Spatial Expression Patterns of Peptide Transporters in the Human and Rat Gastrointestinal Tracts, Caco-2 *In Vitro* Cell Culture Model, and Multiple Human Tissues

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ABSTRACT This study sought to identify the spatial patterns of expression of peptide transporter 1 (PepT1), peptide transporter 3 (PTR3), peptide/histidine transporter 1 (PHT1), and the human peptide transporter 1 (HPT-1) mRNA in complementary DNA (cDNA) libraries of the human and rat gastrointestinal tracts (GIT), Caco-2 *in vitro* cell culture model, and in a human multiple tissue panel. Human PTR3 and PHT1 are putative peptide transporters recently discovered. Using sequence-specific primers designed to amplify regions of PepT1, PTR3, PHT1, and HPT-1, we were able to identify the expression of mRNA for each of these transporters in human cDNA panels (Clontech, Palo Alto, CA), the rat GIT, and in Caco-2 cDNA libraries by the polymerase chain reaction (PCR) and Southern Blot analysis. These studies suggest that in the human GIT, PepT1 appears to be localized predominantly in the duodenum, with decreasing expression in the jejunum and ileum. In contrast, PTR3 and HPT-1 were widely expressed in the human GIT, with predominant expression in the different regions of the colon. PHT1 appeared to be expressed in low levels throughout the human GI tract. Interestingly, the mRNAs for all 4 peptide transporters were expressed in Caco-2 cells throughout 30 days of culture. PepT1, PTR3, PHT1, and HPT-1 were also widely expressed in the rat GIT. Human tissue cDNA panel screening suggests that PTR3 and PHT1 are more uniformly expressed, whereas PepT1 and HPT-1 demonstrated site-specific expression. These results suggest that PepT1, PTR3, PHT1, and HPT-1 all may act to facilitate the diffusion of peptides and peptide-based pharmaceuticals in the GIT. PTR3, PHT1, and HPT-

1 expressions in Caco-2 cell monolayers strongly suggest that their function needs to be further elucidated and their contribution to peptide transport not ignored. Taken together, these results demonstrate the potential for molecular biological characterization in localizing active transporter systems that can potentially be targeted for enhancing the absorption of peptide-based pharmaceuticals.

KeyWords: Peptide Transport, PepT1, PTR3, PHT1, HPT-1, GI Tract, Human Digestive cDNA Panel, Human Tissue cDNA Panel, Caco-2 Cells

INTRODUCTION

The use of *in vitro* and *in situ* models to assess intestinal permeation characteristics of peptides and peptide-based drugs has become commonplace in many laboratories. However, it should not be taken for granted that the actual intestinal enterocyte barrier has differing levels of expression and transcriptional regulation of genes than found in these *in vitro* or *in situ* models. With recent advances in molecular biology leading to the development of several techniques, including microarray technology, reverse transcriptase polymerase chain reaction (RT-PCR), complementary DNA (cDNA) libraries, and multiple-tissue Northern blots, the ability for scientists to screen several tissues for messenger RNA (mRNA) expression has become commonplace in many laboratories. Several commercially available sources of cDNA panels derived from numerous human, rat, and mouse tissues exist. In this study, we have used a recently developed human digestive system cDNA panel (Clontech, Palo Alto, CA) derived from each segment of the human digestive tract (in order: esophagus, stomach, duodenum, jejunum, ileum, ileocecum, cecum, ascending colon, transverse colon, descending colon, and rectum).

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These developments provide pharmaceutical scientists access to strategies for quickly elucidating spatial patterns of expression of mRNAs corresponding to genes/proteins that may be of considerable interest. It is apparent that the formulation scientist can now rapidly and critically assess a target protein's (either receptor or transporter) expression in tissue samples and tailor formulations to optimize delivery of agents to the regions of optimal expression. This may even be expanded to investigate regions where proteins that limit bioavailability of a therapeutic agent could be avoided (ie, multidrug resistant proteins) by delivery of substrates to regions where these proteins are least expressed. We have used such an approach to characterize the molecular expression patterns of peptide transporters in the human and rat gastrointestinal tracts (GIT), Caco-2 cell monolayers, and in multiple human tissues.

The therapeutic use of peptide-based pharmaceuticals for many disorders has been the focus of numerous papers (1-6). Peptides and peptide-based agents, due largely to multiple side chain functionalities, tend to be polar molecules that do not readily traverse biological barriers via passive diffusion (1, 7-11). In the past several years, considerable work has been performed to explore the active peptide transporters that are known to facilitate peptide transport (5,12,13). Several human small oligopeptide transporters are now known, including peptide transporter 1 (PepT1) and human intestinal peptide transporter 1 (HPT1) (5,14-16). PepT1 belongs to a superfamily known as proton oligopeptide transporter (POT), where all the known peptide transporters, with the exception of HPT1 (a cadherin family member), are grouped (13, 27). Of these transporters, PepT1 has gained the most focus and has been theorized to be the predominant peptide transporter in the GIT (1,5,17,18). The focus is on PepT1 because it was the first peptide transporter characterized and because of its high expression in the rat GIT and Caco-2 cell culture model, a human colon-derived adenocarcinoma cell line that has been widely used as an *in vitro* model of the gastrointestinal enterocytes (19-21). More recently, HPT-1 has been identified in Caco-2 cells (16, 22-25), but as yet has not received the same level of research as PepT1.

Until recently, only 2 POT genes had been identified in mammalian species: hPepT1 and hPepT2. Putative peptide transporter 3 (PTR3) is a newly reported transporter (GenBank Accession number AB020598) that has not been exhaustively studied; therefore, little is known about its functional significance. PTR3 is a human protein that shares homology with members of the PTR family (26-30). Release of the human genome has opened the search for new potential drug transporters. A recent report has shown evidence of the presence of 2 potentially new peptide transporters (28). The cDNA sequence predicted for one of these transporters is almost identical to the one submitted in the GenBank for PTR3-Sadee and colleagues (28) named it hPHT2.

Peptide/histidine transporter 1 (PHT1) has been initially identified and cloned from the rat brain (29). Our (30) and Dr. Wolfgang Sadee's (personal communication) laboratories recently cloned the human PHT1 isoform and have begun the analysis of its expression in several human and rat tissues. We included the putative transporters PTR3 and PHT1 in our survey to present a current view of potential peptide transporters in the human GIT and models most often used to assess intestinal peptide permeability. In addition, we cannot rule out that other peptide transporters may exist in the GIT that have yet to be identified.

Elucidating these transporters' gene expression patterns will provide essential information for formulation scientists to develop rationally targeted drug delivery systems. In addition, further functional characterization of these transporters will provide medicinal chemists with insights into the essential functional groups that will enable them to synthesize agents with high specificity for these transporters.

MATERIALS AND METHODS

Materials

Nonessential amino acids, fetal bovine serum, trypsin/EDTA, penicillin and streptomycin, and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Mediatech, Inc. (Herndon, VA). The Human Digestive System and Human Multiple Tissue cDNA Panels were purchased from Clontech (Palo Alto, CA). Taq DNA polymerase was

purchased from New England Biolabs (Beverly, MA). Tri Reagent was obtained from the Sigma Chemical Company (St Louis, MO). RT-PCR kits were obtained from Life Technologies (Gaithersburg, MD). The North2South Biotin Random Prime Kit used in the preparation of DNA probes and the North2South Chemiluminescent Nucleic Acid Hybridization and Detection Kit used for Southern Blot analysis were purchased from Pierce (Rockford, IL).

Animals and tissue collection

Female Holtzman rats (6 months old) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals (200 g) were housed in an environmentally controlled facility, with lights on from 0600 to 2000 hours, and were allowed free access to food and water. Tissue dissections were performed and small and large intestinal segments were obtained according to the standard protocol for identifying the different sections of the GIT (31). After isolation of the tissues, we proceeded to remove the end sections of each sample, saving only the middle portion of each region to avoid contamination from other segments.

On isolation, the tissues were frozen in liquid nitrogen and stored at -80°C until analyzed.

Cell Culture

The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured as described previously (21). The cells were grown in 75 cm² culture flasks (Corning, Inc, Corning, NY), in culture medium that consisted of DMEM with 100 mg/mL penicillin, 100 mg/mL streptomycin, 1% nonessential amino acids, and 10% fetal bovine serum. Cells were maintained at 37°C in an atmosphere of 95% relative humidity and 5% CO₂. The culture medium was replaced every other day for the first week and daily thereafter. Cells used in this study were between passages 30 and 40.

RT-PCR and Electrophoresis

The Human Digestive cDNA Panel was used to determine the spatial patterns of expression of each transporter in the human GIT. Total RNA was isolated from rat dissected tissues and from 4-, 10-, 15-, 20-, 25-, and 30-day-old Caco-2 cells with the

TRI reagent as described by Chomczynski and Sacchi (32). Five µg of total RNA and 0.5 µg of oligo-(dT) were used for the reverse transcriptase reaction. RT-PCR was performed with a portion of the reverse transcriptase-generated cDNA and primers (Table 1) specific for PepT1, PTR3, PHT1, and HPT-1. Human β-actin primers were obtained from Maxim Biotech, Inc (San Francisco, CA). The PCR reaction was performed using optimized PCR conditions (denature: 94°C for 1 minute; annealing: 55°C for 2 minutes; extension: 72°C for 2 minutes for PepT1 and HPT1; and denature: 94°C for 1 minute; annealing: 65°C for 2 minutes; extension: 72°C for 2 minutes for PTR3 and PHT1) using a Perkin-Elmer Thermocycler (Model 480, Norwalk, CT). Reaction products were electrophoretically separated in 1.4% agarose gels. Ethidium-stained bands and densitometry measurements were detected using a NucleoTech 920 Chemiluminescence detection system (NucleoTech Corporation, San Mateo, CA). The molecular weight for each band was calculated using a 100-bp molecular weight ladder as a reference (PanVera Corporation, Madison, WI). Each RT-PCR gene expression analysis was performed at least 3 times.

Southern Analysis

The electrophoretically separated reaction products in the agarose gel were transferred to nylon membranes (Ny+, Millipore, Bedford, MA), and probed with biotin-labeled PepT1, PTR3, PHT1, and HPT1 cDNAs, respectively. The human PepT1 cDNA was kindly donated by Dr Frederick H. Leibach (Medical College of Georgia, Augusta), and HPT-1 full sequence was amplified from Caco-2 cells and confirmed by sequencing analysis, while the PTR3 and PHT1 partial sequence was obtained from clones 668165 and 325557, respectively (I.M.A.G.E. Consortium, <http://image.llnl.gov/>). Rat PepT1 probe was obtained using a 700-bp fragment of the rPepT1 cDNA (kindly donated by Dr Matthias A. Hediger, Harvard Medical School, Boston, MA). Southern analysis of blots for rPTR3, rat HPT1, and rat PHT1 were performed using the same probes as for their human isoforms. Chemiluminescence measurements were analyzed using a NucleoTech 920 Chemiluminescence detection system (San Mateo, CA).

Table 1. Sequence-specific Primers Used for the Amplification of the Human and Rat Peptide Transporter Isoforms and β -actin, a Positive Control, Based on Their Respective, Reported Encoding cDNA Regions

	Sequence (5' to 3')	PCR Product (bp)
Human		
PepT1	Sense: CTACTATGGAATGCGAGCAAT Antisense: ACTTCTTGATACATCCCACTGC	588
PTR3	Sense: TGCCGACCAGGTGATGGATCT Antisense: CACAACATTGGCCAGGAGGAGC	573
PHT1*	Sense: GTTTTACTGGAGCATTAAATTTGGGAGC Antisense: GTAAAACATATGTGGTCTGCATTGG	443
HPT-1	Sense: CATAGAAGTGAAGGACA Antisense: GATGGGGATCTGATCATTG	1004
β -actin	Sense: GCATCCTCACCTGAAGT Antisense: CATCTCTTGCTCGAAGTCC	492
Rat		
PepT1	Sense: ATCTACCATACGTTTGTTGC Antisense: CTGGGGCTGAAACTTCTT	523
PTR3 [†]	Sense: TGCCGACCAGGTGATGGATCT Antisense: CACAACATTGGCCAGGAGGAGC	573
PHT1	Sense: GTTTTACTGGAGCATTAAATTTG Antisense: GTAAAACATATGTGGTCTGC	437
RPT-1	Sense: ATGAACCACTGGCCCA Antisense: GAGGCAAAAGAACTAGCAT	860
β -actin	Sense: TTTGTGCCTTGATAGTTTCG Antisense: AAGTGTGGTGCCAAATCT	375

*Based on preliminary human sequence; see text.

†Based on the human PTR3 sequence.

RESULTS

Table 1 shows the primers designed to specifically amplify cDNA encoding regions of human PepT1 (14), PTR3 (GI: AB020598), PHT1 (28), and HPT-1 (16) in the human GIT. Table 1 also shows the sequence-specific primers for the corresponding homologous rat isoforms of these proteins that were designed to specifically amplify cDNA encoding regions of rat PepT1 (33), rat PHT1 (29), and rat PT-1 (34). All PCR experiments were performed and analyzed in the linear range of amplification for each primer set (data not shown). We were unable to design rat-specific primers for PTR3 since only the human sequence has been reported. However, using the 2 separate sets of human PTR3 primers (data not shown), we were able to amplify fragments that were identical to the size predicted by the human cDNA sequence, respectively. These results suggest a high homology between the human and rat sequences. Through Southern Blot analysis

it was shown that the rat PTR3 hybridizes to the human isoform fragment; we are currently sequencing the rat PTR3 fragments to determine their exact sequence.

Analysis of the human PHT1 expression was first done using rat sequence primers in the human tissues. After identifying that some of these primers amplified products with a sequence highly homologous to the rat PHT1 sequence, we derived a preliminary human PHT1 sequence from human expressed sequences tags highly homologous to the rat PHT1 cDNA (29). The remaining primers were designed based on the reported coding region DNA sequences and their specificity was confirmed by nucleotide BLAST search computer analysis. In each case the primers were confirmed to be highly specific for each particular transporter, with no significant homology to any other known sequences in human or rat tissue. However, HPT-1 PCR analysis amplified several products that may be related to the cadherin family-cell adhesion proteins that are highly homologous to HPT-1 (16). Southern analysis for this particular blot was difficult to perform because of the hybridization of the HPT-1 probe with some other bands present. Identification of the HPT-1-specific PCR product was performed by sequencing analysis of the amplified product either from the cDNA panels or from the Caco-2 cells, respectively (data not shown).

In Figure 1, the results for the RT-PCR amplification and Southern Blot analysis of the human isoforms of PepT1, PTR3, PHT1, and HPT-1 in the human digestive tract cDNA panel are shown. The results of our studies suggest that PepT1 is predominantly expressed in the human duodenum, with decreasing expression found in the jejunum and the ileum (Figure 1). Interestingly, PTR3 expression and HPT-1 expression were demonstrated in several regions of the human GIT, and were not predominantly restricted to the duodenum as was PepT1. Although the results do not infer quantitation or functional significance for direct comparison, they do suggest that PTR3 and HPT-1 should be further characterized to properly assess their respective importance in the facilitation of oligopeptide and peptide-based drug diffusion in the human GIT. According to our RT-PCR analysis, PHT1 is not highly expressed in the GIT as

Figure 1. RT-PCR analysis of human PepT1, PTR3, PHT1, and HPT-1 mRNAs in the human esophagus (A), stomach (B), duodenum (C), jejunum (D), ileum (E), ileocecum (F), cecum (G), ascending colon (H), transverse colon (I), descending colon (J), rectum (K), and in Caco-2 cells (L). RT-PCR was performed with specific primers for each mRNA and amplified products of PepT1, PTR3, PHT1, and HPT-1 were 588, 470, 443, and 1004 bp, respectively. Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Commercially available human β -actin primers were used to generate a mRNA expression positive control, amplifying a product of 303 bp.

Figure 2. RT-PCR analysis of rat PepT1, PTR3, PHT1, and RPT1 mRNAs in the rat stomach (A), duodenum (B), jejunum (C), ileum (D), ileocecal junction (E), cecum (F), and colon (G). RT-PCR was performed using specific primers for rat PepT1, PHT1, and RPT-1 mRNAs amplifying products of 523, 437, and 860 bp, respectively. Analysis of PTR3 mRNA expression in the rat tissues was performed using primers designed from the human PTR3 mRNA sequence (Table 1). Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Rat-specific β -actin primers were designed to generate a mRNA expression-positive control, amplifying a product of 375 bp.

Figure 3. RT-PCR analysis of human PepT1, PTR3, PHT1, and HPT-1 mRNAs in Caco-2 cells at day 4 (A), day 10 (B), day 15 (C), day 20 (D), day 25 (E), and day 30 (F) after being passaged. RT-PCR was performed with the same set of primers used for the analysis of mRNA expression in the human gastrointestinal tract. The Caco-2 cell line was obtained from the American Type Culture Collection (Rockville, MD) and cultured as described previously. Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Commercially available human β -actin primers were used to generate a mRNA expression-positive control, amplifying a product of 303 bp.

Figure 4. RT-PCR analysis of human PepT1, PTR3, PHT1, and HPT-1 mRNAs in the brain (A), colon (B), heart (C), kidney (D), leukocytes (E), liver (F), lung (G), ovary (H), pancreas (I), placenta (J), prostate (K), skeletal muscle (L), small intestine (M), spleen (N), testis (O), and thymus (P). RT-PCR was performed with specific primers for each mRNA, and amplified products of human PepT1, PTR3, PHT1, and HPT-1 were 588, 470, 443, and 1004 bp, respectively. Reaction products were electrophoretically separated in 1.4% agarose gels, stained with ethidium bromide (top panels), and identity confirmed by Southern Blot analysis (lower panels). Commercial available human β -actin primers were used to generate a mRNA expression-positive control, amplifying a product of 303 bp.

confirmed by Southern Blot analyses, which showed a faint band of expression might be present. However, the levels of protein expression and the functional significance of PHT1 need to be determined to properly assess PHT1's role.

The results for the RT-PCR amplification and Southern Blot analysis of the rat isoforms of PepT1, PTR3, PHT1, and PT-1 in the different regions of the rat GIT are shown in Figure 2. These results suggest that PepT1 and PHT1 are widely expressed throughout the rat GIT (Figure 2), in contrast with the regional pattern of expression found in the human GIT panel (Figure 1). Consistent with the expression patterns elucidated in the human GIT cDNA panel, both PTR3 and HPT-1 also were expressed throughout the rat GIT.

In Figure 3, the results for the RT-PCR amplification and Southern Blot analysis of the human isoforms of PepT1, PTR3, PHT1, and HPT-1 are shown in the Caco-2 cell model. Each of these transporters were demonstrated to be present throughout each of the days of culture of the Caco-2 cells. These results suggest that all 4 transporters may act to contribute to the observed transport kinetics measured for peptide and peptide-based pharmaceuticals across Caco-2 monolayers.

Figure 4 represents the results for the RT-PCR amplification and Southern Blot analysis of the human isoforms of PepT1, PTR3, PHT1, and HPT-1 in the multiple tissue human cDNA panels. The results suggest that PepT1 mRNA is predominantly expressed in the human liver and pancreas. Lower levels of PepT1 expression were observed in the kidney, placenta, and prostate. HPT-1 expression was observed in the colon, skeletal muscle, and faintly in the small intestine. Surprisingly, PTR3 and PHT1 expression was determined to be fairly uniform in the human cDNA panel. PTR3 expression was observed in all of the tissues present in the panel with the exception of the prostate, while PHT1 expression was determined particularly in liver, placenta, prostate, and thymus.

DISCUSSION

The transport of most nutrient classes across biological barriers is facilitated by several different transporter classes with overlapping specificities (35-42); however, for oligopeptides and peptide-

based drugs, transport is believed to be predominantly facilitated by either PepT1 or PepT2. Our results suggest that there may actually be other oligopeptide transporters present in these tissues that remain to be functionally characterized. The results of these studies also suggest that more emphasis needs to be placed on identifying other potential peptide transporter isoforms for peptide and peptide-based drug-screening purposes.

The expression patterns of the human and rat isoforms of PepT1, PHT1, and HPT-1 transporters in the GIT have been partially described before (14,25,28,29). However, these studies did not directly compare the regional expression of PepT1, PTR3, PHT1, and HPT-1 simultaneously, due in part to the fact that several of the transporters have only recently been elucidated. In this study, we established the simultaneous expression patterns of several peptide transporters in both whole human and rat GITs, in multiple human tissues, and in the Caco-2 cell culture model. Furthermore, the initial comparison of these transporters' expression by using the same tissue sources and comparable experimental conditions provides a more adequate assessment of their physiological relevancy. The results reported in this article are being used to better establish an understanding and framework for further studies that can completely assess the physiological and functional significance of these peptide transporters. Only on this complete characterization can the advantages and disadvantages of using different models to assess human intestinal peptide absorption by these transporters be fully understood.

In these studies, we demonstrated the human digestive tract mRNA expression of PepT1, PTR3, PHT1, and HPT-1. These results suggest that the relative importance of PepT1 in facilitating peptide and peptide-based drug diffusion may be overstated. HPT-1 and the putative peptide transporters PTR3 and PHT1 are also expressed in the human GIT, and their relative importance in the facilitation of oligopeptide and peptide-based drug transport must be characterized to properly assess their respective importance. These results do not rule out the possibility of the existence of other transporters and do not directly relate to transporter function either; however, they do provide strong suggestive

evidence that more than one peptide transporter is responsible for facilitating peptide and peptide-based drug permeation across the GI barrier, as has been theorized by others (27,28).

RT-PCR is a highly sensitive technique, which is readily used to specifically amplify a signal from mRNA found in a tissue/cell RNA sample. Based on this observation, we used RT-PCR to investigate the expression of several peptide transporters from identical tissue sources. Several differences have been observed between our studies and those already reported by others (14,28,29). These differences may arise in part from the sensitivity of the RT-PCR experiment (when contrasted with Northern Blot analysis) and may be due to differences in tissue sources.

The results in Figure 2 demonstrate that PepT1, PTR3, PHT1, and HPT-1 are widely expressed throughout the rat GIT. In particular, the rPepT1 expression pattern characterized in this study differs with the expression analysis reported by others (42) where a Northern Blot analysis showed localized expression in the small intestine. We attribute these discrepancies to the different sensitivities found between the RT-PCR technique and the Northern Blot analysis. The elucidated patterns of expression of these transporters in the rat GIT suggest that the use of rat intestinal segments for *in situ/in vitro* perfusion studies investigating peptide and peptide-based drug diffusion may not accurately mimic the corresponding regions of the human GIT. Therefore, extrapolation of peptide and peptide-based drug transport values measured in the rat model must be taken in context with the differences in the patterns of expression of the peptide transporters. In addition, because cross-species differences exist in the primary sequences of the different peptide transporters (14,16,29,33,34), one must recognize that there may exist kinetic differences between the ability of human peptide transporter isoforms and their rat counterparts to facilitate peptide and peptide-based drug transport.

Contrasting the expression of PepT1, PTR3, PHT1, and HPT1 in the human GIT with the observed expression in the Caco-2 cell culture model, it becomes readily apparent that the Caco-2 cells also do not appear to be representative of any one region

of the human GIT. The expression of each transporter suggests that peptide and peptide-based drug transport measured across this model is potentially attributable to several transporters. Efforts must be made to completely characterize the function of each transporter and to delineate their respective contributions to an overall assessed permeability.

In addition, using *in vitro* models to assess intestinal permeation characteristics of peptides and peptide-based drugs must be undertaken with the understanding that the actual intestinal enterocyte barrier may have differing levels of expression and transcriptional regulation of transporters than those observed in *in vitro* cell lines. Specifically, our results suggest that expression of these transporters in the Caco-2 cells may not reflect actual expression in different regions of the human GIT, for instance, the duodenum versus the colon for PepT1 expression. However, the significance of our present findings require further investigation into the elucidation and quantitation of the patterns of expression of each transporter's mRNA and protein by Northern and Western Blot analysis, respectively.

In Figure 4, the results for the expression of PepT1, PTR3, PHT1, and HPT1 in several different human tissues in the Human Multiple Tissue cDNA panel (ClonTech) are demonstrated. The faint level of PepT1 expression in the small intestine can be readily explained by the fact that the cDNA source for this panel came from the entire small intestine, and therefore, the high expression observed in the duodenum (Figure 1) was potentially masked by the cDNAs derived and grouped from the other small intestinal regions. In addition, it is somewhat surprising that PTR3 expression and PHT1 expression were found to be so widespread. Although functional assessments of human PTR3's and PHT1's ability to facilitate peptide and peptide-based drug transport remain to be conducted, the widespread expression of both suggests that they may perform a primary role in the peptide transport in several human tissues.

Our results indicated that these transporters may be more widely expressed in certain tissues than currently believed (28). For instance, consider the

expression of PHT1 in the human placenta, a tissue source that has been used for the cloning of the full-sequence of this putative peptide transporter (30). The use of RT-PCR and Southern Blot analysis provides greater sensitivity and also can be used to provide further justification for targeting and assessing regions where greater emphasis needs to be placed in measuring protein levels and functional significance.

The results of our studies provide insights into the potential for an integrated approach toward the rational molecular biological and functional characterization of transporters to aid in the rational design of peptide-based pharmaceuticals and dosage formulations. In addition, we have screened both the rat GIT and Caco-2 cell monolayers for expression of PepT1, PTR3, PHT1, and HPT-1 to assess their ability to mimic human digestive tract for *in vitro* and *in situ* drug screening. Elucidating the patterns of gene expression of these transporters will, in our opinion, provide essential information for formulation scientists to develop rationally targeted drug delivery systems to potentially increase oral bioavailability.

CONCLUSIONS

Our studies provide insights into potential pathways and the regional significance of peptide and peptide-based drug permeation in the human digestive tract. These results suggest that PTR3 and HPT-1 may be the predominant transporters for peptide and peptide-based pharmaceuticals that are formulated in an enteric-coated or controlled-release manner. HPT-1 and the putative peptide transporters PTR3 and PHT1 may also play significant roles in facilitating transport across Caco-2 cell monolayers; they should not be ignored. In addition, PepT1 appeared to be predominantly expressed in the duodenum and may only be important for facilitating peptide and peptide-based pharmaceuticals that are rapidly released in the GI tract. Last, these results demonstrate the potential for using a multiple tissue array technology in the design of targeted formulations of actively transported pharmaceuticals.

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