An update on renal peptide transporters
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Daniel, Hannelore, and Isabel Rubio-Aliaga. An update on renal peptide transporters. Am J Physiol Renal Physiol 284: F885–F892, 2003; 10.1152/ajprenal.00123.2002.—The brush-border membrane of renal epithelial cells contains PEPT1 and PEPT2 proteins that are rhodogenic carriers for short-chain peptides. The carrier proteins display a distinct surface expression pattern along the proximal tubule, suggesting that initially di- and tripeptides, either filtered or released by surface-bound hydrolyases from larger oligopeptides, are taken up by the low-affinity but high-capacity PEPT1 transporter and then by PEPT2, which possesses a higher affinity but lower transport capacity. Both carriers transport essentially all possible di- and tripeptides and numerous structurally related drugs. A unique feature of the mammalian peptide transporters is the capability of proton-dependent electrogenic cotransport of all substrates, regardless of their charge, that is achieved by variable coupling in proton movement along with the substrate down the transmembrane potential difference. This review focuses on the postcloning research efforts to understand the molecular physiology of peptide transport processes in renal tubules and summarizes available data on the underlying genes, protein structures, and transporter function as derived from studies in heterologous expression systems.

PEPT1; PEPT2; renal physiology; localization; functional analysis

RENAL TUBULAR PEPTIDE TRANSPORT activity was originally discovered after intravenous infusion of the resistant dipeptide Gly-Sar in rats that resulted in a high accumulation of the intact dipeptide in renal tissue (1, 3). Studies with renal brush-border membrane vesicles established that renal apical peptide uptake was an electrogenic, proton-dependent uphill transport process for di- and tripeptides and related drugs mediated by two kinetically different systems (for a review, see Ref. 38). The underlying proteins, differing in transport characteristics, now designated as PEPT1 (SLC15A1) and PEPT2 (SLC15A2), have been identified, and the corresponding genes have been cloned from a variety of species (10, 24, 25, 39, 53), and are highly glycosylated. As shown by in vitro translation studies in the presence of microsomes, PEPT1 has a molecular mass of ~75 kDa, whereas digestion with endoglycosidase A shifted the mass to 63 kDa (53). The PEPT2 protein is also glycosylated, with a molecular mass of the mature protein of ~107 kDa and a non-glycosylated mass of 83 kDa (10). Western blot analysis of protein preparations of intestine and kidney identified PEPT1 and PEPT2 immunoreactivity as glycosylated proteins with molecular masses of ~75 and 100 kDa, respectively (47, 52). The Pept2 gene encodes a 729-amino acid protein (10, 41, 52, 54) with 48% amino acid identity to PEPT1 predominantly in transmembrane domains (TMD) and the lowest identity in the large extracellular loop connecting TMD 9 and 10. The genomic organization of the peptide transporter genes has also been elucidated. The murine Pept1 gene is estimated to be ~38 kb long, including a TATA-less promoter region (25), and both murine and human Pept1 genes possess 23 exons and 22 introns (25, 70). The murine Pept2 gene is 34 kb long, consists of 22 exons and 21 introns (52), and also carries a TATA-less promoter. The human Pept1 and Pept2 genes have been mapped to chromosomes 13q33–34 and 3q13.3-q21, respectively (39, 51). The murine Pept2 gene was
localized in a syntenic region with human chromosome 3q13.3-q21, on central mouse chromosome 16 close to D16Mit4 and D16Mit59 (52).

TISSUE DISTRIBUTION OF PEPT1 AND PEPT2 AND THEIR TUBULAR LOCALIZATION

PEPT1 was first identified in the brush-border membrane of the epithelial cells of the small intestine and cloned from an intestinal cDNA library (24, 47). It was then also found in the brush-border membrane of epithelial cells in the kidney proximal tubule S1 segment (60). By immunostaining and transport analysis, PEPT1 has also recently been shown to be present in the apical membrane of bile duct epithelial cells (36).

PEPT2 shows much broader expression and tissue distribution within the organism. It was initially cloned from a renal cDNA library and detected in brush-border membranes of cells in S2 and S3 segments (10, 60). More recently, PEPT2 has been identified in the peripheral nervous system, in the membranes and cytoplasm of satellite glial cells surrounding the ganglionic neurons (29), and in the central nervous system. In situ hybridization analysis revealed its presence in the brain in astrocytes, ependymal and subependymal cells, and in epithelial cells of the choroid plexus (8). In the lung, PEPT2 is expressed in type II pneumocytes and in the apical membrane of tracheal and bronchial epithelial cells (31). Furthermore, a recent study has shown the localization of PEPT2 in the apical membranes of the epithelial cells of the terminal duct and glandules as well as the main and segmental ducts in the lactating mammary gland (30). By RT-PCR, expression of PEPT2 mRNA was also demonstrated in the spleen, colon, and pancreas (20).

Along the nephron, peptide transporters are only found in the proximal tubule (see Fig. 1), both by analysis of function as well as by immunolocalization. PEPT1 has been detected in the brush border of the epithelial cells of the convoluted proximal tubule, in the S1 segment, with progressively weaker expression in deeper cortical regions (60). PEPT2 is localized in the brush-border membrane of S2 and S3 segment cells of the proximal tubule, with strong immunostaining in the outer stripe but not the inner stripe of the outer medulla, including the medullary rays protruding into the deeper cortical regions (60). A recent study employing renal brush-border membrane vesicles has functionally verified this distribution of the peptide transporters in the proximal tubule (40). Whereas in membranes prepared from outer cortex cells, two systems mediated the transport of the model dipeptide Gly-Sar, in membranes from cells of the outer medulla only one system was kinetically detectable. Biochemical studies in the 1980s with brush-border membrane vesicles already showed the presence of a low-affinity, high-capacity and a high-affinity, low-capacity transport system (18). Studies in renal cell lines such as Madin-Darby canine kidney cells that possess PEPT1 activity and LLC-PK1 and SKPT cells that express PEPT2 activity and various heterologous expression systems have established that PEPT1 is the low-affin-

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**Fig. 1. Renal peptide transporters PEPT1 and PEPT2.** PEPT1 and PEPT2 are localized in the apical membrane of epithelial cells of the proximal tubule with a distinct expression pattern. A: comparative transport rates for the substrate O-aminolevulinic acid by PEPT1 or PEPT2 under identical experimental conditions, which demonstrate that PEPT1 is the low-capacity, high-affinity transport system and PEPT2 the high-affinity, low-capacity carrier. B: different affinities of the 2 carriers for a large set of identical Ala-Xaa (filled symbols) and Xaa-Ala (grey symbols) dipeptides. A, D, E, F, G, H, K, L, P, R, S, W, and Y: single amino acids; αA, a-aminobutyric acid; Ai, aminoisobutyric acid; Ni, norleucine; Pi, piperolic acid; Sa, sarcosine. C: simplified model in the role of renal peptide transporters in metabolism of proteins, di- and tripeptides, and amino acids in renal tubular cells. p, Proteins; AT, amino acid transporter.
ity-type transporter. PEPT2 shows under the same experimental conditions much higher affinities for almost all tested substrates. The different kinetic properties of PEPT1 and PEPT2 are shown with δ-amino-levulinic acid as a substrate in Fig. 1A. The maximal transport capacity of PEPT1 appears to be 5–15 times higher than that of PEPT2 for most substrates and in all cellular systems analyzed so far. This supports the contention that peptides are handled sequentially in the kidney, first by the low-affinity, high-capacity transporter PEPT1 and then by the high-affinity, low-capacity transporter PEPT2.

That the transport activity of the renal peptide transporters can also be visualized is shown in Fig. 2 by use of the fluorescent dipeptide conjugate d-Ala-Lys-Ne-7-amino-4-methylcoumarin-3-acetic acid (d-Ala-Lys-AMCA), a known substrate of PEPT2 and PEPT1 (28, 31). Rat kidney perfused with d-Ala-Lys-AMCA reveals strong fluorescent staining only in epithelial cells of the proximal tubule, and staining is almost completely blocked by the dipeptide Gly-Gln when coadministered.

**THE SUBSTRATE SPECIFICITY OF PEPT1 AND PEPT2**

One of the striking features of the mammalian peptide transporters is their apparent promiscuity, because both carriers can transport essentially every possible di- and tripeptide. This means that there are at least 400 different dipeptides and 8,000 different tripeptides that could serve as substrates, and these peptides vary considerably in molecular size, charge, and polarity. Transport occurs in all cases enantioselectively, but di- and tripeptides containing a D-amino acid, particularly when placed in the NH₂ terminus, are also accepted as substrates, although with lower affinity (19). This further increases the number and variety of potential substrates. Moreover, there are drugs such as numerous angiotensin-converting enzyme inhibitors, peptidase inhibitors, and a variety of novel prodrugs that are also taken up by both carriers (for a review, see Refs. 34 and 71). Another puzzling and still not fully explained characteristic of the transporters is that, regardless of the substrates' net charge at a given pH, transport occurs electrogenically and is always associated with the cotransport of protons, as shown by simultaneous recordings of intracellular pH changes. Electrogenicity in the transport of charged substrates is presently best explained by a variable flux coupling ratio for substrate-to-proton cotransport (62). Although there are some differences in the affinities of the different substrate groups, PEPT1 and PEPT2 essentially can transport all di- and tripeptides in an electrogenic mode, and this is important in view of their role in reabsorption and the most efficient conservation of peptide-bound amino acids.

To unravel the secret of the broad substrate spectrum accepted by PEPT1 and PEPT2, numerous studies have been conducted. Large sets of dipeptides, amino acid derivatives, or peptidomimetics have been probed for transport in competition experiments utilizing cells expressing either one of the transporters, by the measurement of substrate-mediated transport currents in Xenopus laevis oocytes expressing PEPT1 or PEPT2, or by competition experiments in the yeast Pichia pastoris heterologously expressing either one of the mammalian transporters (11, 12, 14, 21, 22, 66, 67). One important finding for understanding the "multispecificity" of the peptide transporters was that neither PEPT1 nor PEPT2 requires a peptide bond in a substrate and that the minimal structural requirement in a substrate for binding and transport is a simple carbon chain that separates the oppositely charged NH₂ and COOH head groups by an intramolecular distance of > 500 < 630 picometers (21). In the case of PEPT2, we recently showed by the rational design of a large set of test compounds that the much higher affinity of PEPT2 for the same substrates is based on the requirement of an additional carbonyl function, but...
not COOH, within the backbone of a substrate (67). These minimal substrate requirements are perfectly matched by δ-aminolevulinic acid (ALA), which was shown to be a good substrate of PEPT2 and PEPT1 (20). This compound serves as a precursor of phorphyrin synthesis and is used in the treatment of various cancers by photodynamic therapy, whereby ALA is then administered either exogenously or topically. ALA is known to possess pronounced renal toxicity by selective accumulation in tubular cells (35), to which PEPT1 and PEPT2 might contribute by effective reabsorption of ALA from tubular fluids.

In the case of normal di- and tripeptides, the substrate binding site in both carriers can accept all the various side chains of amino acids, but it appears that the side chains are accommodated in asymmetric binding pockets. This has also been demonstrated by the selectivity of newly identified PEPT1 and PEPT2 inhibitors with \([Z(NO_2)]\) groups attached to the ε-amino group in Lys dipeptides (37, 68). Only when present in a particular position within the dipeptide, the Lys-[Z(NO_2)] derivatives served as inhibitors; otherwise, they were substrates. The bulkiness and/or charge of the amino acid side chains alter the affinity of dipeptides for interaction with PEPT1 and PEPT2. This is shown in Fig. 1B, in which the same substrates have been used to determine the apparent \(K_i\) values for inhibition of D-Phe-Ala transport mediated by PEPT1 or PEPT2 under identical experimental conditions in transgenic \(P. pastoris\) cells expressing either rabbit PEPT1 or PEPT2. It should be emphasized here that affinities for the same substrate determined in different expression systems can vary considerably, and this is most likely due to marked effects of membrane potential and pH on substrate binding affinity. All dipeptides tested have generally higher affinities for PEPT2 than for PEPT1, but the differences can be very modest, as in the case of Gly-Asp and Lys-Glu, or ~20-fold, as in the case of Lys-Gly.

Although there are also differences in the affinities of peptidomimetics, the same classes of xenobiotics such as the various aminoccephalosporins and selected angiotensin-converting enzyme inhibitors are taken up by both carriers. A recently proposed template for binding of a substrate to PEPT1 explains most of the structural and conformational requirements known thus far for the interaction of substrates with the substrate binding domain and possibly also for PEPT2 with similar but not identical structural substrate requirements (6).

INTEGRATING THE PEPTIDE TRANSPORTERS INTO OVERALL AMINO ACID HOMEOSTASIS

Studies of the plasma clearance of dipeptides indicate that the renal tubule is the only tissue capable of accumulating short-chain peptides in concentrations that are greater than their plasma concentrations (for a review, see Refs. 2 and 17). This observation is also in accordance with the recent findings on the localization of the peptide transporters PEPT1 and PEPT2 in extrarenal tissues. Although PEPT1 is found in the intestine and in bile duct epithelium (36, 47), in these locations it does not contribute to the clearance of di- and tripeptides from the circulation. PEPT2 has been localized to the apical membrane of tracheal and bronchial epithelial cells in the lung (31) and ductal cells of the lactating mammary gland (30), but by this localization PEPT2 cannot also play a role in the clearance of short-chain peptides from the blood. Functional studies using isolated choroid plexus suggest that PEPT2 there is located in the apical membrane, where it may mediate the removal of di- and tripeptides from the cerebrospinal fluid to the blood (46) rather than serving as an import system.

In the kidney, the peptide transporters contribute to the homeostasis of amino acids in the organism along with several classes of amino acid transporters located as well in the apical membrane of tubular cells (for a review, see Ref. 48). Di- and tripeptides are delivered to PEPT1 and PEPT2 in epithelial cells either by surface hydrolysis of larger oligopeptides or by glomerular filtration (see Fig. 1). Data from animal studies suggest that up to 50% of circulating plasma amino acids might be peptide bound and of those 25–50% could be di- and tripeptides (27, 57, 58). However, the composition of this di-/tripeptide fraction circulating in plasma or provided in the filtrate is not known. Moreover, only a few individual dipeptides have been analyzed in plasma. The highest concentration of a known individual dipeptide (Cys-Gly) in plasma and, based on its free filtration also in tubular fluids, is \(> 50 < 70 \mu M\) (49). Cys-Gly, which is a breakdown product of glutathione, is obviously cleared quite well in the kidney, with mean concentrations in human urine samples of 7.4 μM. In a concentration range of 50 μM, both peptide transporters may contribute to the reabsorption of Cys-Gly. However, the regional distribution of the peptide transporter along the nephron and recent functional studies suggest that the di- and tripeptides are handled sequentially in the kidney, first by the low-affinity, high-capacity system PEPT1, and then by the high-affinity, low-capacity system, which would dominate at lower substrate concentrations (40). The renal zonation and the different affinities and transport capacities of the two transporters in concert allow maximal reabsorption capacity and highest efficiency for conservation of peptide-bound amino acids and the reduction of renal losses of amino acid nitrogen.

Whereas the apical uptake of di- and tripeptides has been studied extensively and may represent the main route for renal peptide clearance from circulation, recent reports have suggested that a basolateral peptide transporter may also contribute to the selective uptake of peptides into the kidney (see Fig. 1). Studies in Madin-Darby canine kidney cells, which display characteristics of cells of distal tubules or collecting ducts, and LLC-PK1 cells, which display the characteristics of cells of the proximal tubule, express a transport activity for influx across the basolateral membrane that is distinctly different from the peptide transporter type on the apical side (56, 64). These studies suggest that
another peptide transporter has to be found and cloned. However, studies in intact perfused rat kidney in vitro have failed to detect any peritubular dipeptide (Gly-Sar) uptake when glomerular filtration was prevented (43). The basolateral membrane therefore remains as the dark site of epithelial peptide transport.

When peptides are transported into the cell from the apical side, most of them will be cleaved rapidly by cytosolic peptidases because the kidney exhibits the highest intracellular hydrolase activity against short-chain peptides (2) (see Fig. 1C). Dipeptides resistant to hydrolysis or peptidomimetics that cannot be cleaved (i.e., aminopenicillins) may even be released intact back into the circulation across the basolateral membrane (7). Whether this efflux is mediated by an as yet unknown peptide transporter or one of the organic anion and or cation transporters of the OCT and OAT series, which can also transport zwitterionic compounds, remains to be determined. Immunodetection studies with PEPT1- or PEPT2-specific antisera have yet not shown any cross-reactivity with proteins in renal basolateral membranes. Some conflicting results have been obtained for a immunoreactive protein in renal lysosomes. A pH-dependent dipeptide transport activity, similar to PEPT1 but with a lower affinity, has been identified in renal lysosomes, and this system may be responsible for the export of di- and tripeptides from the lysosomes to the cytoplasm (75). In Western blot analysis of lysosomal proteins, a PEPT1 antiserum recognized a specific band with a mass of ~78 kDa. The lysosomal peptide transporter could contribute to renal clearance of short-chain peptides, and the cellular delivery of free amino acids for protein synthesis and metabolism can be anticipated as most of the proteins filtered in the glomerulus are reabsorbed in the renal proximal tubule by endocytosis mediated by binding to endocytic receptor proteins such as megalin or cubilin (16) (see Fig. 1). After endocytosis, the proteins accumulate in lysosomes for degradation and the lysosomal peptide transporter could provide a route for the transfer of released di- and tripeptide from a region of low dipeptidase activity (lysosomes) to a region of high dipeptidase activity (cytosol). This compartmentation could also prevent a premature osmotically induced swelling and rupture of lysosomes (75). The question of whether the lysosomal peptide transporter is PEPT1 or one of the PHT histidine/peptide transporters recently cloned, which are found in lysosomes when expressed heterologously (33, 55, 74), or a completely new transporter remains to be determined.

REGULATION OF EXPRESSION LEVEL AND TRANSPORT ACTIVITY OF PEPT1 AND PEPT2

Most of the studies on regulation of peptide transport have been performed with PEPT1 in view of its intestinal role in the transport of dietary peptides and drugs. Comparative analysis using Northern and Western blotting techniques has shown an increase in the expression of PEPT1 in the small intestine immediately after birth and expression in the colon, which disappeared in adult stage (59). In the kidney, the expression levels of PEPT1 and PEPT2 increased steadily over time to reach maximal levels 2 wk after birth. A differential adaptive response of PEPT1 and PEPT2 has recently been shown in a model of chronic renal failure. The % nephrectomized rat is widely used as a model for the study of the progression of renal damage resulting from the reduction of nephron mass. In this model, a pronounced upregulation of PEPT2 mRNA and protein expression 2 wk after renal ablation occurred, whereas mRNA and protein levels of PEPT1 did not change (63). This is the first study to show the regulation of the peptide transporter PEPT2 in kidney in vivo, and the findings may also be of importance for the pharmacokinetics of the drugs transported by PEPT2 in patients suffering from chronic renal failure.

Dietary regulation of the intestinal peptide transporter PEPT1 has been demonstrated in several studies (23, 61, 72). An increase in the protein content of a diet increased the uptake of a model dipeptide in the small intestine of rats as well as the mRNA and proteins levels of PEPT1 (23, 61). Moreover, addition of selected amino acids or dipeptides to media in cell culture increased dipeptide uptake, and in most cases also the mRNA and proteins levels of PEPT1 (61, 72). This stimulation of gene transcription may be a consequence of an activation of the Pept1 promoter via selected amino acids and dipeptides (61). Promoter analysis studies employing a luciferase reporter assay identified a region that responded to all dipeptides tested and also to selected amino acids, such as Lys, Arg, and especially Phe. This region comprises 254 bp and contains an amino acid response-like element as found in the asparagine synthetase gene (32). Rat and mouse Pept1 and the murine Pept2 promoter regions contain these amino acid response-like elements with a bp substitution in the fifth bp (25, 52). Whether these cis elements play a role in controlling gene expression in the kidney in response to cellular availability of free amino acids and dipeptides needs to be determined. However, studies in humans suggest that starvation reduces renal peptide transport activity, as demonstrated by a reduction in removal of the intravenously infused dipeptide Gly-Gln by the kidney (4, 42). In the yeast Saccharomyces cerevisiae, gene expression of Ptr-2, the yeast peptide transporter (50), is upregulated at the expression level by selected dipeptides that activate an ubiquitin-dependent proteolytic pathway (69). Whether such a mechanism controls the expression levels of mammalian peptide transporters also has to be investigated.

Receptor ligand-induced acute regulation of PEPT1 and PEPT2 transport activities has so far only been studied in cell lines expressing PEPT1, such as intestinal Caco-2 and renal Madin-Darby canine kidney cells, or in renal LLC-PK1 and SKPT cells expressing PEPT2. In Caco-2 cells, insulin (65), leptin (15), α1- and α2-adrenergic receptor ligands such as (+)-pentazocine (26), and clonidine (9) all were shown to stimulate uptake of dipeptides or β-lactams via PEPT1. On the
other hand, either exposure to two immunosuppressive agents, tacrolimus and cyclosporin A (44), or exposure to thyroid hormone (5) or long-term basolateral stimulation with EGF inhibited the uptake of dipeptides via PEPT1 in Caco-2 cells (44). Moreover, it has been demonstrated that activation of signaling pathways that involve protein kinases C and A changes the kinetic properties of PEPT1 and PEPT2 in intestinal and renal cell lines (13, 73). Whether these in vitro observations are of physiological importance in vivo in renal tissues remains to be determined.

CONCLUDING REMARKS

When expression cloning of the mammalian peptide transporters allowed one to relate the transport phenomena observed in intact tissues or tissue preparations to distinct proteins, exciting new findings on the molecular physiology of these unique proton-coupled transporters have been obtained. Heterologous expression studies of cloned transporters yielded a wealth of information on substrate specificity, coupling stoichiometry, and translocation mechanisms, and biophysical analyses of transporter mutants and chimeras provide first clues on the domains within the transporter proteins that contribute to substrate binding and determine specificity. New data on tissue distribution, particularly with respect to PEPT2, have been gathered that are still puzzling in view of the physiological role of the transporter in these tissues. We are now awaiting information on the metabolic consequences of the lack of renal peptide transporters in experimental animals from targeted gene inactivation.

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