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Function, Regulation, and Pathophysiological Relevance of the POT Superfamily, Specifically PepT1 in Inflammatory Bowel Disease

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Abstract

Mammalian members of the proton-coupled oligopeptide transporter family are integral membrane proteins that mediate the cellular uptake of di/tripeptides and peptide-like drugs and couple substrate translocation to the movement of H⁺, with the transmembrane electrochemical proton gradient providing the driving force. Peptide transporters are responsible for the (re)absorption of dietary and/or bacterial di- and tripeptides in the intestine and kidney and maintaining homeostasis of neuropeptides in the brain. These proteins additionally contribute to absorption of a number of pharmacologically important compounds. In this overview article, we have provided updated information on the structure, function, expression, localization, and activities of PepT1 (SLC15A1), PepT2 (SLC15A2), PhT1 (SLC15A4), and PhT2 (SLC15A3). Peptide transporters, in particular, PepT1 are discussed as drug-delivery systems in addition to their implications in health and disease. Particular emphasis has been placed on the involvement of PepT1 in the physiopathology of the gastrointestinal tract, specifically, its role in inflammatory bowel diseases.

Introduction

The solute carrier 15 (SLC15) family of peptide transporters, also known as the H⁺-coupled oligopeptide cotransporter or proton-coupled oligopeptide transporter (POT) family, constitutes a group of membrane transporters mainly involved in the cellular uptake of di- and tripeptides (di/tripeptides). The principal route by which mammals absorb amino acids is transport of di- and tripeptides across the intestinal brush-border membrane (BBM) in the gastrointestinal (GI) tract. This process is mediated by the POT transporter family (152,187). The POT family of membrane transporters, also referred to as peptide transporters, are found in all organisms except archaea, and use the proton electrochemical gradient to drive the uptake of di- and tripeptides across cell membranes (50,68). The human genome contains four members of the POT family, specifically, PepT1, PepT2, PhT2, and PhT1 encoded by SLC15A1, SLC15A2, SLC5A3, and SCL5A4 that belong to the SLC15 gene group. Properties of SLC15 transporters are summarized in Table 1.

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PepT1 and PepT2 mediate the uptake of di or tripeptide substrates into intestinal and renal epithelial cells, respectively. Other sites of functional expression of the two proteins have also been identified, including bile duct epithelium (PepT1), brain, lung, and mammary gland (PepT2). Both proteins can transport essentially every possible di- and tripeptides, regardless of the substrate net charge, but operate in a stereoselective manner. Various drugs and prodrugs with peptide-like structures are transported, allowing efficient intestinal absorption of the compounds via PepT1. In kidney tubules, both peptide transporters can mediate renal reabsorption of the filtered compounds, affecting their pharmacokinetics. The two other peptide transporters, PhT1 and PhT2, identified in mammals possess overall amino acid identity with the PEPT series of 20% to 25%. PhT1 and PhT2 (either or both) are expressed in a variety of tissues, including spleen, intestinal segments, eyes, lung, and thymus, and transport free histidine and certain di- and tripeptides.

Multiple sources in the literature have implicated the H⁺/peptide transporter, PepT1, as a key contributory molecule in the development and progression of inflammatory bowel disease (IBD) (2, 6, 10). Expression of PepT1 in inflamed colon contributes to intestinal inflammatory conditions, stimulating an obvious interest in understanding the physiological functions of this transporter protein. In addition, PepT1 levels tend to be upregulated in a number of colonic diseases, including IBD, contributing to the onset of colonic carcinogenesis in certain cases (132,215). PepT2, PhT1, and PhT2 are additionally expressed in various tissues. While a large number of studies have evaluated the implications of PepT2 in physiopathology of disease, fewer investigations have focused on the roles of PhT1 and PhT2 to date.

IBD is a chronic condition of the GI tract that affects as many as 1.4 million Americans and over 3.5 million people in Europe (26, 117). The prevalence of IBD in Asia, particularly in underdeveloped regions, has increased at an alarming rate over the past decade, becoming a public health burden worldwide (172). IBD is characterized by a number of specific symptoms, notably, epithelial disruption, loss of barrier functionality, and accumulation of immune cells, such as neutrophils that migrate across the intestinal epithelium. In a number of animal models of IBD drastic weight loss, nutrient deficiency, and abnormal development have been frequently reported. The precise causes of IBD are not known at present although several published studies in the literature have attempted to define its etiology and pathological mechanisms. A combination of environmental, genetic, and microbial factors is believed to trigger abnormal immune responses that contribute to initiation of the inflammatory process. However, the specific underlying mechanisms remain to be fully elucidated (227). IBD is classified into two clinical forms, specifically, Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by inflammation and disruption throughout the GI tract whereas UC is localized in the colonic mucosa. Among the common characteristics, both forms of IBD induce significant changes in the intestinal microbiome as a result of genetic or environmental factor (63, 93). Such changes in microbiota composition have been shown to play a role in inflammatory pathogenesis by triggering aberrant immune responses in individuals with IBD (63,135,191,208).

This review covers general knowledge on POT family members, including recent findings on their structure, function, and regulation in both healthy and disease states. In particular, we focus on PepT1 with proven pharmacological relevance in IBD.

History of Discovery

The existence of transmembrane transport of short-chain peptides, as a source of amino acids in many living organisms was proposed more than 30 years ago during investigation of intestinal mechanisms of peptide absorption (128). This hypothesis was coined by researchers examining the appearance/disappearance of oligopeptides in the lumen following consumption of a protein-rich meal containing 50 g of purified bovine serum albumin (3). As early as the 1970s, absorption of di- and tripeptides in human small intestine was demonstrated using glycyl-glycine and glycyl-leucine as probes. These findings were unexpected, since at the time, researchers believed that protein had to be broken down into amino acids before absorption could occur in the gut lumen (1, 4, 5). The identification of mammalian peptide transporters distinct from amino acid transporters for the intestinal absorption and renal reabsorption of nutritional nitrogen was initially performed in studies on BBM vesicles (BBMVs) prepared from intestine and kidney (69, 70). These reports clearly demonstrated that dipeptides, tripeptides, and peptide-like drugs are actively transported into vesicles by a process coupled to the movement of protons down an electrochemical proton gradient.

An *in vitro* study using BBMVs from human small intestine and a proton gradient (pH 8.3/6.7) to stimulate uptake of the glycyl-glutamine (Glyⁿ-Gln) probe was additionally conducted (134). Since this stimulation was not significant, transport of Glyⁿ-Gln was further investigated using a human intestine cell line model (Caco-2). The results showed dipeptide uptake under acidic pH conditions, with optimal transport of Gly-Gln at pH 6 in Caco-2 (2). Caco-2 was additionally employed to investigate whether transport of dipeptides is stimulated by downhill movement of H⁺ across the BBM (active transport) in the cytoplasm of mucosal cells. Intracellular acidification in Caco-2 cells led to highly reduced uptake of glycyl-sarcosine (Gly-Sar), discounting the theory that this transportation is active. Peptide transporters were initially isolated by expression cloning using *Xenopus laevis* oocytes. This technique was used to produce large quantities of specific proteins for further study. PepT1 cDNA encoding a 707-amino acid protein was isolated and screened in a selected rabbit intestinal cDNA library for uptake of Gly-Sar. Significantly increased uptake of Gly-Sar (by 63-fold) was observed in oocytes expressing PepT1, compared to control oocytes (60). With the advent of expression cloning techniques, the molecular basis for recognition, specificity and activity of peptide transporters was further investigated in mammals by the groups of Hediger (60) and Daniel (22). To elucidate the molecular structure of human PepT1, cloned transporter from the rabbit intestine were used to screen the human cDNA library, resulting in the identification of a cDNA that displayed transporter activity of dipeptides and tripeptides but not free amino acids (112). The molecular size of the protein was determined as 78.81 kDa. To characterize the human oligopeptide transporter, PepT1, in complementary RNA-injected *Xenopus laevis* oocytes, researchers used the two-microelectrode voltage clamp technique and Gly-Sar probe. Gly-Sar transport mediated by hPepT1 was electrogenic and coupled to the H⁺ current (123). Orthologs of

PepT1 were subsequently identified in other mammalian species as well as PepT1 paralogs, including PepT2 (encoded by SLC15A2) (115), PhT1 (encoded by SLC15A4) (229), and PhT2 (encoded by SLC15A3) (167). These POTs of the SLC15 family are phylogenetically conserved integral membrane proteins (219) responsible for the symport of protons and peptides/mimetics across biological membranes, as demonstrated in cell cultures, *Xenopus* oocytes, and other heterologous expression systems (24,163).

Several studies to date have addressed the expression, localization, and structure–function relationships of the high-capacity, low-affinity intestinal peptide transporter, PepT1, and the low-capacity, high-affinity renal peptide transporter PepT2, while limited information is available on the peptide/histidine transporters, PhT1 and PhT2. Major discoveries regarding POT transporters are presented in a chronological timeline in Figure 1.

Structure and Homologies

Peptide transport has distinct phylogeny and is well conserved among different species. In mammals, the SCL15 peptide transporters are known as proton-dependent oligopeptide transporters, which are evolutionarily well conserved from bacteria to human (50). POT protein sizes vary from 450 to 700 amino acids (50). This protein family comprises four members, with two being part of the PEPT group (PepT1 and PepT2) that exclusively transports di- and tripeptides and the other two being part of the PHT group (PhT1 and PhT2). Other than mammals, POT transporters have been detected in several species, such as bacteria (*Lactococcus lactis*), yeast (*Saccharomyces cerevisiae*), plants (*Arabidopsis thaliana*, *Hordeum vulgare*), invertebrates (*Caenorhabditis elegans* (*C. elegans*), *Homarus americanus*), fish, amphibians, and birds (61, 81). POT proteins display a high degree of homology across species, as exemplified in Figure 2 showing a protein sequence alignment of PepT1 from human and mouse with ~90% homology. However, the homology between PepT1 and PepT2 for a given species is relatively low (~50%, Fig. 3). Rat PhT1 and PhT2 display amino acid identity of about 50%, but show little homology to either PEPT1 or PEPT2 (<20%). PepT1 in *C. elegans* is expressed in intestinal cells, suggesting a peptide absorption role, similar to the one reported in mammals (129). Uptake of amino acids plays an important role in the growth and development of these animals (83). For *Drosophila*, only one homolog has been cloned. The protein is expressed in the germinal and somatic tissues of both female and male flies, showing the highest expression in nurse cells of the female as well as the midgut and rectum of both sexes (161). In zebrafish, PepT1 is solely expressed in intestinal epithelial cells (IECs) with low affinity but displays high transport capacity at ideal pH (214).

No structures are available for human PepT transporters while those of bacterial homologs have been characterized, such as PepTSo from *Shewanella oneidensis* (*S. oneidensis*) in occluded conformation, PepTSt from *Streptococcus thermophilus* in the inward-open form, PepTSo2 from *S. oneidensis* and GkPOT from *Geobacillus kaustophilus* (78).

Due to the lack of accurate models on the structure of PepT1, the molecular mechanisms underlying the behavior of this protein are poorly understood. This issue has remained an important topic of focus for researchers and pharmacologists. Peptide transporters have been

shown to successfully deliver peptide-like drugs, such as β -lactam antibiotics, angiotensin converting enzyme (ACE) inhibitors, anticancer agents such as bestatin, and antiviral therapeutics, such as valacyclovir, as mentioned previously (130,131). Over the last decade, extensive research has been undertaken to determine the structure of PepT1. Site-directed mutagenesis studies have provided more insights into the crystal structure of PepT1 and facilitated the identification of several key amino acids for substrate binding (130). For instance, in the second transmembrane domain, extracellular histidine plays a role in modulating PepT1 function. PepT1 shares 50% amino acid sequence identity as well as several reported molecular properties with the H⁺/peptide transporter, PepT2. Further studies have revealed the existence of a 200-amino acid hydrophilic segment between transmembrane domains 9 and 10, which is proposed to contain seven N-linked glycosylation sites and two potential protein kinase C-dependent phosphorylation sites.

The cDNA encoding human PepT1 is 2263 bp in length with an open reading frame of 2127 base pairs and encodes a 708-amino acid protein (31) with a presumed molecular weight of ~78 kDa (93). Moreover, POT belongs to the major facilitator superfamily (MFS), which suggests that PepT1 possesses 12 transmembrane alpha helices arranged into two bundles of six resembling a V-shape protein that may be located in the plasma membrane of eukaryotes or inner membrane of bacteria (38, 60) (Fig. 4). The mammalian transporter, PhT1, was characterized in rat tissues and cloned from a rat brain cDNA library. High affinity for histidine and carnosine was documented (229). Rat PhT1 cDNA contains 1719 bp coding for 572 amino acids with a predicted molecular mass of 64.9 kDa. PhT2 shares ~47% identity with PhT1. This homolog was initially cloned from immunocytes and reported to function as a lysosomal histidine transporter in transfected cells (167). The human variant of hPhT2 contains 528 amino acids, and four putative N-linked glycosylation sites have been predicted for both PHT proteins (167).

Analysis of chimeras of with single-point mutations suggests that the transmembrane domains 2 to 5 and 7 play an important role in regulating substrate affinity (50, 142) and the C-terminus is oriented toward the cytoplasm (38). Epitope tagging analysis of mammalian PepT1 revealed that a significant proportion of the protein is positioned outside of the cell. Interestingly, these extracellular proteins are not present in plant and fungal homologs and their specific functions remain unknown (151). The extracellular domain is located within the canonical 12-transmembrane helix of the MFS fold. *In vitro* assays have shown that this domain comes into contact with intestinal trypsin, a protease in the human intestinal mucosa (13). Researchers have determined the extracellular domain in PepT1 using crystal structures of the bacterial POT proteins, PepTSo and PeoTSt, which share <31% identity with the mammalian homolog (142, 184). The extracellular domain in *Mus musculus* was identified between residues 391 and 580. This domain was crystallized and structurally characterized as two monomers forming a head-to-tail dimer. Moreover, analysis of the purified PepT1 extracellular domain revealed a monomeric structure in solution (13). The PepT1 domain also contains two immunoglobulin-like domains that are potentially highly dynamic, compared to the extracellular domain of PepT2.

Function Mode of Action

Peptide transporters 1 and 2 (PepT1 and PepT2) are transmembrane proteins and members of the widely distributed oligopeptide transporter (POT) superfamily. POTs are crucial for transporting amino acids in the peptide form.

PepT1, a H⁺-coupled oligopeptide cotransporter, is powered by the proton motive force and capable of transporting small di and tripeptides composed of two or three amino acids but not free amino acids. The driving force of PepT1 transport activity was determined from different cell-culture studies involving various tissue preparations (such as mammalian cells) and uptake techniques that aided in identifying the specificity and regulation of this transport. Using purified BBMVs and radiolabeled Gly-Pro as a substrate, Ganapathy and Leibach showed that dipeptide uptake via PepT1 is driven by the H⁺ gradient (68, 71, 72). The researchers proposed tertiary-active peptide uptake in renal and IECs. According to this model, the H⁺ gradient is established and maintains pH control of the apical Na⁺/H⁺ antiporter. Therefore, it is speculated that Na⁺ depletion disrupts the H⁺ gradient, affecting PepT1 transport of substrates (204,205).

Relative to other peptide transporters, PepT1 and PepT2 possess distinct functional and physicochemical properties. Functional expression studies have clearly established that PepT1 is a low-affinity transporter. PepT1 is mainly expressed in the small intestine where large amounts of small peptides exist. Therefore, low affinity and high capacity properties of PepT1 are physiologically rational for efficient absorption of di- and tripeptides from the lumen. In contrast to PepT1, PepT2 is a high-affinity transporter for di- and tripeptides (160).

The function of PepT1 is strictly dependent on the transmembrane proton gradient in combination with an inside-negative membrane potential. PepT1 and PepT2 are proton-coupled symporters and deliver di- and tripeptides by utilizing the internally directed proton gradient as the driving force of substrate translocation across the membrane. This feature differentiates them from other transporters that depend on membranous Na⁺/K⁺ gradients. In addition, PepT1 and PepT2 possess broad substrate specificities encompassing over 400 different dipeptides and 8000 different tripeptides (24), all of which vary diversely in chemical structure, polarity, and molecular weight.

As a result of their substrate promiscuities, PepT1 and PepT2 participate in a variety of biological processes in the human body, particularly those aimed at maintaining intestinal homeostasis. Importantly, PepT1 and PepT2 serve as major factors mediating the breakdown and uptake of dietary or endogenous proteins, a process crucial in the digestion of ingested foods and nutrient necessary for cell growth and metabolism.

Uptake of di- and tripeptides or structurally related substrates by PepT1 and PepT2 is accompanied by translocation of proton and thus movement of positive charges. Transport induces a change in the electrical potential of a cell, irrespective of the substrate charge. Neutral and cationic dipeptides are cotransported along with one proton while anionic dipeptides are transported along with two protons by PepT1 (104, 122, 186). In contrast, PepT2 transports neutral substrates with a 2:1 proton-to-substrate stoichiometry and charged

substrates with variable coupling ratios (36). Extracellular pH plays a crucial role in the binding affinity of the substrates. Lower pH values favor the binding of anionic compounds whereas the affinity of cationic substrates increases with higher pH. The substrate species that carry no net charge represent the preferred forms for binding and transport. Neither PhT1 nor PhT2 have been analyzed systematically with respect to driving force, mode of transport, and substrate specificity. However, the pH dependence observed for transport of histidine and the model peptides employed suggest a similar mode of operation as PEPT proteins (229).

A schematic diagram of transport in the intestine is summarized in Figure 5. Substrate uptake at the BBM causes proton influx that, in turn, leads to increased proton efflux back to the lumen by the apical sodium-proton exchanger, NHE3 (206). Sodium influx via NHE3 is compensated by export through the basolateral sodium-potassium ATPase and potassium ions taken up leave the cell through potassium channels. Di- and tripeptides transported into cells by PepT1 undergo rapid intracellular hydrolysis as activity of intracellular dipeptidases is high, and amino acids released leave the cell via basolateral amino acid transporters (Fig. 5). In the intestinal epithelium, proper function of PepT1 requires correct NHE3 activity (99, 154, 221) whereas the function of the renal form, PepT2, is dependent on NHE1 and/or NHE2 (216). As mentioned previously, expression and function of PepT1 are reduced by RNAi gene silencing of NHX-2, an ortholog of NHE3 in the *C. elegans* intestine, that leads to a significant decrease in intracellular pH (14,138).

Substrate

In earlier studies, the structural requirements of substrates with high affinity for PepT1 were not well understood. The only knowledge was that substrates shared structural features, such as a peptide bond. Subsequent analyses showed that these substrates are not required to have a classic peptide bond or C-terminal carboxyl group for recognition by a PepT1 transporter (61). For example, 4-aminophenylacetic acid (197), δ -amino levulinic acid (54), and ω -amino fatty acids such as 8-amino octanoic acid (55), amino acid aryl amide (23), and valacyclovir (79) can be accepted as substrates. It is not known whether PhT1 and PhT2 proteins can transport the same spectrum of di- and tripeptides, but these proteins are reported to accept free histidine as substrate, in contrast to PepT1 and PepT2. Another notable feature is the ability of PepT1 and other POTs to recognize and deliver several important families of peptidomimetic or “peptide-like” drugs, including β -lactam antibiotics, such as cephalosporin and penicillin classes, ACE inhibitors, L-DOPA, ester prodrugs such as enalapril and fosinopril, bestatin, alafosfalin, amino acid-conjugated antiviral drugs (valacyclovir), artificially developed di- and tripeptides such as Gly-Sar, as well as “potential” substrates such as δ -amino-levulonic acid and ω - amino fatty acids that lack obvious peptide-like bonds. POTs have been shown to increase the bioavailability of molecules attached to amino acids or dipeptides and serve as attractive targets for potential therapeutic models in developing and enhancing drug-delivery systems.

POT family members: Drug transporters?

Studies performed in the 1970s and 1980s reported that in addition to physiological substrates, compounds with a resemblance to the backbone of physically secreted di- and tripeptides can be recognized by peptide transporters (24). Preliminary evidence for PepT1 drug transport was obtained from the Phe-Gly interactions observed during absorption in rat jejunum (158). Since then, peptide transporters, such as PepT1, have been investigated as potential vehicles for drug delivery. However, the lack of structural information over a long period of time severely limited the development of drugs with high affinity for PepT1. To determine the kinetics of transport, researchers focused on the specificity of PepT1 and its substrates. Measurements were based on the interference of compounds with uptake of standard substrates. The best-known reference substrate is [^{14}C]-Gly-Sar. The affinity constants of Gly-Sar are in the medium range ($K_t = 0.5\text{--}1.5\text{ mmol/L}$) for PepT1 (24).

Pharmacological studies on PepT1 have considered the structure and dynamics of the transporter protein during interactions with ligands, inhibitors, and substrates. The affinity measurements in these studies are often assessed as IC_{50} and K_i values determined using different assays.

In addition to the physiological substrate, PepT1 can transport a variety of drugs and amino acid-conjugated prodrugs (78). Prodrugs are drug precursors that need to undergo metabolic processes before becoming active pharmacological agents. For instance, PepT1 is reported to contribute to the intestinal permeability of valacyclovir ($K_i = 0.49\text{ mmol/L}$), an ester prodrug used as oral treatment for viral infections. *In vivo* experiments performed on wild-type (WT) and PepT1 $^{-/-}$ mice showed that PepT1 exerts a major influence in improving absorption of valacyclovir and systemic exposure after oral administration in the GI tract (230). Uptake of this drug was clearly increased in different PepT1-expressing cells. Valacyclovir was also identified as a substrate of PhT1 (17). In addition, valganciclovir, a valine ester of ganciclovir, has been identified as a substrate of both PepT1 and PepT2 with varying degrees of affinity (lower and higher, respectively) (188).

D-aminolevulinic acid (ALA), a prodrug used as a photodynamic agent in treatment of GI tumors, was of further interest to researchers as a potential substrate (46, 153). An earlier study showed that ALA uptake occurs via pH gradient-dependent electrogenic cotransport shared by di- and tripeptides (54). Another report concluded that PepT1 contributes to the absorption of oral ALA across the BBM of the human small intestine epithelium (9). Interestingly, other prodrugs, such as nateglinide and glibenclamide, display high-affinity interactions with peptide transporters but are unable to be transported. In addition, these compounds inhibit transporter function and have been characterized as noncompetitive inhibitors. Further research on these antidiabetic drugs revealed potent inhibitory effects on uptake of Gly-Sar by human colon Caco2 and rat PEPT transfectants (200).

D-ALA is transported in the choroid plexus by PepT2, and a number of studies have disclosed a neuroprotective effect of PepT2 on the toxicity of this drug (89, 147, 148). Bestatin, an inhibitor of aminopeptidases, is transferred by peptide transporters in intestinal (209) and renal (88) epithelial cells. The transport of carnosine, a high-affinity substrate for

PepT2 (199,201), by peptide transporters was additionally investigated more than 30 years ago.

PepT1 is also capable of transporting β -lactam antibiotics, such as cefadroxil ($K_i = 7\text{--}14$ mmol/L). Using PepT1^{-/-} mice, researchers confirmed that PepT1 is responsible for small intestine permeability of cefadroxil, since it binds with high affinity to this transporter. PepT1 also plays a role in improving the absorption of this drug and others in this class (156). L-dopa is a dopaminergic precursor used for the treatment of Parkinson's disease. A study utilizing d-phenylglycine as a guide for PepT1-mediated L-dopa transport through the intestine concluded that d-phenylglycine facilitates the transfer of L-dopa through the intestinal PepT1 transporter (218).

Similar to PepT1, PepT2 is able to transport β -lactam antibiotics as substrates. The first affinity study was performed in kidney BBMVs (49). There is a difference between recognition of β -lactam antibiotics by PepT1 and PepT2. PepT2 has higher affinity for these antibiotics due to a hydroxyl group at the N-terminal phenyl ring carrying an alpha-amino acid group (21, 119, 159, 198). For example, Cefadroxil was 13-fold more potent than cyclacillin in competing the dipeptide for uptake via PepT2. Other β -lactam drugs recognized with high affinity by PepT2 are cefaclor, cyclacillin, cephradine, cephalixin, and moxalactam (24). Several other groups have directly demonstrated the uptake of radiolabeled or unlabeled amoxicillin, cefaclor, ceftibuten, or cephalixin in native renal or PepT2-transfected cells.

Another example is maltosine, a compound used for the treatment of metal iron-storage diseases, such as diabetes. Its oral bioavailability is significantly increased through binding of PepT1 (74). ACE inhibitor drugs, such as enalapril ($K_i = 0.15$ mmol/L), are potential substrates of PepT1 and PepT2 with K_i values of 6.2 and 4.3 mmol/L, respectively, and generally applied to treat high blood pressure and heart failure. The oral availability of enalapril can be explained by uptake via intestinal epithelium. Fosinopril interacts with PepT1 and PepT2 with high affinity ($K_i = 110$ μ mol/L and 55 μ mol/L, respectively), as confirmed by studies using Caco2-BBE cells expressing PepT1 ($K_i = 35.5$ mmol/L) and SKPT cells expressing PepT2 ($K_i = 29.6$ mmol/L; (179)). Studies on Fosinopril showed that this drug is transported via a proton-coupled process. Zofenopril is another ACE drug with high affinity for PepT2 with $K_i = 81$ μ mol/L (113). Other drugs interacting with PepT1 and PepT2 are alafosfalin ($K_i = 0.19$ mmol/L), an antibacterial dipeptide (141), and arphamenine A ($K_m = 0.14$ mmol/L) (56), an Arg-Phe analog without a peptide bond, which present high affinity for and are transported by PepT1 (24). Finally, cyclo-trans-4-L-hydroxyprolyl-L-serine (JBP485) is another dipeptide with antihepatitis activity acting as a PepT1 substrate that is rapidly absorbed by the GI tract after oral administration (116). Overall, while the transport activities of PepT1 and PepT2 have gained considerable interest in the pharmaceutical field, many questions remain to be answered in relation to specific mechanisms, fate, and number of carriers per cell. The lists and chemical structures of the compounds transported by PepT1 and PepT2 are presented in Tables 2 and 3, respectively.

Localization and expression

Members of the POT superfamily for which physiological/pathological and pharmacological relevance have largely been determined by their localization and levels are expressed at varying levels throughout the human body (93). Based on various molecular studies, PepT1 has been identified as the exclusive oligopeptide transporter in the brush-border members of intestinal enterocytes whereas PepT2 is predominantly expressed in the kidney.

In the intestine, PepT1 protein is abundantly expressed at the apical membrane of enterocytes in mouse and human duodenum, jejunum, and ileum, with minimal or no expression in the stomach and normal colon (77, 95, 217). While earlier investigations were demonstrating that absorptive enterocytes of all small intestine segments showed uptake of d-Ala-Lys-AMCA, whereas there was a complete lack of fluorescence in colonic samples (77), more recent immunofluorescence experiments from the same group revealed the presence of PepT1 protein in the distal part of the normal colon, suggesting a regionalized expression (225). However, later studies failed to demonstrate transport activity (95, 231). In the small intestine, PepT1 displays a differential expression pattern along the villus-crypt axis, whereby it is concentrated most abundantly in the villus tip and to the lowest extent in the crypt-like regions (112, 233). The acidic nature of the intestinal milieu as a result of low extracellular pH facilitates optimal functionality of PepT1, which has also been detected in the proximal tubes of the kidneys (where it reabsorbs peptides from the primary filtrate) (234), bile duct epithelial cells, ileum, and jejunum (where it is enriched) (47, 102). PepT1 is expressed in immune cells, such as macrophages where it mediates the uptake of the bacterial peptide, N-formylmethionyl-leucyl-phenylalanine (fMLP) (31).

While expression of PepT2 has been reported in glial cells and tissue-resident macrophages of the enteric nervous system (165), it is unlikely that PepT2-mediated absorption is involved in the neuromuscular layer of the GI tract. Transcripts of PhT1 and PhT2 (encoded by SLC15A4 and SLC15A3 genes, respectively) are detectable in human and rat intestinal tissue segments (82), and immunohistochemical analyses have revealed PhT1 expression in small intestine villi (17). Uptake of histidine and carnosine by PhT1, and time, pH, and sodium-dependent uptake in Hpht1-COS-7 cells has been reported (17). However, their relevance in peptide/mimetic absorption in intestine is yet to be established. Results to date suggest that the high-capacity, low-affinity intestinal transporter, PepT1, is solely responsible for absorption of di/tripeptides arising from dietary proteins and GI secretions.

Reverse-transcription PCR of microdissected tubular segments and *in situ* hybridization studies have shown that PepT1 and PepT2 are differentially expressed in rat proximal tubule in kidney (182). SLC15a1 encoding PepT1 transcripts is detected in early parts of the proximal tubule (*pars convoluta*) while SLC15a2 encoding PepT2 transcripts is expressed preferentially (but not exclusively) in latter parts of the proximal tubule (*pars recta*). Neither transporter is expressed anywhere else in the nephron. Definitive confirmation was obtained from immunolocalization studies in rats (174) where PepT1 protein was detected in BBMs of proximal tubule S1 segments with progressively weaker expression in deeper cortical regions. In contrast, PepT2 protein localized primarily in BBMs of proximal tubule S3 segments with strong immunostaining of the outer, but not inner stripe of outer medulla.

PepT1 and PepT2 proteins have also been detected in BBMs of mouse kidney (90). Indirect localization analyses of β -galactosidase expression and fluorophore-conjugated dipeptide accumulation in kidney showed sequential expression of PepT1 and PepT2, respectively, in mouse proximal tubules (164), similar to that in rat (174). Thus, it appears that PepT1 (a high-capacity, low-affinity transporter) and PepT2 (a low-capacity, high-affinity transporter) act in concert to efficiently reabsorb peptide-bound amino acids from tubular fluid.

In the brain, PepT2 mRNAs are expressed in rat astrocytes, subependymal cells, ependymal cells, and epithelial cells of choroid plexus (15). Subsequent immunoblot and immunohistochemical studies in rat showed strongest expression of PepT2 in the cerebral cortex, with strong expression also observed in the olfactory bulb, basal ganglia, cerebellum, hindbrain, epithelial cells of the choroid plexus, and ependymal cells (173). In addition, PepT2 is expressed exclusively on apical membranes of choroid plexus epithelia (i.e., cerebrospinal fluid (CSF)- or CSF-facing) in both adult and neonatal animals. PepT2 protein is found in neurons (adult and neonate) and astrocytes (neonate but not adult), and exhibits an age-related decline in cerebral cortex expression as a function of age (fetal and neonatal tissue > adult). On the other hand, there is no molecular or functional evidence for PepT2 expression in endothelial cells of the blood–brain-barrier or PepT1 expression in brain. Although the peptidehistidine transporters PhT1 (229) and PhT2 (167) have been detected in brain, their functional importance is unknown. A recent study on PhT1-deficient mice investigated the effect of PhT1 on l-His brain disposition using *in vitro* slices and *in vivo* pharmacokinetic approaches (220). Uptake of l-His was reduced in brain slices by 50% in PhT1^{-/-} mice, compared to WT, with decreased distribution in brain parenchyma but not CSF of PhT1^{-/-} mice (220). PhT1 protein was also present in cerebral cortex, cerebellum, and hippocampus of adult but not neonatal mice, and expression increased with age in rats. Clear age-related differences in functional activity were observed, with PepT2 predominantly found in neonatal mice and rats, and PhT1 in adult rodents (91). It appears that PepT2 is involved in the removal of neuropeptides, peptide fragments and peptide-like drugs from CSF, and may act as a regulator of neuropeptide homeostasis in extracellular fluid.

The presence of PepT1 has been also reported in other tissues, such as pancreas, bile duct, and liver, and PepT2 in the lung, mammary gland, vascular smooth muscle, and spleen (6,20,24,163,190). In the nasal epithelium, PepT1 and PepT2 were detected at both mRNA and protein levels and functionally active while PhT1 and PhT2 were only expressed at the mRNA level (6). Expression of functional PepT1 and PepT2 has been reported in prostate cancer cell lines, supporting their potential utility as promising targets for the delivery of tumor-specific drugs (193). PhT1 is expressed in the eye and spleen (190, 229) and PhT2 in the lung, spleen, and thymus (167,190). PepT2, PhT1, and PhT2, but not PepT1, have been detected in mouse and human spleen macrophages and lymphocytes, where PepT2 functions as a transporter of AlaLys-AMCA and Gly-Sar (190). The various sites of expression of the four transporters are summarized in Figure 6. While several new expression sites have been uncovered, the roles and relevance of SLC15 family members in specific tissues remain to be elucidated.

Lipid raft-associated PepT1

Lipid rafts are localized regions of the plasma membrane that function largely in compartmentalizing cellular processes and influencing membrane fluidity. These regions are cholesterol/sphingolipid-enriched and can be isolated from the surrounding bilayer based on their resistance to nonionic detergents and chemical properties. Lipid rafts generally contain three- to fivefold higher amounts of cholesterol compared to the surrounding plasma membrane, and have attracted increasing attention due to their suggested involvement in a number of membrane activities and regulation of organization and function of plasma membrane proteins (25,180). In polarized cells, lipid rafts are primarily found on apical domains, including BBMs of the small intestine, where several types of membrane proteins are located in microvillar lipid rafts. These include digestive enzymes, such as sucrase and isomaltase, and a reported wide range of peripheral membrane proteins, including annexin A2, annexin IV, and XIIIb (80), glutamate receptor, guanine nucleotide-binding proteins $G\alpha_q$ and $G\alpha_{11}$, G protein-coupled receptor 7 (143), galectin-4 (52), epithelial sodium channel (84), melanotransferrin-a glycosylphosphatidyl inositol-linked iron receptor (51), prominin (162), and stomatin (183). Similarly, the Na^+/H^+ exchanger, NHE3, a component of the ileal villus cell type, BBM, is partially associated with lipid rafts but also functionally coupled with human PepT1 (111,206), supporting the feasibility of the presence of PepT1 in lipid rafts. Lipid rafts are also found in nonpolarized cells expressing PepT1, but are not segregated to a particular membrane domain (125,134).

A subsequent study conducted by our group demonstrated expression of PepT1 in lipid rafts of intestinal BBMs (144). This phenomenon was investigated in both polarized and nonpolarized cells. The results showed that association of PepT1 with lipid rafts has functional consequences on peptide delivery. Lipid rafts were thus isolated from purified intestinal BBMs of 6-week-old C57BL6 male mice and cultured Caco-2 BBE cells treated with methyl- β -cyclodextrin. The cholesterol content of the collected gradient fractions for lipid rafts was enzymatically determined. Low-density fractions (LDFs, fractions 4–5) were significantly concentrated with cholesterol but not high-density fractions (HDFs, fractions 9–12). Immunoblot and dot-blot analyses disclosed the presence of high levels of PepT1 and known intestinal lipid raft markers, NAP and GM1, in LDFs, which was confirmed by densitometric analysis of PepT1 immunoblots. Cholesterol depletion conditions induced a complete shift in PepT1 (along with NAP and GM1) from LDFs to HDFs, suggesting that PepT1 localizes in lipid raft-like membranes of mice intestinal BBMs. Similar results were obtained for lipid raft fractions isolated from polarized Caco-2 BBE monolayers, which also contained PepT1 (144). A model of the PepT1 in lipid rafts is presented in Figure 4.

Regulation of PepT1 expression and activity

Nutrient supply-mediated regulation

Multifactorial regulators of PepT1 expression exist at both the mRNA and protein levels. In 1998, Walker and colleagues first demonstrated a peptide-mediated increase in PepT1 mRNA transcription in human colon carcinoma Caco-2 cells (217). Subsequently, the means by which varying concentrations of dipeptide glycyl-phenylalanine in protein diets enhanced PepT1 mRNA/protein expression and transport function was examined in rats (178).

Products of protein digestion act as metabolic signals that modulate transcription of PepT1 mRNA and stability, as revealed by several analyses on PepT1 abundance in the membrane after short-term fasting in rat, mice, chicken, and human (121,124,202,213). Studies showing an increase in PepT1 expression and stability after fasting support the presence of a compensatory regulatory loop that enhances the production of transporters to prepare the intestine to efficiently receive and process nutrients during the postfasting phase. This effect appears to be dependent on peroxisome proliferator-activated receptor alpha (PPAR α), since fasted PPAR α -deficient mice displayed unchanged PepT1 protein abundance compared with mice fed *ad libitum* (176). Benner et al. (14) showed that RNA interference (RNAi) gene silencing of the cytosolic peptidases, ZC416.6 and R11H6.1/PES-9, reduced PepT1 expression and function in the nematode *C. elegans*, and gene silencing of closely related peptidases in mice, LTA4H and CNDP2, yielded similar results. The use of aminopeptidase inhibitors, bestatin and amastatin, led to concentration-dependent suppression of *C. elegans* PepT-1 activity with no effects on mRNA or protein abundance, indicating that the peptidases analyzed modulate the intracellular amino acid pool, in turn, affecting the transport capacity of *C. elegans* PepT-1 (14).

Transcriptional regulation of PepT1

Direct transcriptional regulation of PepT1 gene expression has been documented. Specifically, selected amino acids (leucine and phenylalanine) were shown to control rat PepT1 promoter activity via the amino-acid-responsive element localized 271 bp upstream of the start codon (178). A predicted binding site for the caudal-related homeobox transcription factor, Cdx2, on rat PepT1 promoter, was reported in the same study. Subsequently, Cdx2 was identified as a potential regulator of hPepT1, but the human PepT1 promoter shown to lack the typical binding motifs for Cdx2 (177). Binding of Cdx2 to the human PepT1 promoter is dependent on Sp1 (177) and the short-chain fatty acid (SCFA) butyrate (44). An earlier systematic analysis of the mouse PepT1 promoter revealed the presence of cis-acting elements upstream of the transcription start site, including three GC boxes, which are motifs for binding of the transcription factor Sp1 (62). In humans, basal PepT1 gene expression is also regulated by Sp1, which directly interacts with two GC boxes located within the first 170 bp upstream of the start codon (175).

Regulation of PepT1 expression by intracellular pH

A relationship between PepT1 and the sodium–proton exchanger, NHE3, in intestinal enterocytes has been established. RNAi gene silencing of NHX-2 in *C. elegans in vivo* results in a moderate but significant decrease in intracellular pH (138), leading to reduced protein expression and function of *C. elegans* PepT-1 (14).

Circadian regulation of PepT1 transcription

Circadian rhythms also appear to be involved in regulation of PepT1 transcription and function, as shown in rats maintained over a 12 h photoperiod. Transport of Gly-Sar, a typical substrate of PepT1, was enhanced in the dark, compared to the light phase. PepT1 protein accumulation and mRNA expression levels showed significant variations (150). Subsequent analyses disclosed that the Clock-controlled protein albumin D site-binding

protein can interact with its corresponding binding motif within the PepT1 distal promoter (166).

Inflammation-induced regulation of expression of PepT1 and other POT family members

During chronic inflammation, the expression profile of PepT1 within the GI tract is altered. In patients with chronic diseases, such as IBD and short bowel syndrome, PepT1 expression is upregulated and detectable in the colon (132, 236). This enhanced expression of PepT1 may be explained in several ways. For example, upregulation of proinflammatory cytokine and hormone levels during disease may promote PepT1 expression. Tumor necrosis factor (TNF)- α and interferon- γ (IFN- γ) levels are increased in IBD patients, and both cytokines are capable of enhancing PepT1 expression and transport activity in the human intestinal cell line, Caco-2-BBE (28, 212). The relationship between PepT1 expression and function in the physiopathology of the GI tract is discussed further in the subsection “PepT1 in IBD.”

In a previous study, transcription of PhT1 was upregulated upon inflammation of the colon but not terminal ileum of patients with IBD (109). Importantly, PhT2 was also upregulated in mouse spleen under conditions of LPS-induced acute inflammation (197) suggesting that a common trait of the various members of the POT family is responsiveness to inflammation. In addition to GI tract inflammation, other proinflammatory conditions have been shown to have an impact on expression of members of the POT superfamily. For example, PepT2 levels are increased in the prostate cancer cell line, PC3, treated with TNF- α or IFN- γ (189).

Other PepT1 expression and activity regulatory pathways

Several studies have demonstrated that PepT1 expression and transportation activity are regulated by nutritional status as well as its own substrates (178), pharmacological agents (16), hormones such as insulin (73), epidermal growth factor (EGF) (146), and leptin. For instance, the α 2-adrenergic receptor stimulated PepT1 activity in a clone of the differentiated human intestinal cell line, Caco-2 (Caco-2 3B), engineered to stably express α 2A-adrenergic receptors at a density similar to that found in normal mucosa (16). EGF treatment of Caco-2 cells decreased Gly-Sar transport by suppressing PepT1 expression and thus the number of transporter molecules in the apical membrane (146).

Peptide Transporters and Associations with Pathologies

PepT1 in IBD

While PepT1 is normally poorly expressed in the colon, various studies on human patients and animals have reported enhanced expression in the small intestine and colon during intestinal inflammatory states, including a mouse model of colitis, IBD, and short bowel syndrome (10, 132, 215, 223, 236). The precise mechanisms underlying this upregulation are uncertain, but a number of plausible factors have been suggested. For instance, changes in inflammatory cytokine and hormone levels during the disease state are reported to affect PepT1 expression and function. In patients with IBD, upregulation of TNF- α and IFN- γ levels lead to enhanced hPepT1 expression and consequently, PepT1-mediated uptake of di- and tripeptides in intestinal Caco2-BBE cells (28, 64, 212). The adipocyte-secreted hormone, leptin, has also been shown to increase PepT1 expression in and act on Caco2-

BBE cells. Leptin-mediated upregulation of hPepT1 promoter activity is dependent on expression of the transcription factors CREB and Cdx2 (137). Interestingly, leptin is not expressed in the small or large intestines of healthy individuals, but detected in colonic epithelial cells from inflamed tissues (181). Despite the controversial results obtained regarding colonic PepT1 expression during steady-state, alterations in the expression profiles of PepT1 within the GI tract during chronic inflammation have been well described (132, 236). In patients with chronic diseases, such as IBD and short-bowel syndrome, PepT1 expression is upregulated. While multiple studies have described PepT1 expression in the colon only in inflammatory conditions (132, 236), other reports suggest that PepT1 mRNA is distributed regionally in the colon with little or no expression in proximal colon and high expression in the distal colon. Immunofluorescence analyses demonstrated the presence of PepT1 in the distal part of the colon, where it was suggested to contribute to electrolyte and water absorption (225). In another study, the same authors observed decreased PepT1 expression in the descending colon of patients with IBD during acute inflammation (226). Notably, colonic PepT1 is highly expressed in interleukin (IL)10^{-/-} mice with colitis but not *Lactobacillus plantarum*-treated IL10^{-/-} mice lacking signs of colitis (35). Pathogenic bacteria are also reported to induce colonic PepT1 expression (145), as shown with enteropathogenic *Escherichia coli* (*E. coli*) and the murine pathogen, *Citrobacter rodentium* (*C. rodentium*), both *in vivo* and *in vitro* (145). Interestingly, in cell cultures prepared from colon of transgenic mice that overexpress PepT1 under control of the villin promoter, *C. rodentium* attachment was attenuated, compared with WT colon cultures. In addition, chemokine (C-X-C motif) ligand 1 (or KC) was downregulated at both mRNA and protein levels with increasing PepT1 expression (145). The data collectively suggest that PepT1 plays a protective role against *C. rodentium* infection. In a separate study, *Lactobacillus casei*, a probiotic bacterium, increased PepT1 transporter activity in Caco-2 cells (139).

Using transgenic mice overexpressing hPepT1 in IECs under control of the villin promoter, we previously observed increased inflammation and exacerbated DSS-induced colitis (43). In dextran sodium sulfate (DSS)-treated transgenic mice, the degree of pathology was correlated with increased proinflammatory cytokine production, increased neutrophil infiltration, and greater weight loss, compared with WT mice (43). PepT1-deficient mice (PepT1^{-/-}) developed moderate colitis, compared with WT mice (10). In addition, chemotaxis of immune cells recruited to the intestine during inflammation was decreased in PepT1^{-/-} mice. Phenotypes observed with both transgenic and PepT1^{-/-} mice were attenuated by antibiotic treatment, suggestive of linkage with the presence of gut microbiota (10,41).

The collective findings highlight a number of plausible factors that stimulate PepT1 mRNA and protein expression in the colon during intestinal inflammatory states and demonstrate how alterations in PepT1 can play a role in intestinal inflammation and other pathologies. Upregulation of colonic PepT1 can trigger downstream proinflammatory events through various microbial or immunological mechanisms.

PepT1 delivery of bacterial peptides—Multiple *in vivo* and *in vitro* studies have reported the ability of PepT1 to transport a broad range of di- and tripeptides from bacteria

and microbial agents across the intestinal epithelial membrane, a phenomenon believed to contribute significantly to the pathogenesis of intestinal inflammation.

Restriction of PepT1 expression to the small intestine limits its uptake of bacterial peptides due to the normally low concentrations of bacteria in this region (207). In healthy colon, mucosal surfaces of epithelial cells are constantly exposed to bacterial flora and small peptides generated by these microorganisms in the intestinal lumen. These peptides, which sometimes exert proinflammatory effects, are only marginally present in the cytoplasm of colonic epithelial cells (32). The normal expression pattern is altered in patients with IBD. Thus, in cases of chronic UC or CD, PepT1 expression becomes more pronounced in the colon. Commensal bacteria colonizing the human colon produce significant amounts of di/tripeptides, and several studies have shown that PepT1 can transport these small molecules into various cell types. Therefore, expression of PepT1 in the colon may lead to increased intracellular accumulation of prokaryotic peptides, triggering downstream proinflammatory effects.

Upregulation of PepT1 in the colon can promote the transport of luminal N-formyl peptides and di/tripeptides into epithelial cells (Fig. 5). For instance, PepT1 has been shown to transport fMLP, a tripeptide produced by *E. coli* that is commonly present in the intestinal lumen (133). Entry of fMLP, a polymorphonuclear leukocyte chemotactic factor of neutrophils, into both IECs and the human monocyte cell line, KG-1, has been documented in the literature (31,133). Moreover, PepT1-mediated transport of this peptide into Caco2-BBE cells is modulated by IFN- γ (31). Along with other researchers, our group has also identified muramyl dipeptide (MDP) and L-Ala- γ -D-Glu-meso-diaminopimelic acid (tri-DAP) as PepT1 substrates (42, 211). MDP is a constituent of peptidoglycan, a component of the cell walls of both Gram-negative and -positive bacteria, whereas tri-DAP is a peptidoglycan degradation product from Gram-negative bacteria.

Penetration of bacterial peptides into epithelial cells via PepT1-mediated transport can trigger the activation of intracellular inflammatory signaling pathways, exerting downstream proinflammatory effects that exacerbate the disease state. Several studies have examined the downstream effects of these peptides in IECs. Merlin et al. (132) showed enhanced expression of MHC 1 molecules upon uptake of fMLP by epithelial cells. Previous studies on animal models have also revealed the presence of fMLP on cellular surfaces of MHC 1 molecules (37). These results suggest that transport of fMLP via upregulation of PepT1 leads to increased antigenic presentation of bacterial products by IECs, in turn, promoting greater sensitivity of epithelial cells to bacterial peptides and rapid triggering of inflammatory responses. Mechanistic insights into the downstream activity resulting from internalization of bacterial peptides were obtained with the aid of human or murine epithelial and immune cell lines. Treatment of Caco2-BBE cells with fMLP stimulated activation of nuclear factor kappa B (NF- κ B) and activator protein-1 transcription factors (29). MDP and tri-DAP initiated NF- κ B activity in Caco2-BBE cells, similar to fMLP in intestinal epithelia (42,211). Additionally, tri-DAP-mediated activation of the mitogen-activated protein kinase (MAPK) pathway and upregulation of IL-8 in Caco2-BBE cells (42). MDP-stimulated Caco2-BBE cells exhibited upregulation of IL-8 and monocyte chemoattractant protein (MCP)-1 (211). Interestingly, mucosal IL-8 and MCP-1, chemoattractants for neutrophils

and monocytes, respectively, were shown to be upregulated in colonic IBD regions (12). In a separate contrasting study, primary murine macrophages transported MDP in a PepT1-independent manner, suggesting that PepT1 transport may be cell and/or species specific (127). Overall, the data suggest that PepT1-mediated transport of bacterial peptides by IECs results in downstream activation of inflammatory pathways, and consequent migration of activated immune cells toward regions with higher bacterial loads, including the colon. Our proposed model of activation of inflammatory pathways induced by PepT1-mediated uptake of small bacterial peptides is presented in Figure 7. Earlier studies indicate that PepT1 transports bacterial di/tripeptides into cells to initiate inflammatory functions. Recent findings by our group and others suggest that PepT1-mediated transport of bacterial di/tripeptides leads to interactions between these bacterial peptides and intracellular innate immune receptors, initiating a proinflammatory response. Chemical structures of tri-DAP, fMLP, and MDP are displayed on Table 2.

The PepT1-NOD2 axis—Commensal bacteria that colonize the human colon produce significant amounts of di/tripeptides. Previous studies have clearly shown that PepT1 can transport bacteria-derived peptides, such as the formylated bacterial peptide, fMLP, MDP, and tri-DAP (28, 42, 211). Sensing of pathogens is the first step in mounting an effective immune response required for elimination of the invading organism and establishing protective immunity. Nucleotide binding oligomerization domain (NOD)-like receptors consisting of more than 20 related family members are present in the cytosol and recognize intracellular ligands (65, 76, 108, 196). Binding of bacterial products to NOD receptors triggers activation of the downstream proinflammatory NF- κ B pathway. NOD1 is activated by peptides that contain a diaminopimelic acid, such as the PepT1 substrate tri-DAP, while NOD2 recognizes MDPs, including the PepT1 substrate MDP. Thus, PepT1 transport activity is proposed to play an important role in determining the intracellular levels of ligands for NOD1 and NOD2, which, in turn, control the extent of activation of downstream inflammatory pathways (65, 76, 108, 196). Previous work of our group using surface plasmon resonance and atomic force microscopy specifically identified a direct binding of tri-DAP on leucine-rich region of NOD1 with a K_d value of 34.5 μ mol/L (107). Genetic variants of the NOD2 gene have been identified in patients with CD by two independent groups (92, 149). These polymorphisms are present in 8% to 17% of Caucasian patients with CD, and homozygous individuals have 20% to 40% higher risk of developing CD (but not UC) (39). Several studies have shown a link between PepT1 transport of bacterial peptides into IECs and enhancement of inflammatory effector functions, supporting the possibility of interactions between PepT1 and innate immune receptors, such as NOD receptors.

To investigate the potential role of PepT1/NOD2 signaling in colitis, we generated transgenic mice in which hPepT1 expression was driven either by the villin promoter leading to specific expression in IECs or the β -actin promoter leading to systemic expression (43). These mouse models were used to determine the role of PepT1 in DSS-induced colitis or crossed with NOD2-deficient mice (*Nod2*^{-/-}) to establish whether PepT1 and NOD2 act in concert during colitis. Colitis was exacerbated in β -actin- and villin-hPepT1 transgenic mice, compared with WT littermates, as evident from greater weight loss, enhanced neutrophil infiltration, and more pronounced upregulation of proinflammatory cytokine

mRNA levels upon DSS treatment, an established murine model of colitis. The aggravation of colitis in β -actin-hPepT1 and villin-hPepT1 transgenic mice appeared dependent on the presence of bacteria in the colon and NOD2 expression, since this effect was attenuated or abrogated in presence of antibiotics and in β -actin-hPepT1/*Nod2*^{-/-} and villin-hPepT1/*Nod2*^{-/-} mice (43). Our data suggest that PepT1 acts as an amplifier of inflammation by transporting bacterial peptides, such as fMLP, MDP, and tri-DAP, and increasing their intracellular concentrations and subsequent interactions with NOD2 to mediate downstream inflammatory functions. A recent study assessed the proposed interplay of PepT1 with NOD2 by measuring the expression levels of PepT1 in *Nod2*^{-/-} mice (226). Interestingly, PepT1 levels did not differ in the distal colon of *Nod2*^{-/-} mice. In addition, colonic tissue cultures from WT and PepT1-deficient mice exposed *in vivo* to MDP contained similar levels of proinflammatory cytokines (226). However, these results were obtained in healthy mice where colitis was not induced, suggesting that the pathological role of PepT1 transport of bacterial products is only associated with a state of illness, such as colitis. The data additionally highlight the potential of PepT2 as an alternative gate for intracellular MDP entry (191). A subsequent investigation performed on rats showed that perfusion of MDP induces inflammatory cell accumulation, increase in mucosal *Nod2* and receptor-interacting serine-threonine kinase 2, *Rip2* (activator of NF- κ B) transcript expression, NF- κ B activity and inflammatory cytokine expression, clearly indicating a causal link between sensing of PepT1-transported bacterial peptides by Nod2 and the development of inflammatory disorders (120).

PepT1 is not the only POT family member that functions in the intracellular transport of ligands activating innate immunity. Earlier *in vitro* knockdown of PhT1 in HEK293T cells led to decreased NF- κ B activity upon stimulation with the NOD1 ligand, tri-DAP (109). PhT1-deficient mice experienced less severe DSS-induced colitis than control mice and were impaired in terms of NOD1-dependent cytokine production (168). A separate study showed that mutation of SLC15A4, which encodes PhT1, causes a decrease in IFN type I production triggered by toll-like receptor (TLR)-7- and TLR9-dependent signaling pathways in plasmacytoid dendritic cells (19). PepT2 is also reported to act as an MDP transporter in human myeloid cells, where its expression is associated with phagosomes (33). Finally, γ -iE-DAP, a peptide derived from breakdown of the cell walls of Gram-negative bacteria, was recognized by NOD1 in lung epithelial cells after PepT2-specific transport in association with receptor interacting protein-2 activity. PepT2/NOD1-mediated signaling led to increased NF- κ B activity and augmented production of proinflammatory cytokines (192). Interestingly, preliminary clinical studies have shown upregulation of PhT1 mRNA levels in patients with UC and CD (109), suggesting a potential role of PhT1 in intestinal inflammation, in addition to PepT1. The importance of PhT1 in the regulation of innate immune responses was later validated by experiments showing that SLC15A4 positively regulates TLR9- dependent production of Th1 cytokines, such as IL-12 and IL-15 (168). In addition, SLC15A4^{-/-} mice displayed a less severe form of Th1-dependent colitis than control mice. This mouse model also highlights a role of SLC15A4 in NOD1-dependent cytokine production, potentially by transporting the NOD1 ligand, tri-DAP, from lysosomes to the cytosol. However, PhT1 does not appear to be important for recognition of the NOD2 ligand, MDP (168). Recently, significant advances have been made in understanding the

function of PhT1 and 2 in presentation of bacterial peptides to innate immunity receptors, as highlighted by Nakamura et al. (136). This study demonstrated that MDP transport from endosomes and lysosomes is dependent on the two dendritic cell- and macrophage-specific endolysosomal transporters, PhT2 and PhT1. Importantly, MDP sensing also required the recruitment of NOD1 and NOD2, and subsequently receptor-interacting serine/threonine-protein kinase 2 (RIPK2), to transporter-containing compartments (136). The above studies collectively highlight the significance of PepT1 and other POT family members in the transport of bacterial peptides into cells, triggering the initiation of inflammatory responses via recognition of these peptides by innate immune receptors.

Although PepT1 activity has been implicated in both cellular and animal models of intestinal inflammation, its genetic linkage to human disease has only recently been uncovered.

Polymorphisms of PepT1—In an effort to investigate the potential utility of PepT1 as a drug-delivery system, the functional consequences of genetic variations of PepT1 were initially analyzed using a DNA polymorphism discovery panel of 44 ethnically diverse individuals (232). The researchers identified 13 single nucleotide polymorphisms (SNPs) in PepT1 and analyzed their functions *in vitro*. All PepT1 nonsynonymous variants displayed conserved substrate recognition. A single variant (P586L) was associated with significantly reduced uptake capacity (232). Another study examined whether the genetic polymorphisms affect the peptidomimetic drug transporter activity of PepT1 (8), focusing on not only the coding region but sequencing of the 23 exons and adjoining intronic sections of PepT1. A cohort of 247 individuals of various ethnic origins was examined, leading to the identification of 38 SNPs (21 in intron and noncoding regions and 17 in the exon coding region), of which nine were nonsynonymous. These results confirmed earlier findings (232) and additionally identified a novel SNP inducing a functional change, specifically, F28Y with similar expression levels as WT but altered affinity (i.e., higher K_m value for dipeptide transport) (8).

In the context of IBD and short bowel syndrome patients, PepT1 has been shown to be upregulated (132,236). However, limited evidence showing direct association of PepT1 polymorphisms with human intestinal disease has been obtained. Following the discovery of several genes that mediate susceptibility to IBD, including NOD2 (92, 149), and accumulating evidence implicating PepT1-mediated transport of NOD ligands and other bacterial peptides in IBD, Zucchelli et al. (237) examined 12 hPepT1 polymorphisms for associations with IBD. Using subsets of individuals not carrying common NOD2 mutations (which provide a strong CD predisposing background), among two cohorts of Swedish and Finnish patients, functional hPepT1 SNP (rs2297322) was significantly associated with CD. Interestingly, in Swedish cohorts, rs2297322 was associated with increased risk of IBD while in Finnish cohorts, the same SNP was shown to be protective against IBD (237). This is a significant finding, suggesting that this PepT1 mutation may play a role in IBD pathology in some populations. However, more genome-wide association studies are required to establish the populations at risk or protected and the underlying mechanisms by which this specific hPepT1SNP confers either protection or susceptibility in different populations. A recent study with a German cohort failed to identify an association between the rs2297322 variant and IBD susceptibility (226) and the link between PepT1

polymorphisms and IBD therefore remains unclear. While the former study (237) focused on the significance of the association in a subset of patients devoid of NOD2 mutation and speculated that the rs2297322 variant has higher penetrance in the absence of a strong CD-predisposing background, the latter study (226) did not specify whether patients with NOD2 mutations were isolated. Moreover, in both investigations, the differences in allele frequency of rs2297322 between Scandinavian and German cohorts were discussed (226,237).

The antiinflammatory lysine-proline-valine tripeptide—The utility of antiinflammatory PepT1 ligands in the treatment of intestinal inflammation has been confirmed. Lysine-proline-valine (KPV), a tripeptide from the COOH terminus of α -melanocyte-stimulating hormone, possesses antiinflammatory properties (85, 98). In particular, KPV has shown significant antiinflammatory efficacy in DSS-induced colitis and naive T-cell transfer model of chronic colitis (97). Dalmasso et al. (41) initially reported that KPV is transported by PepT1 *in vitro*. KPV inhibited NF- κ B activation and attenuated the production of proinflammatory cytokines by both IL-1 β -stimulated Caco2-BBE and TNF- α -stimulated Jurkat, an immortalized human T lymphocyte cell line (41). *In vivo*, KPV reduced the level of intestinal inflammation in mice treated with DSS or 2,4,6-trinitrobenzene sulfonic acid, which are both well-established models of inducible colitis. Attenuation of colitis was histologically detected and proinflammatory cytokine mRNA expression levels were decreased, confirming that inflammation is reduced by KPV (41). In a separate study, nanoparticles (NPs) were employed to target KPV to the colon. Specifically, KPV NPs were encapsulated in a polysaccharide gel that collapsed and released the drug load primarily in the colon (106). Oral administration of KPV-loaded NPs attenuated colitis in mice treated with DSS. Animals receiving KPV throughout DSS treatment had lower neutrophil activity and proinflammatory cytokine levels than their control counterparts (106). In a subsequent study, KPV-loaded hyaluronic acid (HA)-functionalized polymeric NPs were shown to be a more efficient delivery system with the capacity for release in the colonic lumen and subsequent penetration of colitis tissues, enabling KPV to be internalized into target cells. In addition to their efficacy in targeted delivery, HA-KPV-NPs exerted combined effects against colitis in mice by accelerating mucosal healing and simultaneously alleviating inflammation (228). Moreover, our group demonstrated that KPV can prevent colon tumorigenesis in a azoxymethane (AOM)/DSS-induced colitis-associated cancer (CAC) model (215). In PepT1-deficient mice, KPV did not protect against tumorigenesis, indicating that the presence of PepT1 is required for protective activity, which corroborates with the previous finding that KPV is taken up by PepT1 (41, 215). The soy tripeptide valine-proline-tyrosine (VPY) has also showed some antiinflammatory potency in intestinal epithelial and immune cells and *in vitro* as it reduced the severity of colitis in mice. VPY transport and activity was abrogated in presence of Gly-Sar showing that VPY is a substrate of PepT1 (105).

Associations between miRNAs and PepT1 and their relevance in intestinal physiopathology—MicroRNAs (miRNAs) are small noncoding RNAs (18–25 nucleotides) that play important regulatory roles in various biological processes by acting as posttranscriptional gene expression modulators via binding to the 3'-untranslated regions of specific targeted mRNAs (7). MiRNAs contribute to multiple processes, such as immunity,

cell growth and proliferation, and intestinal epithelial differentiation. Specific colonic miRNAs are differentially expressed in patients with IBD (59). In view of the emergence of miRNAs as a new class of gene expression regulators, the role of specific miRNAs in the posttranscriptional control of PepT1 was examined. Dalmasso et al. (45) were the first to demonstrate that a specific miRNA, miRNA-92b, suppresses PepT1 expression at both the mRNA and protein levels in Caco2-BBE cells by directly targeting the 3'-untranslated region. Furthermore, intracellular communications between IECs that overexpress PepT1 under intestinal inflammatory conditions affect miRNA expression in other cells that are in contact with IECs. Accordingly, it is important to investigate the causal relationship between PepT1 expression and miRNA expression as well as crosstalk between miRNAs and their target proteins under disease states. In a previous study by our group, transgenic mice overexpressing hPepT1 in IECs driven by the villin promoter were employed to examine the effects of colonic hPepT1 expression on miRNA synthesis. Ten colonic miRNAs were upregulated in villin-hPepT1 mice, compared to WT animals (11). DSS-induced colitis also induced alterations in colonic miRNA expression in both WT and villin-hPepT1-overexpressing mice. More specifically, the results indicated that hPepT1 overexpression enhances colonic secretion of miRNA-23b, a miRNA previously associated with IBD (224) and shown to directly target macrophage myristoylated alanine-rich C kinase substrate (Marcksl-1), a nonepithelial protein that potentially suppresses intestinal inflammation (57). Levels of miRNA 23b were significantly higher in DSS-treated villin hPepT1 mice, compared to untreated villin hPepT1 and DSS-treated WT mice. Marcksl-1 levels were also lower in DSS-treated villin hPepT1 mice compared to other groups, suggesting a causal effect of miRNA 23b on this gene during intestinal inflammation. The overall findings encourage further investigation of the effects of specific colonic miRNAs regulated by PepT1 on cell-cell communication during colitis (11).

A recent study demonstrated that in patients with active UC, miR-193a-3p targets PepT1 and reduces its expression and activity, leading to suppression of the NF- κ B pathway (40). Intracolonic delivery of miR-193a-3p significantly ameliorated DSS-induced colitis whereas overexpression of colonic PepT1 via PepT1 3'-untranslated region mutant lentivirus vector abolished the antiinflammatory effect of miR-193a-3p. Data obtained from antibiotic treatment of DSS-induced colitis mice further suggested that miR-193a-3p regulation of PepT1 mediates uptake of bacterial products and is potentially a key mechanism of colonic inflammation (40).

Maintenance of intestinal homeostasis is vital for sustaining physiological equilibrium as its disruption can trigger inflammatory conditions, such as IBD. hPepT1 is known to play a crucial role in regulating specific miRNAs that influence such conditions, and therefore, clarification of the functions of this protein in the physiological environment is necessary. A recent study conducted by our group focused on the expression levels of various miRNAs and their target proteins along the crypt-villi axis in the jejunum of PepT1^{-/-} mice (233). The results demonstrated alterations in miRNA expression along the crypt-villus axis as well as levels of certain protein targets in PepT1^{-/-} mice, although the underlying mechanisms were not established. In addition, we observed significant changes in morphology of the crypt-villus axis in the PepT1^{-/-} group, with higher levels of apoptosis and proliferation of IECs, compared to WT mice. Decreased microvillus size observed in the PepT1^{-/-} group is

suggested to underlie the significant weight loss observed in these mice (233). Accordingly, PepT1 is proposed to function in the maintenance of intestinal homeostasis. However, the observed changes and dysregulation of miRNAs triggered by this protein require further analysis.

Colitis-associated cancer

A number of human studies have demonstrated how chronic intestinal inflammatory conditions can lead to carcinogenic effects. CAC is one such example of a cancerous malignancy associated with colitis. Since PepT1 functions in maintaining intestinal homeostasis and breach of this stability triggers an inflammatory response, the protein may contribute to the development of CAC. A recent study showed that tumor growth and size are increased significantly in mice overexpressing PepT1 (villin-hPepT1), compared to their WT counterparts (215). Conversely, tumor number and size and intestinal inflammation were decreased significantly in PepT1^{-/-} mice. Notably, colonic epithelial cell proliferation was increased in villin-hPepT1 mice but decreased in PepT1^{-/-} mice. Analysis of human colonic biopsy specimens revealed increased expression of PepT1 in patients (215). In view of the implicated effects of PepT1 on colonic tumorigenesis, further research should be performed to examine this linkage.

MicroRNA and PepT1 interplay in CRC—The involvement of miRNAs in various aspects of cancer biology has been documented in the literature. Evidence on the ability of aberrant miRNA expression in inducing colorectal cancer (CRC) has been obtained from multiple studies (30). Specific miRNAs exhibit either tumor suppressing or oncogenic properties. The reduction or elevation of such miRNAs leads to changes in expression patterns, subsequently affecting transcriptional regulation of protein-coding genes. Studies examining the association of aberrant miRNAs with CRC have demonstrated upregulation of the majority of miRNAs, suggestive of an oncogenic role.

Variations in PepT1 levels during inflammatory states can modulate the expression patterns of specific miRNAs in the colon (11). Upregulated PepT1 is reported to induce tumorigenesis (215). Changes in miRNAs as a result of PepT1 alterations may contribute to the potential development of cancerous malignancies, along with intestinal inflammation. The interactions and associated pathways contributing to the carcinogenic state remain to be determined.

PepT1 and obesity

The potential connection between PepT1 and obesity was initially predicted in a study reporting that stomach-derived leptin could reach the intestine in its active form and control intestinal tract functions, such as absorption and secretion (27). Gastric leptin has been shown to be involved in the control of intestinal peptide transport via PepT1 *in vitro* in Caco-2 cells that exhibit enterocyte-like differentiation and *in vivo* using a rat jejunal perfusion model (27). Leptin, a protein hormone, is a major product of adipose tissue, and its regulatory role in appetite and metabolism has been described in detail (66). PepT1 activity and expression are significantly reduced in jejunum of leptin-deficient mice *ob/ob*, a mouse strain that develops obesity and diabetes (86). Earlier studies have demonstrated that leptin

enhances PepT1 expression and activity in the jejunum and absorption of small peptide products of protein digestion through PepT1 (86). In mice with diet-induced obesity, reduced PepT1-specific transport is associated with leptin receptor downregulation (87). In a model characterized by progressive long-term hyperleptinemia, central desensitization to leptin is associated with obesity. PepT1 regulation is dependent on the duration of leptin treatment and diet (87). A contribution of PepT-1 to development of obesity has also been shown in *Caenorhabditis elegans*, whereby loss of PepT1 induced a decrease in intestinal proton influx, leading to higher uptake of free fatty acids with fat accumulation (185). PepT1^{-/-} mice are resistant to high fat diet-induced obesity owing to a slight reduction in food intake but mainly originating from a limited capacity to digest and absorb fat in the small intestine that increases fecal energy loss. This energy loss is not compensated by increased bacterial fermentation in the colon and caecum that would allow SCFA energy redelivery to the host (103). Overall, the results indicate that PepT1 is involved in energy uptake and contributes to the development of obesity through various pathways, among which the most well characterized is leptin-dependent activation of PepT1 activity and expression.

PepT1 and diabetes

Diabetes and insulin levels affect PepT1 activity and protein expression (18). Studies on Caco-2 cells have shown that insulin upregulates PepT1, suggesting converse downregulation of PepT1 in diabetes (203). Assessment of PepT1 activity in BBMV's prepared from jejunum of diabetic rats deprived of insulin revealed lower activity under these conditions (73). Kinetic analyses showed that the basis for this increased activity is not a change in K_m but a significant increase in V_{max} , suggesting the presence of a larger number of transporters. This theory was verified at both the protein and mRNA levels in the BBM of jejunal mucosal cells (73). To ascertain whether the effect of diabetes on PepT1 is specific to the intestine or also occurs in other tissues, similar studies were performed on PepT1 located in the BBM of renal tubules. The results showed that, as in the intestine, uncontrolled diabetes promotes protein and mRNA expression of PepT1.

Importantly, antidiabetic drugs, such as Glibenclamide or Nateglinide, widely used to treat noninsulin-dependent diabetes mellitus, inhibited the transport activity of PepT1 and PepT2 in a noncompetitive manner (169, 200). It is speculated that upregulation of PepT1 or PepT2 leads to increased availability of substrates for enhanced gluconeogenesis in diabetes (73).

Conclusion

Over the years, substantial progress has been made in elucidating the roles of POT superfamily members in physiopathology. Expression of PepT1 and PepT2 has been detected in numerous tissues and organs in animals and humans, providing novel insights into their pharmacological potential. In addition, new knowledge on the subsequently discovered peptide/histidine transporters, PhT1 and PhT2, has facilitated their characterization as potential key factors in several physiological processes. For instance, PhT1 is involved in chick embryogenesis and posthatch development (238). However, the physiological roles of PhT1 and PhT2 in disease are yet to be established. Given their

expression in multiple tissue types, including brain, intestine segments, eyes, spleen, lung, and thymus, further investigation of the functions of these two peptide/histidine transporters in health and disease is necessary to determine their pharmacological potential. A thorough analysis of the expression profile of PepT2, PhT1, and PhT2 in all the tissues will be a first step in the characterization of their role in physiology and will help to determine the diseases in which they could be involved. Experiments that involve the use of animal models deficient for either one or the other of the transporters will help deciphering their role in various diseases. For example, experiments performed with PhT1-deficient mice have provided insights into the function of this protein in histidine transport and homeostasis regulation in the brain (220). Further studies using this mouse model should aid in elucidating other relevant functions of the transporter protein, including its potential relevance in IBD pathogenesis and pharmacology.

Given the important role of PepT1 in intestinal physiopathology, another crucial avenue of investigation is the complex interrelationship of gut microbiota with aberrant expression and polymorphisms of PepT1 in the progression of IBD. Indeed, dysbiosis, defined as a change in normal microbiota composition, is accepted as the determining event in IBD pathogenesis (75, 227). Animal models have been used to illustrate the effects of bacteria on this process. For example, IL-10-knockout (IL10^{-/-}) mice do not develop colitis under germ-free conditions, and the severity of colitis in mice housed under normal conditions is decreased upon administration of antibiotics (34, 96, 171). Alterations in gut microbiota patterns are observed in patients with CD (126), and some IBD patients have reportedly shown improvement following antibiotic treatment (100). A number of studies have established that PepT1 can transport bacterial peptides into cells. This internalization promotes interactions between bacterial peptides and innate immune receptors (including NOD), in turn, triggering proinflammatory events. From a pharmacological perspective, aberrant expression of colonic PepT1 offers a strategy for antiinflammatory targeting. Future potential therapies for IBD (and CAC) may also target inflammation foci using ligands of PepT1 such as KPV, chemically modified prodrugs transported by PepT1 or probiotics that downregulate PepT1 expression in the small intestine and colon. The uptake of bacterial peptides is one mode of interaction between PepT1 and microbiota. More generally, it would be interesting to investigate the overall microbiota. For instance, we speculate that in the same manner as IBD patients or a mouse model of intestinal inflammation, PepT1-deficient and villin-PepT1 mice that are protected against and more susceptible to colitis, respectively, display altered microbiota compositions, one inducing protection and the other inflammation. The diet affects gut microbiota (118,210), and differences in intestinal absorption due to overexpression or deficiency of PepT1 could lead to alterations in the substrate to be “digested” by the microbial population, thus modifying its composition and/or function. Future studies linking PepT1 and gut microbiota may facilitate the identification of novel specific interactions between host and microbiota that are highly relevant in the context of intestinal inflammation.

Finally, the findings on PepT1 in relation to inflammation processes of IBD occurring in the gut may be extrapolated to other transporters in lung or brain, thereby supporting the utility of PepT2 and other POT members as viable targets for therapy in the organs in which they are expressed.

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Didactic Synopsis

Major teaching points

- The proton-coupled oligopeptide transporter (POT) family comprises PepT1, PepT2, PhT2, and PhT1 encoded by SLC15A1, SLC15A2, SLC5A3, and SCL5A4 genes, respectively (belonging to the solute carrier gene group).
- POT family transporters mediate the uptake of di- or tripeptide through the membrane using the transmembrane proton gradient as the driving force.
- The primary sites of functional expression of PepT1 and PepT2 are the intestine and kidney, respectively. Other localization sites have additionally been identified.
- Expression of PepT1 is regulated by a variety of parameters, including extracellular pH, circadian cycles, dietary supply, and inflammation.
- PepT1 has significant physiopathological relevance in inflammatory bowel disease.

Figure 1. Major events in the discovery of the members of the SLC15 family.

Main discoveries that identified the different members of POT family, their expression and their function is displayed on the time line in a chronological manner.

Figure 2. Homology between human and mouse PepT1.
Protein sequence alignment of *Homo sapiens* and *Mus musculus* PepT1 transporter.
Identical residues are underlined with gray and show a 90% homology.

Figure 3. Homology between human PepT1 and PepT2.
Sequence alignment of *Homo sapiens* PepT1 and PepT2 showing. Identical residues are underlined with green and show a 50% homology.

Figure 4. Schematic model of hPepT1 in the membrane and particularly the lipid raft.

The structure of the transporter itself is presented within the lipid raft but is the same when localized in nonlipids raft membrane regions.

Figure 5. Model for PepT1 interaction with different peptides in the epithelial cell of the gut. The transporter activity of PepT1 is driven by the electrochemical proton gradient established by the apical Na^+/H^+ antiporter for the pH balance from the peptide-transport-induced intracellular acid load (center cell). This mechanism depends on the basolateral Na^+/K^+ ATPase (right cell). The uptake of di- and tripeptides will occur rapidly and they will be hydrolyzed in the cytosol, free amino acids will be released into the blood stream by different amino acid transporters located in the basolateral membrane. PepT1 transport of bacterial di- and tripeptides in the lumen, such as N-formylmethionyl-leucyl-phenylalanine (fMLP), muramyl dipeptide (MDP), and L-Ala- γ -D-Glu-meso-diaminopimelic acid (tri-DAP), into the intestinal epithelial cells (left cell). Due to the accumulation of bacterial di- and tripeptides, the NFkB pathway is stimulated leading to the activation of proinflammatory cytokines. Di- and tripeptides are common substrates cotransported with protons by PepT1.

Figure 6. Overview of the organs where members of the SCL15 family are expressed. Tissues expressing PepT1 are labeled in green, PepT2 in orange, PhT1 in purple, and PhT2 in light blue.

Figure 7. Model for PepT1 transport of bacterial peptide and downstream activation of proinflammatory pathway.

PepT1 is upregulated during inflammatory bowel disease (IBD) causing the transport of bacterial di- and tripeptides in the lumen, such as N-formylmethionylleucyl-phenylalanine (fMLP), muramyl dipeptide (MDP), and L-Ala-γ-D-Glu-meso-diaminopimelic acid (tri-DAP), into the intestinal epithelial cells. Due to the accumulation of bacterial di- and tripeptides, the NFκB pathway is stimulated leading to the activation of proinflammatory cytokines. In addition, IBD can cause the disruption of barrier function leading the transport of bacterial di-tripeptides (fMLP, MDP, tri-DAP) via paracellular pathway. Once they are in the lamina propria, the di-tripeptides can be taken by macrophages which also express PEPT1. The binding of PEPT1-Di-Tripeptide will signal the macrophage to upregulate major histocompatibility class I molecules. MCP, monocyte chemoattractant protein. Redrawn from (93).

Table 1

SLC15 Proton Oligopeptide Cotransporter Family

Human gene name	Protein name	Name	Predominant substrates	Tissue and subcellular expression	Link to disease	Human gene locus	Sequence ID
SLC15A1	PEPT1	Oligopeptide transporter 1, H ⁺ -peptide transporter 1	Di- and tripeptides, protons, beta-lactam, Bacterial peptides	Apical surface of epithelial cells from: Small intestine, Kidney, Pancreas, Bile duct and liver	Inflammatory bowel disease (Ser117Asn SNP and colonic upregulation)	13q32.3	NM_005073.3
SLC15A2	PEPT2	Oligopeptide transporter 2, H ⁺ -peptide transporter 2	Di- and tripeptides, protons, beta-lactam, MDP	Apical surface of epithelial cells from: Kidney, Choroid plexus, Neurons, Astrocytes (neonates), Lung, Mammary gland, Spleen, Enteric nervous system	Lead exposure (2 haplotype associated with higher blood lead burden in male children)	3q21.1	NM_021082
SLC15A3	PHT2	Peptide/histidine transporter 2, PTR3	Di- and tripeptides, protons, histidine	Lung, Spleen, Thymus, Intestine (faintly in brain, liver, adrenal gland, heart)	none	11q12.2	NM_016582
SLC15A4	PHT1	Peptide/histidine transporter 1, PTR4	Di- and tripeptides, protons, histidine, Tri-DAP, MDP	Brain, Eye, Intestine (faintly in lung and spleen)	Inflammatory bowel disease	12q24.32	NM_145648

Table 2

Molecules That Interact with H⁺/Peptide Symporters PEPT1

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT1	Compound	Chemical structure	References
Prodrugs	Valacyclovir		(58,94,230)
	Aminolevulinic acid		(9,46,54,140,153)
ACE inhibitor	Enalapril		(235)
	Fosinopril		(179)
β-lactam antibiotics	Cefadroxil		(22,153,155,222)
	Cephalexin		(53,58,159,179,198)
	Penicillin G		(157)
	Cyclacillin		(67,198)

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT1			Compound	Chemical structure	References
			Ceftibuten		(166,198)
			Loracarbef		(49,67,198)
			Cefixime		(53,194,222)
			Cephradine		(58,159,198)
			Cefroxadine		(101)
			L-alanyl-L-D-glutamyl-L-meso-diaminopimelic acid (tri-DAP)		(42,107,211)
			Muramyl dipeptide (MDP)		(211)
			N-Formylmethionine-leucyl-phenylalanine (fMLP)		(28,133)

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT1			Compound	Chemical structure	References
Other compounds			L-Dopa-Phe		(159,195,218,231)
			Alafosfalin		(141)
			Arphamenine A		(56)
			cyclo-trans-4-L-hydroxypropyl-L-serine (JBP485)		(114,116)

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT1	Compound	Chemical structure	References
	Maltosine		(74)
	Ochratoxin A		(170)

Table 3

Molecules That Interact with H⁺/Peptide Symporters PEPT2

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT2	Compound	Chemical structure	References
β-lactam antibiotics	Cefaclor		(24,49,110,119)
	Cefadroxil		(21,49,67,198)
	Cephalexin		(49,67,119,198)
	Cyclacillin		(67,119,198)
	Loracarbef		(49)
ACE inhibitor	Moxalactam		(119)
	Fosinopril		(113,179)

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT2			References
Other compounds	Compound	Chemical structure	
	Zofenopril		(113)
	Alafosfalin		(141)
Arphamenine A			(48)
	Arphamenine B		(48)
Bestatin			(49,199)

Agents and other compounds interacting with H ⁺ /peptide symporters PEPT2	Compound	Chemical structure	References
	Carnosine		(199,201)
	Val-ganciclovir		(188)