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PEPTIDE TRANSPORTERS IN THE INTESTINE AND THE KIDNEY

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ABSTRACT

Even though the existence of a transport process for intact peptides in the brush border membrane of intestinal and renal absorptive epithelial cells has been known for almost three decades, it is only recently that the molecular nature of the proteins responsible for the transport process has been elucidated. Two peptide transporters, PEPT 1 and PEPT 2, have been cloned. The cloned transporters catalyze active transport of intact di- and tripeptides and utilize a transmembrane electrochemical H^+ gradient as the driving force. The characteristic of H^+ coupling makes PEPT 1 and PEPT 2 unique among the transporters thus far identified in mammalian cells. In addition, the peptide transporters have immediate pharmacologic relevance because a number of peptide-like drugs are recognized as substrates by these transporters. Recently, cultured cell lines of intestinal and renal origin that express PEPT 1 and PEPT 2 have been identified. These cell lines are likely to facilitate studies on the regulatory aspects of the peptide transporters.

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INTRODUCTION

Peptide transporters mediate the cellular uptake of small intact peptides consisting of two or three amino acids. These transporters are distinct from those responsible for the uptake of free amino acids. Transmembrane transport of peptides is a phenomenon widely distributed throughout nature. It is present in animals including man, in bacteria, in yeast, in molds, and in germinating seeds of higher plants (66). A special feature of peptide transport is that it is not only conserved as a process throughout evolution, its operational mechanism has also been conserved. In biological systems, active transport of organic solutes is coupled primarily to transmembrane ion gradients. In the microbial kingdom, the principal inorganic ion used for this purpose is H^+. The electrochemical H^+ gradient (proton-motive force) in these systems is the primary energy source for the transport of amino acids and sugars, as well as of peptides. In contrast, in the animal kingdom the principal coupling ion for active transport appears to be Na^+ rather than H^+. The electrochemical Na^+ gradient (sodium-motive force) provides the energy for active transport of most organic solutes, including amino acids and sugars in animal cells. But the peptide transport process is an exception, because the evolutionary shift in the coupling ion, i.e. from H^+ to Na^+, that occurred in the case of amino acids and sugars did not occur in the case of peptides. It is the proton-motive force, not the sodium-motive force, that energizes active transport of peptides across the animal cell plasma membrane. Thus, the energetics of the peptide transport process in animal cells makes it unique and distinct from most other solute transport processes.

IMPORTANCE OF INTESTINAL AND RENAL PEPTIDE TRANSPORTERS

In mammals, the peptide transporters are expressed primarily, though perhaps not exclusively, in the intestine and the kidney (29, 35). The functional importance of the peptide transport process in these two organs can be divided into three categories—nutritional, pharmacological, and clinical. Numerous

studies have convincingly shown that absorption of protein digestion products in the small intestine occurs primarily in the form of small peptides (for reviews, see References 42, 65, 67). The investigations carried out in Hartnup disease and Cystinuria, two distinct genetic disorders of amino acid transport, have emphasized the nutritional importance of the peptide transport process. Affected individuals rarely exhibit clinical symptoms of protein malnutrition. In these genetic disorders, the affected amino acids are absorbed normally if presented to the intestine in the form of small peptides. The apparently normal protein nutritional status of the affected individuals suggests that the intestinal absorption of peptides is quantitatively more important than the absorption of free amino acids. A kinetic advantage is also conferred by the transport of intact peptides versus the transport of free amino acids. Furthermore, the peptide transport process is relatively resistant to alterations by various conditions that affect the amino acid transport process. Recent studies have shown that the intestinal absorption of the trace element Zn^{2+} can occur at least partly in the form of peptide-Zn^{2+} complex, which is recognized as a substrate by the peptide transporter (91). In addition to the small intestine, the absorptive cells of the renal proximal tubule also possess active transport mechanisms for small peptides. This organ may play a significant role in conserving peptide-bound amino nitrogen via the peptide transport process, which might otherwise be lost in the urine. The physiologic significance of such a role, however, was viewed with considerable doubt for a long time because it was generally assumed, with little supporting data, that the concentrations of small peptides in the circulation are very low. But recent studies (39, 66, 83, 84) have provided clear evidence to the contrary, demonstrating unequivocally that greater than 50% of the plasma amino acid pool is in peptide-bound form, of which one fourth to one half represent di- and tripeptides. The concentration of small peptides in the renal tubular lumen is likely to be higher than in the plasma, because larger peptides and small proteins that are filtered by the glomerulus are expected to be hydrolyzed by the highly active peptidases (e.g. dipeptidylpeptidase IV and aminopeptidase N) of the renal brush border membrane to generate smaller peptides. The urinary excretion of acid-soluble, peptide-bound amino acids is very low (74). Therefore, it is highly likely that the renal peptide transport system does indeed serve an important function in the conservation of peptide-bound amino nitrogen.

The peptide transport systems present in the small intestine and the kidney also have pharmacologic relevance. Many orally active peptide antibiotics possess structural features similar to those of the physiologic substrates of the peptide transport system. The intestinal peptide transport system accepts these antibiotics as substrates and acts as a vehicle for their effective absorption (75, 104, 105). The relative efficacy of these antibiotics, therefore, is determined by the relative affinity of the intestinal peptide transport system for them. Their

pharmacologic potency is determined not only by their rate of absorption in the small intestine, but also by their half-life in the circulation. The peptide transport system in the kidney is responsible for active reabsorption of these antibiotics from the glomerular filtrate (50, 59) and, hence, increases their half-life in the circulation. Thus, the intestinal and renal peptide transport systems play an important pharmacologic role in determining the efficacy of these antibiotics. It seems conceivable that the peptide antibiotics are not the only pharmacologically relevant compounds that use the peptide transport system as a vehicle for cellular uptake. The transport system also participates in the transport of many other therapeutically and biologically active peptides, such as angiotensin-converting enzyme inhibitors (90), renin inhibitors (57), and anticancer drugs (51). It has been suggested that the peptide transporters, in concert with the action of cytosolic peptidases, can be exploited for systemic delivery of certain drugs in the form of peptide prodrugs (2). For example, the dipeptide analogues of α-methyldopa, L-α-methyldopa-phe (106), and L-α-methyldopa-pro (48) are absorbed in the intestine much more efficiently than α-methyldopa is because the peptide analogues serve as substrates for the intestinal peptide transport system whereas α-methyldopa does not. Once transported into the intestinal cell, the peptide analogues are hydrolyzed by cytosolic peptidases to release α-methyldopa.

The clinical relevance of the peptide transport system in the small intestine and the kidney has received increasing attention in recent years. In current clinical practice, short-chain peptides are being seriously considered as viable substitutes for free amino acids in enteral and parenteral solutions (1, 26, 40, 42). The rationale for the attempt to replace amino acids with small peptides in the enteral solutions commonly used in patients with severely impaired gastrointestinal function is based on a question: Since the transport of peptides in the small intestine is the primary mode of nitrogen assimilation, is it logical to employ only free amino acids as the source of nitrogen in enteral solutions in these patients? Available evidence in laboratory animals and in man strongly suggests that enteral solutions containing small peptides may provide an absorptive advantage to patients with severely reduced intestinal absorptive area and to patients who are acutely catabolic (trauma, sepsis, and burns). Moreover, synthetic di- and tripeptides offer an effective alternative means to include in enteral solutions those amino acids that are unstable or sparingly soluble in free form (e.g. tyrosine, cystine, glutamine). Expanding knowledge about extraintestinal peptide assimilation in animals, especially the extraordinary ability of the mammalian kidney to extract small peptides and hydrolyze them into free amino acids, is encouraging the view that small peptides can substitute for free amino acids not only in enteral solutions but also in parenteral solutions. Recent studies have shown that it is possible to supply daily nitrogen requirements, intravenously, in the form of small peptides in animals and in

man (41, 89). Low osmolality of peptide-based parenteral solutions is another advantage, especially in patients with severe fluid restriction. Furthermore, evidence is strong that glutamine may be of importance to the septic or traumatized patients (22); unfortunately, the free amino acid is relatively unstable in solution and is converted during heat sterilization to pyrrolidone carboxylic acid. That amino acid can, however, be included in the form of a dipeptide, alanylglutamine, in intravenous solutions because, unlike free glutamine, it is stable. Human studies have shown that the metabolic utilization of alanylglutamine is highly efficient and that the supplementation of the parenteral solutions with this peptide significantly reduces postoperative nitrogen losses and prevents the postoperative reduction in muscle glutamine content (88). Thus, the peptide-based parenteral solutions may offer a variety of advantages in a clinical setting, and the basis for the clinical efficacy of these solutions is the ability of extraintestinal tissues, primarily the kidneys, to utilize peptides via peptide transport and hydrolysis.

FUNCTIONAL AND STRUCTURAL CHARACTERISTICS OF PEPTIDE TRANSPORTERS

Substrate Specificity and Stereoselectivity

The substrate specificity of the peptide transport systems in the intestine and the kidney has been determined primarily based on competition experiments. Most of these studies have used dipeptide substrates to measure the transporter activity. Whether or not peptides containing three or more amino acids act as substrates for the peptide transport systems was assessed by determining the ability of these peptides to inhibit the uptake of the dipeptide substrate. The consensus from these competition experiments is that the intestinal and renal peptide transporters accept di- and tripeptides as substrates and exclude larger peptides. Recently, direct evidence has been provided for the transport of intact tripeptides by the renal peptide transport system (98, 100, 101). Detailed kinetic analyses have revealed that a common transporter is responsible for the transport of di- and tripeptides (99).

Several studies have indicated that the intestinal and renal peptide transporters are stereoselective (4, 16, 21, 31, 62, 92). Peptides containing L-amino acids interact with the peptide transporters with greater affinity than do peptides containing D-amino acids. The same is true with peptidomimetic drugs, which also serve as substrates for the peptide transporters. The side chains of amino β-lactam antibiotics contain an asymmetric carbon atom, and the L-isomers of these drugs are better substrates for the peptide transporters than the D-isomers are. Lister et al (62) carried out a detailed investigation of the stereoselectivity of the intestinal peptide transport system. With dipeptides

comprising the L- and D-isomers of alanine and phenylalanine, they determined the relative importance of the chiral form of each of these amino acids at the N- and the C-termini of the dipeptide. Interestingly, the influence of the L-isomer versus the D-isomer on the affinity toward the transport system appears to vary markedly with each individual amino acid. Furthermore, the placement of the isomers, i.e. N- versus C-terminus, also has marked effects on the affinity. Similar detailed studies were conducted by Daniel et al (16) on the determinants of substrate affinity for the renal peptide transport system. These studies led to essentially similar results. Substitution of the amino and carboxy groups of the peptide substrates also greatly influences affinity. For example, blocking of these groups reduces affinity to a significant extent (31). However, several β-lactam antibiotics that are not zwitterions because of a lack of protonatable amino groups (e.g. cefixime and cefdinir) have been shown to be transported via the intestinal peptide transport system (49, 107, 108), indicating that these compounds are recognized as substrates, though with low affinity.

Driving Force

The transport of peptides via the peptide transporters in the intestinal and renal brush border membrane vesicles is not affected by a Na^+ gradient. Instead, the transport process is markedly influenced by a H^+ gradient. In isolated brush border membrane vesicles, imposition of an inwardly directed H^+ gradient leads to active uphill transport of peptides, as evidenced from the overshoot phenomenon. The H^+ gradient–dependent transport is stimulated by an inside-negative membrane potential and inhibited by an inside-positive membrane potential, implying that the transport of peptides across the membrane is associated with the transfer of positive charge. Thus, in the normal intestinal and renal absorptive cells, the H^+ gradient as well as the membrane potential provide the driving force for active transport of peptides. The possible involvement of an electrochemical H^+ gradient in the energization of intestinal and renal peptide transport systems was originally hypothesized based on studies from our laboratory (30, 32). Direct supporting evidence for this hypothesis subsequently came from several other laboratories, as well as from our own (for reviews, see References 29, 33–36, 47, 66). These findings are physiologically relevant because an inwardly directed electrochemical H^+ gradient is known to exist across the brush border membrane of intestinal and renal absorptive cells under in vivo conditions (3, 64). This gradient is generated and maintained by the combined action of the Na^+-H^+ exchanger in the brush border membrane and the Na^+-K^+-adenosine triphosphatase (ATPase) in the basolateral membrane.

Structural Features

The essential role of specific amino acid residues in the catalytic activity of the intestinal and renal peptide transport systems has been studied by assessing the ability of amino acid group–specific reagents to modify the catalytic activity of the transport system. Evidence points to involvement of histidyl groups in the activity of the intestinal (55, 58) and renal (68) peptide transporters. Chemical modification of histidyl groups with diethylpyrocarbonate leads to inactivation of the H^+-coupled peptide transport activity in intestinal as well as in renal brush border membrane vesicles. This is not surprising because histidyl groups have been shown to be essential for the activity of several other H^+-coupled transport systems in bacterial as well as mammalian systems (28, 46, 54, 80). The imidazole residue in the side chain of histidine is best suited for the role as a proton acceptor/proton donor under physiologic conditions. There is also evidence for a critical role for thiol groups in the catalytic function of the renal peptide transporter (68, 70). It has been suggested that a dithiol-disulfide interchange may be involved in the function of the transporter (70). A recent study demonstrated that Zn^{2+}, Mn^{2+}, and Cu^{2+} selectively enhance H^+/peptide cotransport catalyzed by the renal peptide transporter (14). The stimulatory effect is claimed to be the result of direct interaction of the metals with the transporter proteins. These results have led to the intriguing speculation that the renal peptide transporter may be a metalloprotein containing Zn^{2+} or a closely related transition metal as an integral part.

CLONING OF THE H^+-COUPLED PEPTIDE TRANSPORTERS

Intestinal H^+/Peptide Cotransporter PEPT 1

Even though considerable progress over the last decade has been made in the delineation of the functional aspects of the intestinal peptide transport system, little information has been available on the molecular nature of the protein component(s) involved in the transport process. With photolabile substrate analogues, it has been shown that a 127,000-kDa protein is specifically labeled in the intestinal brush border membrane (56). This protein has been suggested as a possible component of the intestinal peptide transport system. But the association between this protein and H^+-coupled peptide transport activity has not been demonstrated unequivocally.

Encouraged by the recent explosion of information on use of the expression cloning strategy with *Xenopus laevis* oocytes in the investigation of membrane transporters at the molecular level, we initiated studies, using this approach, to probe the molecular nature of the intestinal peptide transporter. These studies led to the successful expression of the intestinal H^+/peptide

cotransporter in *Xenopus* oocytes following microinjection of messenger (m) RNA isolated from the rabbit intestine (69). This work was followed by success in other laboratories in the functional expression of rat and human intestinal H^+/peptide cotransporters in *Xenopus* oocytes (82, 93, 94). Size fractionation of mRNA prior to injection into the oocytes has indicated that the functional expression of the peptide transport activity is associated with mRNA 1.8–3.6 kb in size (93, 94). These studies indicated clearly the feasibility of cloning the intestinal H^+/peptide cotransporter using this expression cloning strategy. Application of this strategy has recently led to the isolation of a complementary (c) DNA from the rabbit intestine that codes for a H^+-coupled peptide transporter (PEPT 1) (9, 23). The predicted protein consists of 707 amino acids and possesses 12 putative transmembrane domains. Consensus sequences for protein phosphorylation by protein kinase A and protein kinase C are also present. Multiple sites for potential N-glycosylation are evident as well. Subsequently, the human homologue has also been cloned (60). The human intestinal H^+/peptide cotransporter consists of 708 amino acids and is highly homologous to the rabbit intestinal H^+/peptide cotransporter. However, important differences exist in the phosphorylation sites. Although the rabbit intestinal H^+/peptide transporter possesses a site for protein kinase A-dependent phosphorylation, the human homologue does not. Thus, no potential site exists for phosphorylation by protein kinase A in the human intestinal H^+/peptide cotransporter. In addition, the human transporter contains two potential sites for protein kinase C-dependent phosphorylation, whereas the rabbit transporter contains only one.

Independent efforts by Dantzig et al have led to the isolation of a different cDNA from a human colon carcinoma cell line (Caco-2), which also has been shown to be associated with H^+/peptide cotransporter activity (19). This protein, designated HPT-1, is distinct from PEPT 1. The HPT-1 protein consists of 832 amino acids and, interestingly, possesses a single putative transmembrane domain. Since membrane transporters generally possess multiple transmembrane domains, it has been suggested that the HPT-1 protein may oligomerize to form a functionally active peptide transporter, or it may associate with other membrane protein(s) as a prerequisite to elicit peptide transport activity. In this respect, there is a striking analogy between HPT-1 and the protein (BAT or D2) associated with the transport of neutral and cationic amino acids (7, 95, 109). No information is available on the relationship between HPT-1 and PEPT 1.

Renal H^+/Peptide Cotransporter PEPT 2

Studies with isolated brush border membrane vesicles have indicated that the peptide transport systems expressed in the intestine and the kidney, though

similar in several aspects, are distinct in kinetic properties (15, 30, 99). Although the intestinal H^+/peptide cotransporter is of a low-affinity/high-capacity type, the kidney H^+/peptide cotransporter is of a high-affinity/low-capacity type. However, the similarity between the two transport systems in certain functions—such as recognition of di- and tripeptides as substrates, energization by a transmembrane H^+ gradient, and rheogenicity—strongly suggests that the transporters may share considerable homology in amino acid sequence. This rationale led to the screening of a human kidney cDNA library by cross-hybridization using probes derived from the PEPT 1 cDNA (63). These studies have resulted in successful cloning of the H^+/peptide cotransporter expressed in the kidney. The full-length cDNA, designated PEPT 2, is 2685 bp long, with an open reading frame of 2190 bp (including termination codon). The predicted protein consists of 729 amino acids. Hydropathy analysis identifies the presence of 12 putative transmembrane domains. Five potential sites exist for protein kinase C-dependent phosphorylation but none exists for protein kinase A–dependent phosphorylation. Comparison of the amino acid sequence between PEPT 1 (human intestine) and PEPT 2 (human kidney) reveals 50% identity and 70% similarity. PEPT 2 is 21 amino acids longer than PEPT 1 is. The extent of similarity is much higher in transmembrane domains than in the hydrophilic regions between the transmembrane domains.

Homology of PEPT 1 and PEPT 2 to Other Cloned Transporters

The amino acid sequences of the intestinal and renal H^+/peptide cotransporters, PEPT 1 and PEPT 2, do not show strong homology with any other cloned mammalian transporter proteins. PEPT 1 and PEPT 2, thus, represent a novel gene family. Interestingly, two other H^+-dependent transporters, namely the folate transporter (77, 110, 111) and the monocarboxylate transporter (38), which have recently been cloned from human tissue, do not exhibit significant homology to PEPT 1 and PEPT 2. However, a weak but definite homology exists with certain transport proteins cloned from nonmammalian sources. These proteins are the peptide transporter from *Saccharomyces cerevisiae* (yeast Ptr 2) (76), the chlorate/nitrate transporter from *Arabidopsis thaliana* (CHL 1) (103), and the di- and tripeptide transporter from *Lactococcus lactis* (L. Lact. Ptr) (44).

Chromosomal Localization of the PEPT 1 and PEPT 2 Genes

The chromosomal location of the genes encoding PEPT 1 and PEPT 2 have been mapped by somatic cell hybrid analysis and by in situ hybridization to human metaphase chromosome spreads (60, 78). The PEPT 1 gene is located

at chromosome 13q24-q33 and the PEPT 2 gene is located at chromosome 3q13.3-q21.

FUNCTIONAL CHARACTERISTICS OF CLONED PEPT 1 AND PEPT 2

Interaction with Peptides

PEPT 1 cDNA has been functionally expressed in *X. laevis* oocytes by microinjection of cRNA (23) or in HeLa cells using the vaccinia virus expression system (60), and the interaction of the transporter with peptides has been investigated. The rabbit as well as human PEPT 1 accept a variety of di- and tripeptides as substrates. Free amino acids and peptides consisting of four or more amino acids are excluded by PEPT 1. The peptide substrates of PEPT 1 may contain neutral amino acids, anionic amino acids, cationic amino acids, or imino acids. PEPT 2 cDNA has been expressed in HeLa cells, and substrate specificity studies have shown that PEPT 2 also prefers dipeptides and tripeptides as substrates (63, 78). Larger peptides and free amino acids are not recognized as substrates. As in the case of PEPT 1, the peptide substrates of PEPT 2 also may contain structurally diverse amino acids. Thus, both PEPT 1 and PEPT 2 exhibit broad substrate specificity. This characteristic is essential for their normal physiologic function. With the 20 different amino acids normally present in proteins, it is theoretically possible to generate 400 different dipeptides and 8000 different tripeptides. The amino acids that constitute these endogenous peptides are chemically and structurally diverse. These diverse peptides should be recognized as substrates by the peptide transporters for effective absorption in the intestine and kidney. Therefore, the physiologic advantage of the broad substrate specificity of PEPT 1 and PEPT 2 is quite obvious.

Functional expression studies have also clearly established that PEPT 1 is a low-affinity transporter whereas PEPT 2 is a high-affinity transporter. For example, the K_t for glycylsarcosine is 1.9 mM for the rabbit PEPT 1 and 0.3 mM for the human PEPT 1. In contrast, the human PEPT 2 has a K_t of 0.07 mM for glycylsarcosine. The relatively greater affinity of PEPT 2 versus PEPT 1 for endogenous peptides is evident not only for glycylsarcosine but also for a variety of chemically diverse di- and tripeptides (23, 60, 78).

Interaction with Peptidomimetic Drugs

Several studies have shown that the cloned PEPT 1 and PEPT 2 effectively transport peptidomimetic drugs (9, 23, 60, 63). These drugs include cephalosporins (e.g. cephalexin, cepharadine, cefadroxil), penicillins (e.g. cyclacillin, ampicillin), and inhibitors of angiotensin converting enzyme (e.g. cap-

topril). A recent study (27) comparing the interaction of β-lactam antibiotics (i.e. cephalosporins and penicillins) with cloned human PEPT 1 and PEPT 2 under identical experimental conditions has revealed that β-lactam antibiotics are differentially recognized by PEPT 1 and PEPT 2. PEPT 1 exhibits greater affinity for cyclacillin (a penicillin) than for cefadroxil (a cephalosporin). In contrast, the affinities are reversed in the case of PEPT 2. PEPT 2 shows much greater affinity for cefadroxil than for cyclacillin.

Electrophysiology of PEPT 1

Detailed studies of steady-state kinetics of human PEPT 1 have been recently carried out with *X. laevis* oocytes using electrophysiologic approaches (64a). Since the process of H^+/peptide cotransport catalyzed by PEPT 1 is rheogenic, associated with the transfer of positive charge across the membrane, the process results in depolarization of the membrane. The activity of the cloned PEPT 1 can be measured easily by monitoring membrane currents following functional expression of PEPT 1 in the *X. laevis* oocytes. Membrane currents are monitored while transmembrane potential is clamped at any particular value using the two-microelectrode voltage clamp technique. Addition of a transportable substrate to the medium bathing the oocyte alters the membrane potential as a result of the PEPT 1–mediated transport. The membrane potential is, however, maintained at the predefined value by passing current through one of the electrodes, and this current is the measured parameter of PEPT 1 activity. Since H^+/peptide cotransport results in the transfer of positive charge into the oocyte, an inward current has to be passed to maintain the membrane potential. With this technique, the transport mechanism of human PEPT 1 has been investigated with regard to voltage dependence and to steady-state kinetics. At pH 5.0, glycylsarcosine-induced concentration-dependent currents bear a nonlinear relationship with membrane potential. Clamping the membrane potential at hyperpolarized values results in an increase in the magnitude of PEPT 1–induced currents. The Hill coefficient for activation of PEPT 1 by H^+ is 1, suggesting a H^+/glycylsarcosine coupling ratio of 1. The K_t for the peptide at pH 5.0 decreases as the clamped membrane potential is changed from −150–0 mV. In contrast, the K_t for H^+ increases with a similar maneuver.

Differential Expression of PEPT 1 and PEPT 2 in the Intestine and the Kidney

The expression of PEPT 1 and PEPT 2 mRNAs in the human kidney and in the human intestine has been investigated by reverse transcription polymerase chain reaction (63). These studies have revealed that PEPT 1 is expressed in

the intestine as well as in the kidney. In contrast, PEPT 2 is expressed in the kidney but not in the intestine.

In situ hybridization with PEPT 1–specific probes in the rabbit intestine has shown that the transporter is expressed all along the small intestine (25). The expression level is very low in the colon and is nondetectable in the stomach and the cecum. In the small intestine, the expression of PEPT 1 is limited to the mucosal cells. Along the crypt-villus axis, the PEPT 1 mRNA is not detectable in the crypt, starts appearing at the crypt-villus junction, and increases rapidly towards the villus tip. The mRNA is most abundant in the duodenum and jejunum and is present at low levels in the ileum.

The kidney expresses primarily PEPT 2 and, to a much lesser degree, PEPT 1. The specific regions of the renal tubular system that express these transporters have not been identified. Microperfusion and microinfusion studies have indicated that the peptide reabsorptive capacity is present not only in the proximal convoluted tubule but also in the proximal straight tubule (6, 85). Most probably, the descending and ascending limbs of Henle's loop may also possess the ability to reabsorb peptides, at least to a small degree. PEPT 1 is a low-affinity/high-capacity transporter whereas PEPT 2 is a high-affinity/low-capacity transporter. Based on these characteristics, it has been speculated that these two transporters may be differentially expressed along the length of the nephron, PEPT 2 primarily in the proximal parts and PEPT 1 primarily in the distal (10). It is likely that the concentration of small peptides in the lumen of the nephron increases significantly along the length of the nephron because of the action of highly active brush border membrane peptidases such as dipeptidylpeptidase IV on larger peptides and proteins. The expression of the high-affinity system PEPT 2 in the proximal regions and the low-affinity system PEPT 1 in the distal regions would be advantageous under these physiologic conditions.

PEPTIDE TRANSPORTERS IN CULTURED CELL LINES OF INTESTINAL AND RENAL ORIGIN

Even though the intestinal and renal peptide transport systems have been investigated in detail using different tissue preparations, availability of cell lines of intestinal and renal origin that constitutively express the peptide transport activity will potentially be useful in investigations of the regulatory aspects of the transport system. Several studies have demonstrated, using either β-lactam antibiotics (17, 18, 20, 52, 81) or peptides (12, 96, 97) as substrates, that the human colon carcinoma cell line Caco-2 expresses H^+-coupled peptide transport activity. These studies have established that the transport activity is expressed in the apical membrane as well as in the basolateral membrane of the cell line. In both membranes, the transport activity is coupled to H^+.

Although some evidence indicates that the transport systems present in these two membranes may be distinct (81, 96), the evidence is equivocal; further work is needed to resolve this issue. Kinetic analysis has revealed that the transport system expressed in the apical membrane is of a low-affinity type. Of the two H^+/peptide cotransporters thus far cloned from human tissues (i.e. PEPT 1 and PEPT 2), the Caco-2 cells possess mRNA transcripts only for PEPT 1 (27). The mRNA transcripts for PEPT 2 are not detectable in this cell line. Since the normal intestine expresses PEPT 1 and not PEPT 2, the Caco-2 cells provide a suitable model system for intestinal peptide transport studies.

In contrast to the intestine, the predominant H^+/peptide cotransporter expressed in the kidney is PEPT 2. Therefore, identification of a renal cell line that expresses PEPT 2 is important for studies of the renal peptide transport process. Renal cell lines that are being used widely for transport studies are the LLC-PK1 cell line isolated from porcine kidney, the OK cell line derived from opossum kidney, and the Madin-Darby canine kidney (MDCK) cell line originating from dog kidney. Interestingly, the LLC-PK1 and OK cell lines do not possess measurable H^+/peptide transport activity (11). The MDCK cells do possess peptide transport activity that is H^+ coupled, but the kinetic characteristics of the activity indicate that the transporter present in these cells may be PEPT 1 and not PEPT 2 (11). Recently, a cell line derived from rat kidney (SKPT) has been shown to express constitutively a H^+-coupled peptide transporter that is of a high-affinity type and resembles PEPT 2 (10). Northern blot analysis has subsequently revealed that the SKPT cells contain mRNA transcripts that are hybridizable to the PEPT 2 cDNA probe (27). The PEPT 1–specific mRNA transcripts are not detectable in these cells. Thus, SKPT cells are ideal as an experimental model system for investigations of the renal peptide transport process.

REGULATION OF THE INTESTINAL AND RENAL PEPTIDE TRANSPORTERS

Significant information is available on the developmental, dietary, and hormonal regulation of the intestinal peptide transport system (29). The ability of the intestine to absorb intact peptides varies with age. In several animal species including man, the peptide transport system is present in the small intestine even before birth (43, 45, 79). The peptide absorptive capacity is maximal at birth and then decreases with age to reach adult levels. The intestinal peptide transport system is also regulated by diet. A high-protein diet enhances the ability of the intestine to absorb peptides (24). Similarly, short-term restriction of diet also increases the intestinal peptide transport activity (61). Our present knowledge on the regulation of the intestinal peptide transport system by hormones is limited. Activators of protein kinase C have been shown to inhibit

the peptide transporter in Caco-2 cells (12). This effect apparently involves phosphorylation of the transporter protein. The cloned human PEPT 1, which is known to be responsible for peptide transport in Caco-2 cells, does possess two putative sites for phosphorylation by protein kinase C, thus supporting the suggestion that the protein kinase C–mediated modulation of the peptide transporter in Caco-2 cells may involve direct phosphorylation of the transporter protein.

A more recent study (73) has shown that elevation of cyclic adenosine monophosphate (cAMP) levels in Caco-2 cells induced by cholera toxin and by *Escherichia coli* heat-labile enterotoxin results in the inhibition of the peptide transporter activity. Available evidence indicates that the cAMP-induced effect may in fact be mediated by protein kinase C. Supporting this mechanism are the findings that the human PEPT 1, which is expressed in Caco-2 cells, does not possess a consensus sequence for protein kinase A-dependent phosphorylation. The inhibition of the peptide transporter in Caco-2 cells by cholera toxin and by *E. coli* heat-labile enterotoxin may have clinical significance. Both these toxins are diarrheagenic and cause malabsorption of nutrients in the intestine. The studies with Caco-2 cells suggest that the intestinal ability to absorb peptides may be compromised during infection with *Vibrio cholerae* and with the enterotoxigenic strains of *E. coli*.

Little is known about the regulation of peptide transport in the kidney. The peptide transporter expressed in MDCK cells, which is presumably PEPT 1, is inhibited by calmodulin antagonists (11). This inhibition is primarily associated with a decrease in the maximal velocity without a significant change in the affinity of the transport system for its peptide substrates. The regulation of PEPT 2, the principal peptide transporter expressed in the kidney, by hormones and/or second messengers has not been studied.

NONPLASMA MEMBRANE PEPTIDE TRANSPORTERS

Lysosomes

Lysosomes participate in the digestion of macromolecules, including proteins that enter the organelle by endocytosis, pinocytosis, and other mechanisms. We speculated several years ago that, in analogy with the digestion and absorption of the dietary proteins in the intestine, intralysosomal digestion of proteins may not be complete, and that the process may result in the generation of free amino acids as well as small peptides that may enter the cytoplasm via specific transporters in the lysosomal membrane (37). In recent years, several amino acid transport systems have been characterized in the lysosomal membrane (13). Similarly, evidence has been presented for selective transport of di- and tripeptides across the lysosomal membrane (8, 53). Whether or not the

lysosomal peptide transport system is coupled to H^+ is not known. However, since there is a transmembrane H^+ gradient across the lysosomal membrane in the lysosome-to-cytoplasm direction, it is likely that the H^+ gradient provides the driving force for the lysosomal peptide transport system. The molecular identity of the lysosomal peptide transport system and its relationship to the plasma membrane peptide transporters remain to be established.

Endoplasmic Reticulum

The endoplasmic reticulum in antigen-presenting cells has been shown to possess a transport mechanism for peptides (72). Antigen presentation by MHC (major histocompatibility complex) class I molecules involves cytoplasmic degradation of viral proteins and translocation of the resultant antigenic peptides into the lumen of the endoplasmic reticulum for subsequent complex formation with the MHC class I molecule prior to movement to the cell surface. The translocation of the peptides across the endoplasmic reticulum membrane is mediated by two proteins that form a functional heterodimer (87). These proteins are called TAP 1 and TAP 2 for transporter associated with antigen processing. The molecular nature of this transporter complex has been elucidated (5, 86, 102). This transporter belongs to the family of ABC (ATP binding cassette) transporters, which mediate active transport in association with ATP hydrolysis. The peptide substrates for the TAP heterodimer usually consist of nine or ten amino acids (71). There is no structural similarity between this peptide transporter and the H^+-coupled peptide transporters found in the brush border membrane of the intestine and kidney.

CONCLUDING REMARKS

In the last few years, a number of transporters of mammalian origin that serve for the uptake of organic solutes have been cloned. Based on the ionic dependence, these transporters fall into four major classes: Na^+-independent, Na^+-dependent, (Na^+, Cl^-)-dependent, and (Na^+, K^+)-dependent. Interestingly, no apparent homology exists between these different classes of transporters, but significant homology exists among the members within each of these classes. Thus, each class of transporters appears to represent a distinct gene family. The H^+/peptide cotransporters are distinct from all the above-mentioned transporters because of the involvement of H^+ as the cotransported ion. It is most likely that the H^+-coupled peptide transporters belong to a totally different gene family. Recent successful cloning of these transporters is highly significant because it provides the opportunity for detailed investigations of the H^+-dependent transporter gene family at the molecular level. Comparison of the structural features of these transporters with those of the transporters

coupled to Na^+, Na^+ plus Cl^-, or Na^+ plus K^+ has the potential to reveal the structural determinants of the preference of a particular ion(s) for cotransport with the organic substrate.

A number of issues regarding the H^+/peptide cotransporters still need to be investigated. Are there other transporters that belong to this gene family? Are there additional H^+/peptide cotransporters other than PEPT 1 and PEPT 2? What is the functional relationship, if any, between HPT 1 and PEPT 1/PEPT 2? Another important aspect that needs to be studied in the future is whether or not the H^+/peptide cotransport system is expressed in the plasma membrane of mammalian tissues other than the intestine and kidney. Northern blot analysis reveals the presence of mRNA transcripts that hybridize to the PEPT 1 cDNA probe in liver, pancreas, and brain (23, 60). Since no information is available on H^+-coupled peptide transport in these organs, whether the mRNA transcripts code for peptide transporters or for different transporters with homology to the peptide transporters remains to be seen.

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