. (1984) Kinetics and mechanism of *in vitro* uptake of amino-ß-lactam antibiotics by rat small intestine and relation to the intact-peptide transport system. *Biochem. Pharmacol*. **33**: 3345–3352

Neumann, J., Brandsch, M. (2003) *δ*-aminolevulinic acid transport in cancer cells of the human extrahepatic biliary duct. *J. Pharmacol. Exp. Ther*. **305**: 219–224

Neumann, J., Bruch, M., Gebauer, S., Brandsch, M. (2004) Transport of the phosphonodipeptide alafosfalin by the H^+/peptide cotransporters PEPT1 and PEPT2 in intestinal and renal epithelial cells. *Eur. J. Biochem*. **271**: 2012–2017

Newey, H., Smyth, D. H. (1959) The intestinal absorption of some dipeptides. *J. Physiol*. **145**: 48–56

Nicklin, P. L., Irwin, W. J., Timmins, P., Morrison, R. A. (1996) Uptake and transport of the ACE-inhibitor ceronapril (SQ 29852) by monolayers of human intestinal absorptive (Caco-2) cells *in vitro*. *Int. J. Pharm*. **140**: 175–183

Nielsen, C. U., Andersen, R., Brodin, B., Frokjaer, S., Taub, M. E., Steffansen, B. (2001) Dipeptide model prodrugs for the intestinal oligopeptide transporter. Affinity for and transport via hPepT1 in the human intestinal Caco-2 cell line. *J. Control. Release* **76**: 129–138

Nielsen, C. U., Brodin, B., Jorgensen, F. S., Frokjaer, S., Steffansen, B. (2002a) Human peptide transporters: therapeutic applications. *Expert Opin. Ther. Patents* **12**: 1329–1350

Nielsen, C. U., Supuran, C. T., Scozzafava, A., Frokjaer, S., Steffansen, B., Brodin, B. (2002b) Transport characteristics of L-carnosine and the anticancer derivative 4-toluenesulfonyl-ureido-carnosine in a human epithelial cell line. *Pharm. Res*. **19**: 1337–1344

Nielsen, C. U., Brodin, B. (2003) Di/tri-peptide transporters as drug delivery targets: regulation of transport under physiological and patho-physiological conditions. *Curr. Drug Targets* **4**: 373–388

Novotny, A., Xiang, J., Stummer, W., Teuscher, N. S., Smith, D. E., Keep, R. F. (2000) Mechanisms of 5-aminolevulinic acid uptake at the choroid plexus. *J. Neurochem*. **75**: 321–328

Nozawa, T., Toyobuku, H., Kobayashi, D., Kuruma, K., Tsuji, A., Tamai, I. (2003) Enhanced intestinal absorption of drugs by activation of peptide transporter PEPT1 using proton-releasing polymer. *J. Pharm. Sci*. **92**: 2208–2216

Ocheltree, S. M., Shen, H., Hu, Y., Xiang, J., Keep, R. F., Smith, D. E. (2004a) Role of PEPT2 in the choroid plexus uptake of glycylsarcosine and 5-aminolevulinic acid: studies in wild-type and null mice. *Pharm. Res*. **21**: 1680–1685

Ocheltree, S. M., Shen, H., Hu, Y., Xiang, J., Keep, R. F., Smith, D. E. (2004b) Mechanisms of cefadroxil uptake in the choroid plexus: studies in wild-type and PEPT2 knockout mice. *J. Pharmacol. Exp. Ther*. **308**: 462–467

Ocheltree, S. M., Shen, H., Hu, Y., Keep, R. F., Smith, D. E. (2005) Role and relevance of peptide transporter 2 (PEPT2) in the kidney and choroid plexus: *in vivo* studies with glycylsarcosine in wild-type and PEPT2 knockout mice. *J. Pharmacol. Exp. Ther*. **315**: 240–247

Ogihara, H., Saito, H., Shin, B. C., Terado, T., Takenoshita, S., Nagamachi, Y., Inui, K., Takata, K. (1996) Immuno-localization of H^+/peptide cotransporter in rat digestive tract. *Biochem. Biophys. Res. Commun*. **220**: 848–852

Okano, T., Inui, K., Maegawa, H., Takano, M., Hori, R. (1986) H^+ coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. *J. Biol. Chem*. **261**: 14130–14134

Palacin, M., Nunes, V., Font-Llitjos, M., Jimenez-Vidal, M., Fort, J., Gasol, E., Pineda, M., Feliubadalo, L., Chillaron, J., Zorzano, A. (2005) The genetics of heteromeric amino acid transporters. *Physiology* **20**: 112–24

Pan, W., Kastin, A. J. (2004) Polypeptide delivery across the blood-brain barrier. *Curr. Drug Targets CNS Neurol. Disord*. **3**: 131–136

Pardridge, W. M. (1992) Recent developments in peptide drug delivery to the brain. *Pharmacol. Toxicol*. **71**: 3–10

Pardridge, W. M., Frank, H. J., Cornford, E. M., Braun, L. D., Crane, P. D., Oldendorf, W. H. (1981) Neuropeptides and the blood-brain barrier. *Adv. Biochem. Psychopharmacol*. **28**: 321–328

Paulsen, I. T., Skurray, R. A. (1994) The POT family of transport proteins. *Trends Biochem. Sci*. **19**: 404

Payne, J. W., Payne, G. M., Gupta, S., Marshall, N. J., Grail, B. M. (2001) Conformational limitations of glycylsarcosine as a prototypic substrate for peptide transporters. *Biochim. Biophys. Acta* **1514**: 65–75

Petzinger, E., Wickboldt, A., Pagels, P., Starke, D., Kramer, W. (1999) Hepatobiliary transport of bile acid amino acid, bile acid peptide, and bile acid oligonucleotide conjugates in rats. *Hepatology* **30**: 1257–1268

Pinsonneault, J., Nielsen, C. U., Sadee, W. (2004) Genetic variants of the human H^+/dipeptide transporter PEPT2: analysis of haplotype functions. *J. Pharmacol. Exp. Ther*. **311**: 1088–1096

Prasad, P. D., Mahesh, V. B., Leibach, F. H., Ganapathy, V. (1994) Functional coupling between a bafilomycin A1-sensitive proton pump and a probenecid-sensitive folate transporter in human placental choriocarcinoma cells. *Biochim. Biophys. Acta* **1222**: 309–314

Quay, J. F. (1972) Transport interaction of glycine and cephalexin in rat jejunum. *The Physiologist* **15**: 241

Quay, J. F., Foster, L. (1970) Cephalexin penetration of the surviving rat intestine. *The Physiologist* **13**: 287

Raeissi, S. D., Li, J., Hidalgo, I. J. (1999) The role of an alpha-amino group on H^+-dependent transepithelial transport of cephalosporins in Caco-2 cells. *J. Pharm. Pharmacol*. **51**: 35–40

Rochat, B., Audus, K. L. (1999) Drug disposition and targeting. Transport across the blood-brain barrier. *Pharm. Biotechnol*. **12**: 181–200

Rubio-Aliaga, I., Daniel, H. (2002) Mammalian peptide transporters as targets for drug delivery. *Trends Pharmacol. Sci*. **23**: 434–440

Rubio-Aliaga, I., Frey, I., Boll, M., Groneberg, D. A., Eichinger, H. M., Balling, R., Daniel, H. (2003) Targeted disruption of the peptide transporter Pept2 gene in mice defines its physiological role in the kidney. *Mol. Cell Biol*. **23**: 3247–3252

Rühl, A., Hoppe, S., Frey, I., Daniel, H., Schemann, M. (2005) Functional expression of the peptide transporter PEPT2 in the mammalian enteric nervous system. *J. Comp. Neurol*. **490**: 1–11

Sai, Y., Tsuji, A. (2004) Transporter-mediated drug delivery: recent progress and experimental approaches. *Drug Discov. Today* **9**: 712–720

Said, H. M., Blair, J. A., Lucas, M. L., Hilburn, M. E. (1986) Intestinal surface acid microclimate *in vitro* and *in vivo* in the rat. *J. Lab. Clin. Med*. **107**: 420–424

Saito, H., Inui, K. (1993) Dipeptide transporters in apical and basolateral membranes of the human intestinal cell line Caco-2. *Am. J. Physiol*. **265**: G289–G294

Saito, H., Terada, T., Okuda, M., Sasaki, S., Inui, K. (1996) Molecular cloning and tissue distribution of rat peptide transporter PEPT2. *Biochim. Biophys. Acta* **1280**: 173–177

Saito, H., Motohashi, H., Mukai, M., Inui, K. (1997) Cloning and characterization of a pH-sensing regulatory factor that modulates transport activity of the human H+/peptide cotransporter, PEPT1. Biochem. Biophys. Res. Commun. 237: 577–582

Saitoh, H., Gerard, C., Aungst, B. J. (1996) The secretory intestinal transport of some ß-lactam antibiotics and anionic compounds: a mechanism contributing to poor oral absorption. *J. Pharmacol. Exp. Ther*. **278**: 205–211

Sakata, K., Yamashita, T., Maeda, M., Moriyama, Y., Shimada, S., Tohyama, M. (2001) Cloning of a lymphatic peptide/histidine transporter. *Biochem. J*. **356**: 53–60

Sala-Rabanal, M., Loo, D. D., Hirayama, B. A., Turk, E., Wright, E. M. (2006) Molecular interactions between dipeptides, drugs and the human intestinal H^+-oligopeptide cotransporter hPEPT1. *J. Physiol*. **574**: 149–166

Sawada, K., Terada, T., Saito, H., Hashimoto, Y., Inui, K. (1999a) Effects of glibenclamide on glycylsarcosine transport by the rat peptide transporters PEPT1 and PEPT2. *Br. J. Pharmacol*. **128**: 1159–1164

Sawada, K., Terada, T., Saito, H., Hashimoto, Y., Inui, K. I. (1999b) Recognition of L-amino acid ester compounds by rat peptide transporters PEPT1 and PEPT2. *J. Pharmacol. Exp. Ther*. **291**: 705–709

Seal, C. J., Parker, D. S. (1991) Isolation and characterization of circulating low molecular weight peptides in steer, sheep and rat portal and peripheral blood. *Comp. Biochem. Physiol. B* **99**: 679-685

Seow, H. F., Bröer, S., Bröer, A., Bailey, C. G., Potter, S. J., Cavanaugh, J. A., Rasko, J. E. (2004) Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. *Nat. Genet*. **36**: 1003–1007

Shen, H., Smith, D. E., Yang, T., Huang, Y. G., Schnermann, J. B., Brosius, F. C. 3rd (1999) Localization of PEPT1 and PEPT2 proton-coupled oligopeptide transporter mRNA and protein in rat kidney. *Am. J. Physiol*. **276**: F658–F665

Shen, H., Smith, D. E., Keep, R. F., Xiang, J., Brosius, F. C. 3rd (2003) Targeted disruption of the PEPT2 gene markedly reduces dipeptide uptake in choroid plexus. *J. Biol. Chem*. **278**: 4786–4791

Shen, H., Ocheltree, S. M., Hu, Y., Keep, R. F., Smith, D. E. (2007) Impact of genetic knockout of PEPT2 on cefadroxil pharmacokinetics, renal tubular reabsorption, and brain penetration in mice. *Drug Metab. Dispos*. **35**: 1209–1216

Shepherd, E., Lister, N., Affleck, J., Bronk, J., Kellett, G., Collier, I., Bailey, P., Boyd, C. A. (2002) Identification of a candidate membrane protein for the basolateral peptide transporter of rat small intestine. *Biochem. Biophys. Res. Commun*. **296**: 918–922

Shu, C., Shen, H., Hopfer, U., Smith, D. E. (2001) 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**: 1307–1315

Silbernagl, S., Ganapathy, V., Leibach, F. H. (1987) H^+ gradient-driven dipeptide reabsorption in proximal tubule of rat kidney. Studies *in vivo* and *in vitro*. *Am. J. Physiol*. **253**: F448–F457

Smith, D. E., Johanson, C. E., Keep, R. F. (2004) Peptide and peptide analog transport systems at the blood-CSF barrier. *Adv. Drug Delivery Rev*. **56**: 1765–1791

Snyder, N. J., Tabas, L. B., Berry, D. M., Duckworth, D. C., Spry, D. O., Dantzig, A. H. (1997) Structure-activity relationship of carbacephalosporins and cephalosporins: antibacterial activity and interaction with the intestinal proton-dependent dipeptide transport carrier of Caco-2 cells. *Antimicrob. Agents Chemother*. **41**: 1649–1657

Song, X., Lorenzi, P. L., Landowski, C. P., Vig, B. S., Hilfinger, J. M., Amidon, G. L. (2005a) Amino acid ester prodrugs of the anticancer agent gemcitabine: synthesis, bioconversion, metabolic bioevasion, and hPEPT1-mediated transport. *Mol. Pharmacol*. **2**: 157–167

Song, X., Vig, B. S., Lorenzi, P. L., Drach, J. C., Townsend, L. B., Amidon, G. L. (2005b) Amino acid ester prodrugs of the antiviral agent 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole as potential substrates of hPEPT1 transporter. *J. Med. Chem*. **48**: 1274–1277

Stark, M., Jornvall, H., Johansson, J. (1999) Isolation and characterization of hydrophobic polypeptides in human bile. *Eur. J. Biochem*. **266**: 209–214

Steffansen, B., Nielsen, C. U., Brodin, B., Eriksson, A. H., Andersen, R., Frokjaer, S. (2004) Intestinal solute carriers: an overview of trends and strategies for improving oral drug absorption. *Eur. J. Pharm. Sci*. **21**: 3–16

Steffansen, B., Nielsen, C. U., Frokjaer, S. (2005) Delivery aspects of small peptides and substrates for peptide transporters. *Eur. J. Pharm. Biopharm*. **60**: 241–245

Steiner, H. Y., Naider, F., Becker, J. M. (1995) The PTR family: a new group of peptide transporters. *Mol. Microbiol*. **16**: 825–834

Steinhardt, H. J., Adibi, S. A. (1986) Kinetics and characteristics of absorption from an equimolar mixture of 12 glycyl-dipeptides in human jejunum. *Gastroenterology* **90**: 577–582

Su, Y., Sinko, P. J. (2006) Drug delivery across the blood-brain barrier: why is it difficult? How to measure and improve it? *Expert Opin. Drug Deliv*. **3**: 419–435

Sugawara, M., Huang, W., Fei, Y. J., Leibach, F. H., Ganapathy, V., Ganapathy, M. E. (2000) Transport of valganciclovir, a ganciclovir prodrug, via peptide transporters PEPT1 and PEPT2. *J. Pharm. Sci*. **89**: 781–789

Sugawara, M., Ogawa, T., Kobayashi, M., Miyazaki, K. (2003) Uptake of dipeptide and ß-lactam antibiotics by the basolateral membrane vesicles prepared from rat kidney. *Biochim. Biophys. Acta* **1609**: 39–44

Swaan, P. W., Stehouwer, M. C., Tukker, J. J. (1995) Molecular mechanism for the relative binding affinity to the intestinal peptide carrier. Comparison of three ACE-inhibitors: enalapril, enalaprilat, and lisinopril. *Biochim. Biophys. Acta* **1236**: 31–38

Tamai, I., Nakanishi, T., Hayashi, K., Terao, T., Sai, Y., Shiraga, T., Miyamoto, K., Takeda, E., Higashida, H., Tsuji, A. (1997) The predominant contribution of oligopeptide transporter PepT1 to intestinal absorption of ß-lactam antibiotics in the rat small intestine. *J. Pharm. Pharmacol*. **49**: 796–801

Tamai, I., Nakanishi, T., Nakahara, H., Sai, Y., Ganapathy, V., Leibach, F. H., Tsuji, A. (1998) Improvement of L-dopa absorption by dipeptidyl derivation, utilizing peptide transporter PepT1. *J. Pharm. Sci*. **87**: 1542–1546

Taub, M. E., Moss, B. A., Steffansen, B., Frokjaer, S. (1998) Oligopeptide transporter mediated uptake and transport of D-Asp(OBzl)-Ala, D-Glu(OBzl)-Ala and D-Ser(Bzl)-Ala in filter-grown Caco-2 monolayers. *Int. J. Pharm*. **174**: 223–232

Terada, T., Inui, K. (2004) Peptide transporters: structure, function, regulation and application for drug delivery. *Curr. Drug Metab*. **5**: 85–94

Terada, T., Saito, H., Mukai, M., Inui, K. I. (1996) Identification of the histidine residues involved in substrate recognition by a rat H^+/peptide cotransporter, PEPT1. *FEBS Lett*. **394**: 196–200

Terada, T., Saito, H., Mukai, M., Inui, K. (1997a) Characterization of stably transfected kidney epithelial cell line expressing rat H^+/peptide cotransporter PEPT1: localization of PEPT1 and transport of ß-lactam antibiotics. *J. Pharmacol. Exp. Ther*. **281**: 1415–1421

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

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

Terada, T., Sawada, K., Saito, H., Hashimoto, Y., Inui, K. (1999) Functional characteristics of basolateral peptide transporter in the human intestinal cell line Caco-2. *Am. J. Physiol*. **276**: G1435–G1441

Terada, T., Saito, H., Sawada, K., Hashimoto, Y., Inui, K. (2000a) N-terminal halves of rat H^+/peptide transporters are responsible for their substrate recognition. *Pharm. Res*. **17**: 15–20

Terada, T., Sawada, K., Irie, M., Saito, H., Hashimoto, Y., Inui, K. (2000b) Structural requirements for determining the substrate affinity of peptide transporters PEPT1 and PEPT2. *Pflügers Arch*. **440**: 679–684

Terada, T., Sawada, K., Ito, T., Saito, H., Hashimoto, Y., Inui, K. (2000c) Functional expression of novel peptide transporter in renal basolateral membranes. *Am. J. Physiol*. **279**: F851–F857

Terada, T., Sawada, K., Saito, H., Hashimoto, Y., Inui, K. (2000d) Inhibitory effect of novel oral hypoglycemic agent nateglinide (AY4166) on peptide transporters PEPT1 and PEPT2. *Eur. J. Pharmacol*. **392**: 11–17

Terada, T., Irie, M., Okuda, M., Inui, K. (2004) Genetic variant Arg57His in human H^+/peptide cotransporter 2 causes a complete loss of transport function. *Biochem. Biophys. Res. Commun*. **316**: 416–420

Teuscher, N. S., Novotny, A., Keep, R. F., Smith, D. E. (2000) Functional evidence for presence of PEPT2 in rat choroid plexus: studies with glycylsarcosine. *J. Pharmacol. Exp. Ther*. **294**: 494–499

Teuscher, N. S., Keep, R. F., Smith, D. E. (2001) PEPT2-mediated uptake of neuropeptides in rat choroid plexus. *Pharm. Res*. **18**: 807–813

Teuscher, N. S., Shen, H., Shu, C., Xiang, J., Keep, R. F., Smith, D. E. (2004) Carnosine uptake in rat choroid plexus primary cell cultures and choroid plexus whole tissue from PEPT2 null mice. *J. Neurochem*. **89**: 375–382

Thamotharan, M., Lombardo, Y. B., Bawani, S. Z., Adibi, S. A. (1997) An active mechanism for completion of the final stage of protein degradation in the liver, lysosomal transport of dipeptides. *J. Biol. Chem*. **272**: 11786–11790

Theis, S., Hartrodt, B., Kottra, G., Neubert, K., Daniel, H. (2002a) Defining minimal structural features in substrates of the H^+/peptide cotransporter PEPT2 using novel amino acid and dipeptide derivatives. *Mol. Pharmacol*. **61**: 214–221

Theis, S., Knütter, I., Hartrodt, B., Brandsch, M., Kottra, G., Neubert, K., Daniel, H. (2002b) Synthesis and characterization of high-affinity inhibitors of the H^+/peptide transporter PEPT2. *J. Biol. Chem*. **277**: 7287–7292

Thwaites, D. T., Anderson C. M. (2007) H^+-coupled nutrient, micronutrient and drug transporters in the mammalian small intestine. *Exp. Physiol*. **92**: 603–619

Thwaites, D. T., Hirst, B. H., Simmons, N. L. (1994) Substrate specificity of the di/tripeptide transporter in human intestinal epithelia (Caco-2): identification of substrates that undergo H^+-coupled absorption. *Br. J. Pharmacol*. **113**: 1050–1056

Thwaites, D. T., Cavet, M., Hirst, B. H., Simmons, N. L. (1995) Angiotensin-converting enzyme (ACE) inhibitor transport in human intestinal epithelial (Caco-2) cells. *Br. J. Pharmacol*. **114**: 981–986

Thwaites, D. T., Ford, D., Glanville, M., Simmons, N. L. (1999) H^+/solute-induced intracellular acidification leads to selective activation of apical Na^+/H^+ exchange in human intestinal epithelial cells. *J. Clin. Invest*. **104**: 629–635

Thwaites, D. T., Kennedy, D. J., Raldua, D., Anderson, C. M., Mendoza, M. E., Bladen, C. L., Simmons, N. L. (2002) H^+/dipeptide absorption across the human intestinal epithelium is controlled indirectly via a functional Na^+/H^+ exchanger. *Gastroenterology* **122**: 1322–1333

Tomita, Y., Katsura, T., Okano, T., Inui, K., Hori, R. (1990) Transport mechanisms of bestatin in rabbit intestinal brush-border membranes: role of H^+/dipeptide cotransport system. *J. Pharmacol. Exp. Ther*. **252**: 859–862

Tsuda, M., Terada, T., Irie, M., Katsura, T., Niida, A., Tomita, K., Fujii, N., Inui, K. (2006) Transport characteristics of a novel peptide transporter 1 substrate, antihypotensive drug midodrine, and its amino acid derivatives. *J. Pharmacol. Exp. Ther*. **318**: 455–460

Tsuji, A. (1999) Tissue selective drug delivery utilizing carrier-mediated transport systems. *J. Control. Release* **62**: 239–244

Tsuji, A., Tamai, I., Hirooka, H., Terasaki, T. (1987) β-lactam antibiotics and transport via the dipeptide carrier system across the intestinal brush-border membrane. *Biochem. Pharmacol*. **36**: 565–567

Tsuji, A., Tamai, I., Nakanishi, M., Amidon, G. L. (1990) Mechanism of absorption of the dipeptide alpha-methyldopa-phe in intestinal brush-border membrane vesicles. *Pharm. Res*. **7**: 308–309

Tsuji, A., Tamai, I., Nakanishi, M., Terasaki, T., Hamano, S. (1993) Intestinal brush-border transport of the oral cephalosporin antibiotic, cefdinir, mediated by dipeptide and monocarboxylic acid transport systems in rabbits. *J. Pharm. Pharmacol*. **45**: 996–998

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

Uebel, S., Kraas, W., Kienle, S., Wiesmuller, K. H., Jung, G., Tampe, R. (1997) Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries. *Proc. Natl Acad. Sci. USA* **94**: 8976–8981

Ugolev, A. M. (1971) Membrane digestion and peptide transport. *Ciba Found. Symp*. 1971, 23–143

Vabeno, J., Lejon, T., Nielsen, C. U., Steffansen, B., Chen, W., Ouyang, H., Borchardt, R. T., Luthman, K. (2004) Phe-Gly dipeptidomimetics designed for the di-/tripeptide transporters PEPT1 and PEPT2: synthesis and biological investigations. *J. Med. Chem*. **47**: 1060–1069

Vernaleken, A., Veyhl, M., Gorboulev, V., Kottra, G., Palm, D., Burckhardt, B. C., Burckhardt, G., Pipkorn, R., Beier, N., van Amsterdam, C., Koepsell, H. (2007) Tripeptides of RS1 (RSC1A1) inhibit a monosaccharide-dependent exocytotic pathway of Na^+-D-glucose cotransporter SGLT1 with high affinity. *J. Biol. Chem*. **282**: 28501–28513

Vig, B. S., Stouch, T. R., Timoszyk, J. K., Quan, Y., Wall, D. A., Smith, R. L., Faria, T. N. (2006) Human PEPT1 pharmacophore distinguishes between dipeptide transport and binding. *J. Med. Chem*. **49**: 3636–3644

Wanchana, S., Yamashita, F., Hara, H., Fujiwara, S., Akamatsu, M., Hashida, M. (2004) Two- and three-dimensional QSAR of carrier-mediated transport of β-lactam antibiotics in Caco-2 cells. *J. Pharm. Sci*. **93**: 3057–3065

Weitz, D., Harder, D., Casagrande, F., Fotiadis, D., Obrdlik, P., Kelety, B., Daniel, H. (2007) Functional and structural characterization of a prokaryotic peptide transporter with features similar to mammalian PEPT1. *J. Biol. Chem*. **282**: 2832–2839

Wenzel, U., Thwaites, D. T., Daniel, H. (1995) Stereoselective uptake of β-lactam antibiotics by the intestinal peptide transporter. *Br. J. Pharmacol*. **116**: 3021–3027

Wenzel, U., Gebert, I., Weintraut, H., Weber, W. M., Clauss, W., Daniel, H. (1996) Transport characteristics of differently charged cephalosporin antibiotics in oocytes expressing the cloned intestinal peptide transporter PepT1 and in human intestinal Caco-2 cells. *J. Pharmacol. Exp. Ther*. **277**: 831–839

Whitaker, C. J., Battah, S. H., Forsyth, M. J., Edwards, C., Boyle, R. W., Matthews, E. K. (2000) Photosensitization of pancreatic tumour cells by delta-aminolaevulinic acid esters. *Anticancer Drug Des*. **15**: 161–170

Yamashita, T., Shimada, S., Guo, W., Sato, K., Kohmura, E., Hayakawa, T., Takagi, T., Tohyama, M. (1997) Cloning and functional expression of a brain peptide/histidine transporter. *J. Biol. Chem*. **272**: 10205–10211

Yeung, A. K., Basu, S. K., Wu, S. K., Chu, C., Okamoto, C. T., Hamm-Alvarez, S. F., von Grafenstein, H., Shen, W. C., Kim, K. J., Bolger, M. B., Haworth, I. S., Ann, D. K., Lee, V. H. (1998) Molecular identification of a role for tyrosine 167 in the function of the human intestinal proton-coupled dipeptide transporter (hPEPT1). *Biochem. Biophys. Res. Commun*. **250**: 103–107

Yuasa, H., Fleisher, D., Amidon, G. L. (1994) Noncompetitive inhibition of cephradine uptake by enalapril in rabbit intestinal brush-border membrane vesicles: an enalapril specific inhibitory binding site on the peptide carrier. *J. Pharmacol. Exp. Ther*. **269**: 1107–1111

Zhang, E. Y., Fu, D. J., Pak, Y. A., Stewart, T., Mukhopadhyay, N., Wrighton, S. A., Hillgren, K. M. (2004) Genetic polymorphisms in human proton-dependent dipeptide transporter PEPT1: implications for the functional role of Pro586. *J. Pharmacol. Exp. Ther*. **310**: 437–445

Zhou, X., Thamotharan, M., Gangopadhyay, A., Serdikoff, C., Adibi, S. A. (2000) Characterization of an oligopeptide transporter in renal lysosomes. *Biochim. Biophys. Acta* **1466**: 372–378

Zhu, T., Chen, X. Z., Steel, A., Hediger, M. A., Smith, D. E. (2000) Differential recognition of ACE inhibitors in *Xenopus laevis* oocytes expressing rat PEPT1 and PEPT2. *Pharm. Res*. **17**: 526–532

Zlokovic, B. V. (1995) Cerebrovascular permeability to peptides: manipulations of transport systems at the blood-brain barrier. *Pharm. Res*. **12**: 1395–1406