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Biochimica et Biophysica Acta 1235 (1995) 461–466

BBA Biochimica et Biophysica Acta

Rapid Report

Molecular cloning of PEPT 2, a new member of the H^+/peptide cotransporter family, from human kidney

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Received 27 January 1995; accepted 3 February 1995

Abstract

Mammalian kidney is known to express a transport system specific for small peptides and pharmacologically active aminocephalosporins. This system is energized by a transmembrane electrochemical H^+ gradient. Recently, a H^+-coupled peptide transporter has been cloned from rabbit and human intestine (Fei et al. (1994) Nature 368, 563–566; Liang et al., J. Biol. Chem., in press). Functional studies have established that the renal peptide transport system is similar but not identical to its intestinal counterpart. Therefore, in an attempt to isolate the renal H^+/peptide cotransporter cDNA, we screened a human kidney cDNA library with a probe derived from the rabbit intestinal H^+/peptide cotransporter cDNA. This has resulted in the isolation of a positive clone with a 2190 bp long open reading frame. The predicted protein consists of 729 amino acids. Hydropathy analysis of the amino acid sequence indicates the presence of twelve putative transmembrane domains. The primary structure of this protein exhibits 50% identity and 70% similarity to the human intestinal H^+/peptide cotransporter. Functional expression of the kidney cDNA in HeLa cells results in the induction of a H^+-coupled transport system specific for small peptides and aminocephalosporins. Reverse transcription-coupled polymerase chain reaction demonstrates that the cloned transporter is expressed in human kidney but not in human intestine. This transporter, henceforth called PEPT 2, represents a new member in the growing family of H^+-coupled transport systems in the mammalian plasma membrane.

Keywords: Proton/peptide cotransport; cDNA cloning; Functional expression; β-Lactam antibiotic; (Human kidney)

Mammalian kidney expresses a transport system that is specific for small peptides consisting of 2–4 amino acids [1–4]. The physiological role of the transport system is to absorb amino nitrogen from the tubular filtrate in the form of small peptides. The pharmacological relevance of this transporter is the recognition of β-lactam antibiotics and other peptide-like drugs as its substrates [5–7]. Due to its role in the reabsorption of these compounds, the transporter is an important component in determining the half-life of these pharmacologically active drugs in the circulation and hence holds great potential for therapeutic applications. The driving force for the transporter is a transmembrane electrochemical H^+ gradient [4,8]. A H^+-coupled peptide transport system is also expressed in the mammalian small intestine where it functions in the absorption of peptides and β-lactam antibiotics [9,10]. Functional studies however have established that the renal peptide transport system is similar but not identical to its intestinal counterpart [11–13]. Recently, we have isolated a cDNA encoding a H^+-coupled peptide transporter from the small intestine of the rabbit [14] and the human [15]. Here we report the cloning of a H^+-coupled peptide transporter from human kidney. This transporter is expressed in the kidney but not in the small intestine. This kidney-specific peptide transporter, designated as PEPT 2, represents a new member in the growing family of H^+-coupled transport systems.

A human kidney λgt10 cDNA library, obtained from Graeme Bell, University of Chicago, was screened by plaque hybridization using VCS 257 host cells. The probe was a 560 bp fragment released from the 5′ end coding region of the rabbit intestinal peptide transporter cDNA by digestion with *Afl*III and *Hin*dIII. The probe was labeled

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SSDI 0005-2736(95)00049-6

with [α-^{32}P]dCTP using the oligolabeling kit from Pharmacia. Hybridization was carried out at 35° C in a solution containing 50% formamide, 6 × SSC, 5 × Denhardt's solution, 0.1% SDS and 100 μg/ml of salmon sperm DNA. Washing was done under low stringency conditions. Positive clones were identified and plaque-purified by secondary and tertiary screening.

Digestion of the phage DNA from the positive clone

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   1  CGAGGAGAGAGAGAGTAAGGAGCCAGCCATGAATCCTTTCCAGAAAAATGAGTCCAAG   60
                                  M  N  P  F  Q  K  N  E  S  K
  61  GAAACTCTTTTTTCACCTGTCTCCATTGAAGAGGTACCACCTCGACCACCTAGCCCTCCA  120
      E  T  L  F  S  P  V  S  I  E  E  V  P  P  R  P  P  S  P  P
 121  AAGAAGCCATCTCCGACAATCTGTGGCTCCAACTATCCACTGAGCATTGCCTTCATTGTG  180
      K  K  P  S  P  T  I  C  G  S  N  Y  P  L  S  I  A  F  I  V
 181  GTGAATGAATTCTGCGAGCGCTTTTCCTATTATGGAATGAAAGCTGTGCTGATCCTGTAT  240
      V  N  E  F  C  E  R  F  S  Y  Y  G  M  K  A  V  L  I  L  Y
 241  TTCCTGTATTTCCTGCACTGGAATGAAGATACCTCCACATCTATATACCATGCCTTCAGC  300
      F  L  Y  F  L  H  W  N  E  D  T  S  T  S  I  Y  H  A  F  S
 301  AGCCTCTGTTATTTTACTCCCATCCTGGGAGCAGCCATTGCTGACTCGTGGTTGGGAAAA  360
      S  L  C  Y  F  T  P  I  L  G  A  A  I  A  D  S  W  L  G  K
 361  TTCAAGACAATCATCTATCTCTCCTTGGTGTATGTGCTTGGCCATGTGATCAAGTCCTTG  420
      F  K  T  I  I  Y  L  S  L  V  Y  V  L  G  H  V  I  K  S  L
 421  GGTGCCTTACCAATACTGGGAGGACAAGTGGTACACACAGTCCTATCATTGATCGGCCTG  480
      G  A  L  P  I  L  G  G  Q  V  V  H  T  V  L  S  L  I  G  L
 481  AGTCTAATAGCTTTGGGGACAGGAGGCATCAAACCCTGTGTGGCAGCTTTTGGTGGAGAC  540
      S  L  I  A  L  G  T  G  G  I  K  P  C  V  A  A  F  G  G  D
 541  CAGTTTGAAGAAAAACATGCAGAGGAACGGACTAGATACTTCTCAGTCTTCTACCTGTCC  600
      Q  F  E  E  K  H  A  E  E  R  T  R  Y  F  S  V  F  Y  L  S
 601  ATCAATGCAGGGAGCTTGATTTCTACATTTATCACACCCATGCTGAGAGGAGATGTGCAA  660
      I  N  A  G  S  L  I  S  T  F  I  T  P  M  L  R  G  D  V  Q
 661  TGTTTTGGAGAAGACTGCTATGCATTGGCTTTTGGAGTTCCAGGACTGCTCATGGTAATT  720
      C  F  G  E  D  C  Y  A  L  A  F  G  V  P  G  L  L  M  V  I
 721  GCACTTGTTGTGTTTGCAATGGGAAGCAAAATATACAATAAACCACCCCCTGAAGGAAAC  780
      A  L  V  V  F  A  M  G  S  K  I  Y  N  K  P  P  P  E  G  N
 781  ATAGTGGCTCAAGTTTTCAAATGTATCTGGTTTGCTATTTCCAATCGTTTCAAGAACCGT  840
      I  V  A  Q  V  F  K  C  I  W  F  A  I  S  N  R  F  K  N  R
 841  TCTGGAGACATTCCAAAGCGACACGACTGGCTAGACTGGGCGGCTGAGAAATATCCAAAG  900
      S  G  D  I  P  K  R  H  D  W  L  D  W  A  A  E  K  Y  P  K
 901  CAGCTCATTATGGATGTAAAGGCACTGACCAGGGTACTATTCCTTTATATCCCATTGCCC  960
      Q  L  I  M  D  V  K  A  L  T  R  V  L  F  L  Y  I  P  L  P
 961  ATGTTCTGGGCTCTTTTGGATCAGCAGGGTTCACGATGGACTTTGCAAGCCATCAGGATG 1020
      M  F  W  A  L  L  D  Q  Q  G  S  R  W  T  L  Q  A  I  R  M
1021  AATAGGAATTTGGGGTTTTTTGTGCTTCAGCCGGACCAGATGCAGGTTCTAAATCCCCTT 1080
      N  R  N  L  G  F  F  V  L  Q  P  D  Q  M  Q  V  L  N  P  L
1081  CTGGTTCTTATCTTCATCCCGTTGTTTGACTTTGTCATTTATCGTCTGGTCTCCAAGTGT 1140
      L  V  L  I  F  I  P  L  F  D  F  V  I  Y  R  L  V  S  K  C
1141  GGAATTAACTTCTCATCACTTAGGAAAATGGCTGTTGGTATGATCCTAGCATGCCTGGCA 1200
      G  I  N  F  S  S  L  R  K  M  A  V  G  M  I  L  A  C  L  A
1201  TTTGCAGTTGCGGCACGTGTAGAGATAAAAATAAATGAAATGGCCCCAGCCCAGCCAGGT 1260
      F  A  V  A  A  R  V  E  I  K  I  N  E  M  A  P  A  Q  P  G
1261  CCCCAGGAGGTTTTCCTACAAGTCTTGAATCTGGCAGATGATGAGGTGAAGGTGACAGTG 1320
      P  Q  E  V  F  L  Q  V  L  N  L  A  D  D  E  V  K  V  T  V
1321  GTGGGAAATGAAAACAATTCTCTGTTGATAGAGTCCATCAAATCCTTTCAGAAAACACCA 1380
      V  G  N  E  N  N  S  L  L  I  E  S  I  K  S  F  Q  K  T  P
1381  CACTATTCCAAACTGCACCTGAAAACAAAAAGCCAGGATTTTCACTTCCACCTGAAATAT 1440
      H  Y  S  K  L  H  L  K  T  K  S  Q  D  F  H  F  H  L  K  Y
1441  CACAATTTGTCTCTCTACACTGAGCATTCTGTGCAGGAGAAGAACTGGTACAGTCTTGTC 1500
      H  N  L  S  L  Y  T  E  H  S  V  Q  E  K  N  W  Y  S  L  V
1501  ATTCGTGAAGATGGGAACAGTATCTCCAGCATGATGGTAAAGGATACAGAAAGCAGAACA 1560
      I  R  E  D  G  N  S  I  S  S  M  M  V  K  D  T  E  S  R  T
1561  ACCAATGGGATGACAACCGTGAGGTTTGTTAACACTTTGCATAAAGATGTCAACATCTCC 1620
      T  N  G  M  T  T  V  R  F  V  N  T  L  H  K  D  V  N  I  S
1621  CTGAGTACAGATACCTCTCTCAATGTTGGTGAAGACTATGGTGTGTCTGCTTATAGAACT 1680
      L  S  T  D  T  S  L  N  V  G  E  D  Y  G  V  S  A  Y  R  T
1681  GTGCAAAGAGGAGAATACCCTGCAGTGCACTGTAGAACAGAAGATAAGAACTTTTCTCTG 1740
      V  Q  R  G  E  Y  P  A  V  H  C  R  T  E  D  K  N  F  S  L
1741  AATTTGGGTCTTCTAGACTTTGGTGCAGCATATCTGTTTGTTATTACTAATAACACCAAT 1800
      N  L  G  L  L  D  F  G  A  A  Y  L  F  V  I  T  N  N  T  N
1801  CAGGGTCTTCAGGCCTGGAAGATTGAAGACATTCCAGCCAACAAAATGTCCATTCGGTGG 1860
      Q  G  L  Q  A  W  K  I  E  D  I  P  A  N  K  M  S  I  R  W
1861  CAGCTACCACAATATGCCCTGGTTACAGCTGGGGAGGTCATGTTCTCTGTCACAGGTCTT 1920
      Q  L  P  Q  Y  A  L  V  T  A  G  E  V  M  F  S  V  T  G  L
1921  GAGTTTTCTTATTCTCAGGCTCCCTCTAGCATGAAATCTGTGCTCCAGGCAGCTTGGCTA 1980
      E  F  S  Y  S  Q  A  P  S  S  M  K  S  V  L  Q  A  A  W  L
1981  TTGACAATTGCAGTTGGGAATATCATCGTGCTTGTTGTGGCACAGTTCAGTGGCCTGGTA 2040
      L  T  I  A  V  G  N  I  I  V  L  V  V  A  Q  F  S  G  L  V
2041  CAGTGGGCCGAATTCATTTTGTTTTCCTGCCTCCTGCTGGTGATCTGCCTGATCTTCTCC 2100
      Q  W  A  E  F  I  L  F  S  C  L  L  L  V  I  C  L  I  F  S
2101  ATCATGGGCTACTACTATGTTCCTGTAAAGACAGAGGATATGCGGGGTCCAGCAGATAAG 2160
      I  M  G  Y  Y  Y  V  P  V  K  T  E  D  M  R  G  P  A  D  K
2161  CACATTCCTCACATCCAGGGGAACATGATCAAACTAGAGACCAAGAAGACAAAACTCTGA 2220
      H  I  P  H  I  Q  G  N  M  I  K  L  E  T  K  K  T  K  L  *
2221  TGACTCCCTAGATTCTGTCCTAACCCCAATTCCCTGGCCCTGTCTTGAAGCATTTTTTTT 2280
2281  CTTCTACTGGATTAGACAAGAGAGATAGCAGCATATCAGAGCTGATCTCCTCCACCTTTC 2340
2341  TCCAATGACAGAAGTTCCAGGACTGGTTTTCCAGTACATCTTTAAACAAGGCCCCAGAGA 2400
2401  CTCTATGTCTGCCCGTCCATCAGTGAACTCATTAAAACTTGTGCAGTGTTGCTGGAGCTG 2460
2461  GCCTGGTGTCTCCAAATGACCATGAAAATACACACGTATAATGGAGATCATTCTCTGTGG 2520
2521  GTATGCAAAGTTATGGGAATTCCTTTATAGGTAACTGCCATTTAGGACTGATGGCCCTAA 2580
2581  TTTTTGAGGTGCTGATTTAGAGGCAAAATTGCAGAATAACAAAGAAATGGTATTTCAAGT 2640
2641  TTTTTTTTTTATAAGCAATGTAATTATGCTATTCACAGGGGCCCG                 2685
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Fig. 1. Nucleotide sequence of the human kidney H^+/peptide cotransporter (PEPT 2) cDNA and the predicted amino acid sequence. Putative transmembrane domains are underlined.

with *Eco*RI yielded four fragments, 1.85, 0.5, 0.2, and 0.15 kb in size. The digestion pattern indicated that there were three internal *Eco*RI sites in the cDNA insert and that the size of the full-length insert was approx. 2.7 kb. In order to obtain the full-length cDNA insert for subsequent subcloning and functional expression, the phage DNA was subjected to partial digestion with *Eco*RI. This procedure yielded five new fragments (2.7, 2.5, 2.35, 2.0 and 0.65 kb in size) in addition to the above-described four fragments. Attempts to subclone the 2.7 kb fragment into pBluescript SKII were not successful. However, we succeeded in subcloning the 2.5 kb fragment into this vector. The fragments arising from complete digestion with *Eco*RI were also subcloned into pBluescript SKII for nucleotide

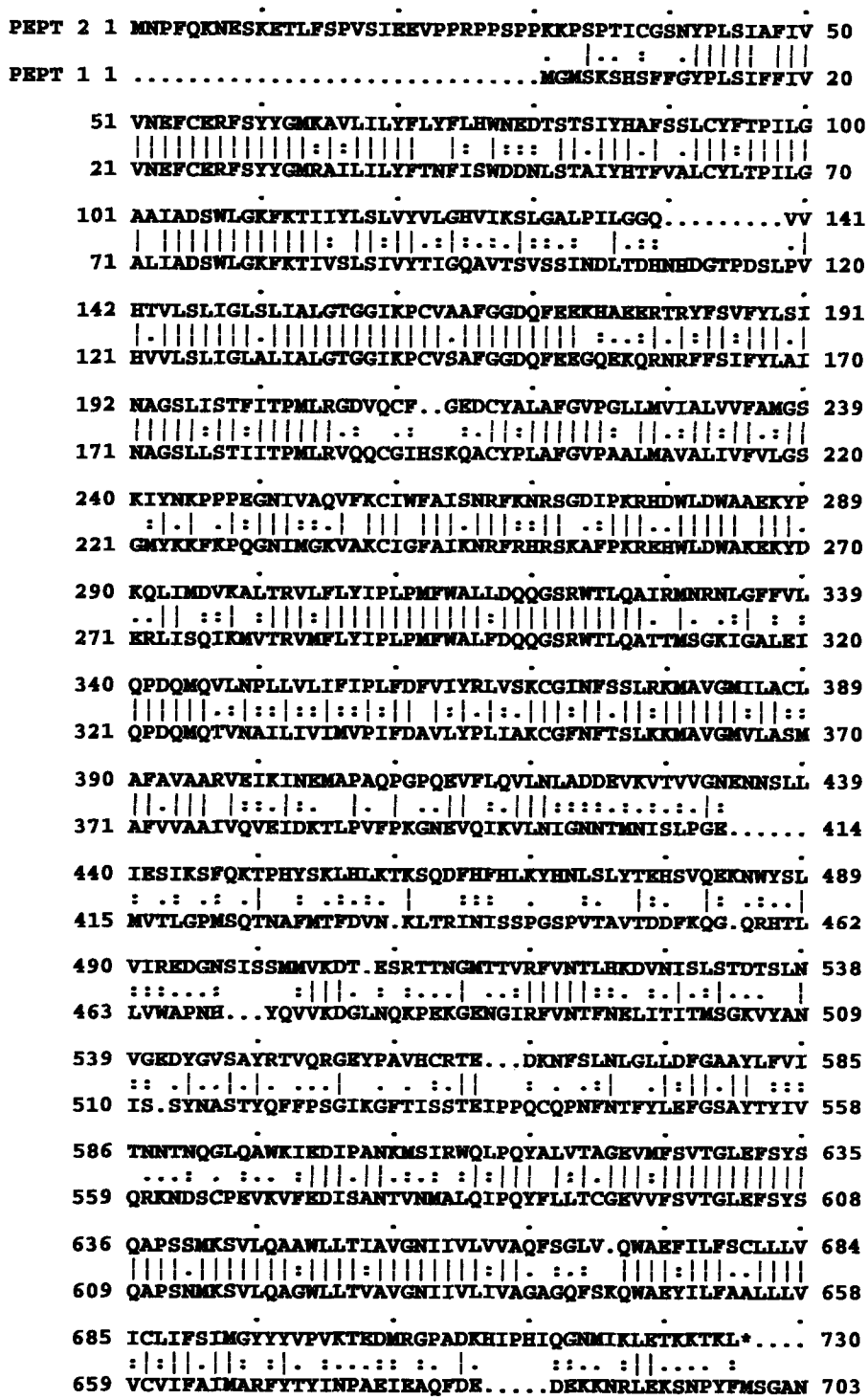

Fig. 2. Comparison of amino acid sequence between the human intestinal (PEPT 1) and kidney (PEPT 2) H^+/peptide cotransporters. Solid lines denote identical amino acids and dots denote conserved amino acid substitutions.

sequencing. Complete sequencing of these fragments and comparison of the predicted amino acid sequence with that of the human intestinal H^+/peptide transporter enabled us to determine the order of alignment of the *Eco*RI fragments in the kidney cDNA insert. The 5′ end of the insert was the 0.2 kb fragment followed by 1.85 kb, 0.5 kb, and 0.15 kb fragments. Thus, the 2.5 kb clone lacked the 5′ end 0.2 kb fragment.

We used the following strategy to obtain the 2.7 kb full-length clone. Two PCR primers, one corresponding to the 5′ end of the cDNA insert (upstream primer) with an adaptor containing the site for *Xho*I and the other corresponding to a region located approximately in the middle of the 1.85 kb fragment (downstream primer) were designed. With these primers and the phage DNA as the template, a 0.9 kb PCR product was obtained. This product and the 2.5 kb clone in pBluescript SKII were digested with *Xho*I and *Nsi*I and the full-length clone was constructed from the resultant fragments. This cDNA was under the control of the T7 promotor in the vector, suitable for expression in HeLa cells using the vaccinia virus expression system.

Sequencing was done by the dideoxy chain termination method [16]. Synthetic oligonucleotide primers were used whenever necessary to complete the sequencing of both the sense and the antisense strands. Sequence analysis was performed by the software package GCG version 7.B (Genetics Computer Groups, Madison, WI). Sequence alignment was done using the GenBank program GAP.

The cDNA was functionally expressed in HeLa cells using the vaccinia virus expression system [17] as described previously [18,19]. HeLa cells were first infected with a recombinant (VTF_{7-3}) vaccinia virus encoding T7 RNA polymerase and then transfected with the cDNA construct or empty vector. After 8–10 h following transfection, uptake of radiolabeled glycylsarcosine (30 μM), glycine (50 nM) or proline (50 nM) was measured. The uptake medium was 25 mM Mes/Tris (pH 6.0) or 25 mM Hepes/Tris (pH 8.0) containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. The incubation time for uptake measurements was 3 min.

For RT-PCR, two sets of PCR primers were designed based on the nucleotide sequences of the cloned human kidney cDNA and the human intestinal H^+/peptide cotransporter (PEPT 1) cDNA. The kidney cDNA-specific primers corresponded to nucleotide positions 900–917 and 1760–1777 of the cDNA and the PEPT 1 cDNA-specific primers corresponded to nucleotide positions 342–360 and 1575–1592 of the cDNA. The specificity of each pair of primers was established by PCR using respective cDNAs as templates. RNA samples derived from human kidney (cortex) and human small intestine (jejunum) were subjected to RT-PCR with each set of these primers and the products were analyzed by agarose gel electrophoresis.

The full-length cDNA, designated as PEPT 2, is 2685 bp long with an open reading frame of 2190 bp (including termination codon) (Fig. 1). The initiation codon is in a Kozak consensus sequence, GCCATGA [20]. The coding sequence is flanked by a 30 bp long sequence on the 5′ end and a 465 bp long sequence on the 3′ end. The cDNA is predicted to code for a protein consisting of 729 amino acids. The encoded protein is expected to have a core molecular size of 81 940 Da and an isoelectric point of 8.26. Hydropathy analysis of the amino acid sequence according to Eisenberg et al. [21], keeping the transmembrane domains to 20–23 residues/span, identifies the presence of 12 transmembrane domains, with a long hydrophilic segment ($\sim$ 165 amino acids) between the transmembrane domains 9 and 10. There are three putative *N*-glycosylation sites in this exoplasmic hydrophilic segment. There are five potential sites for protein kinase C-dependent phosphorylation, but no site for protein kinase A-dependent phosphorylation. Comparison of the amino acid sequence between the kidney clone (PEPT 2) and the human intestinal H^+/peptide cotransporter (PEPT 1) reveals significant homology, with 50% identity and 70% similarity (Fig. 2). PEPT 2 is 21 amino acids longer than PEPT 1. The extent of similarity is much higher in transmembrane domains than in the large hydrophilic loop carrying the *N*-glycosylation sites and in the amino- and carboxy-termini.

To establish the identity of PEPT 2 as a H^+/peptide cotransporter, the clone was functionally expressed in HeLa cells using the vaccinia virus expression system. The H^+/peptide cotransporter activity was measured by determining the uptake of the hydrolysis-resistant dipeptide glycylsarcosine in control cells transfected with empty vector and in cells transfected with the PEPT 2 cDNA. When this construct was transfected into HeLa cells expressing a recombinant vaccinia virus T7 RNA polymerase, the cells were able to transport the dipeptide in a H^+-dependent manner (Fig. 3). The transport in PEPT 2 cDNA-transfected cells was 4-fold higher than in cells transfected with the vector lacking the cDNA insert. The H^+-dependence of the cDNA-induced peptide transport activity was evident from the significant increase in the activity when measured at pH 6.0 instead of pH 8.0. However, the activity was unaffected by the pH change in control cells. The transport of glycylsarcosine observed in control HeLa cells was not carrier-mediated because this activity was not saturable.

We also determined the transport of glycine and proline in control and PEPT 2 cDNA-transfected HeLa cells. The radiolabel in glycylsarcosine used in transport measurements was present in the glycyl moiety as well as in the sarcosyl moiety. If the dipeptide is hydrolyzed to any significant extent during incubation with the cells, transport of the radiolabel may occur in the form of free amino acids mediated by transport systems other than the H^+/peptide cotransporter. Mammalian kidney is known to express H^+-coupled transport systems for amino acids such as glycine and proline [22,23]. Therefore, in order to

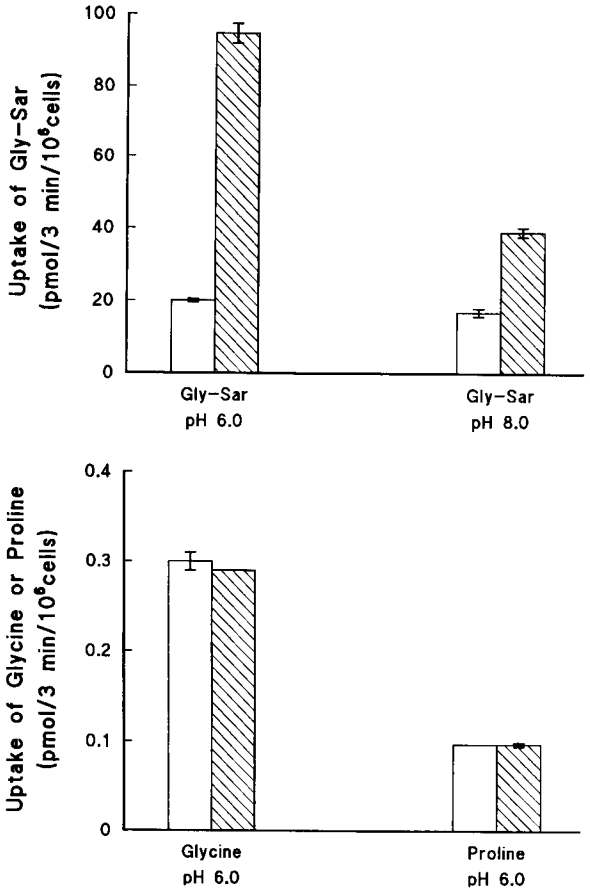

Fig. 3. Transport of glycylsarcosine, glycine, and proline in cells transfected with empty vector and in cells transfected with PEPT 2 cDNA. HeLa cells were transfected with either empty vector (open bars) or PEPT 2 cDNA (striped bars) and 8–10 h following transfection transport of radiolabeled glycylsarcosine (30 μM), glycine (50 nM), or proline (50 nM) was measured at pH 6.0 or 8.0 using a 3 min incubation period. Values are means ± S.E.

Table 1
Substrate specificity of PEPT 2

Inhibitor	[^{14}C]Gly-Sar transport	
	pmol/10^6 cells per 3 min	%
None	52.6 ± 2.5	100
Glycine	48.3 ± 2.0	92
Sarcosine	46.3 ± 3.0	88
Gly-Sar	0.8 ± 0.2	1
Gly-Phe	0	0
Ala-Gly-Pro	0	0
Cyclacillin	0.7 ± 0.0	1
Cephadroxil	0	0
Cephalexin	0	0

HeLa cells were transfected with either the empty vector or PEPT 2 cDNA and 8–10 h following transfection, transport of [^{14}C]glycylsarcosine (30 μM) was measured at pH 6.0 using a 3 min incubation. Concentration of unlabeled amino acids, peptides, and β-lactam antibiotics was 10 mM. Data represent PEPT 2 cDNA-induced transport, calculated by subtracting the transport in vector-transfected cells from the transport in PEPT 2 cDNA-transfected cells. In the absence of unlabeled compounds, the transport in vector-transfected cells represented 25 ± 3% of the transport in PEPT 2 cDNA-transfected cells. Values are means ± S.E.

establish unequivocally that the cloned kidney cDNA is indeed a H^+/peptide cotransporter, it was necessary to compare the transport of glycine and proline in control and PEPT 2 cDNA-transfected cells. The results given in Fig. 3 show that there was no difference in the transport activities of these two amino acids in control cells versus cDNA-transfected cells, clearly establishing the identity of PEPT 2 as a H^+-coupled peptide transporter.

That the kidney clone represents a H^+/peptide cotransporter is also supported by the substrate specificity of the induced transport activity (Table 1). Transport of radiolabel from [^{14}C]glycylsarcosine (30 μM) induced by PEPT 1 cDNA (i.e., transport in PEPT 1 cDNA-transfected cells minus transport in cells transfected with empty vector) was completely inhibitable by excess (10 mM) unlabeled dipeptides, tripeptides, and β-lactam antibiotics. Free amino acids had minimal effect on the transport of the radiolabel.

Mammalian kidney and small intestine are the primary tissues in which H^+-coupled peptide transport activity is expressed. Northern analysis of mRNA derived from human kidney and intestine with PEPT 2 cDNA as the probe failed to detect hybridizable transcripts in these tissues. Therefore, we used RT-PCR to determine the expression of the PEPT 2 mRNA in the human kidney and small intestine. RNA isolated from these tissues was subjected to RT-PCR using primers which were specific for PEPT 2 and the products were analyzed. RNA from human kidney produced a PCR product of expected size (~ 0.9 kb) (Fig. 4). In contrast, RNA from human intestine failed to generate this product. These results show that PEPT 2 is specifically expressed in the kidney. Small intestine, a major site for peptide transport, does not express PEPT 2. The failure to detect hybridizing signals in the kidney RNA samples is

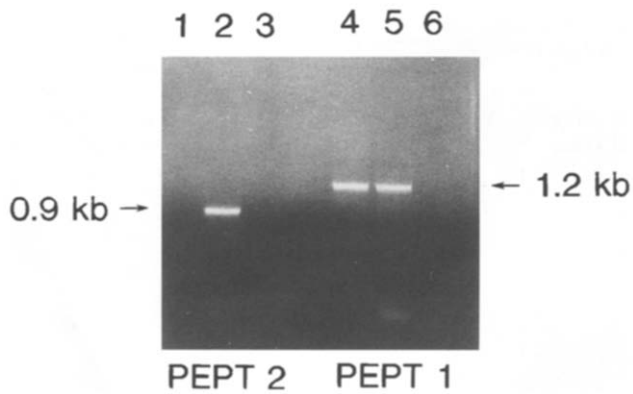

Fig. 4. Differential expression of PEPT 1 and PEPT 2 in human intestine and kidney. RNA from human intestine (lanes 1 and 4) and human kidney (lanes 2 and 5) was subjected to RT-PCR using primer pairs specific for either PEPT 1 (lanes 4–6) or PEPT 2 (lanes 1–3). Lanes 3 and 6 are negative controls run in parallel in the absence of RNA. The RT-PCR products were analyzed by agarose gel electrophoresis.

most likely due to low abundance of the transcripts. Northern analysis with PEPT 1 cDNA as the probe has indicated previously that, in contrast to PEPT 2, the PEPT 1 transcripts are present in the small intestine as well as kidney [15]. In the present study, we investigated the expression of PEPT 1 transcripts in these two tissues by RT-PCR using primers specific for PEPT 1. RNA from both tissues produced PCR products of expected size ($\sim$ 1.2 kb) (Fig. 4). It is clear from these experiments that while PEPT 1 is expressed in both intestine and kidney, PEPT 2 is expressed only in kidney.

Studies on peptide transport mechanisms in the kidney are of importance for physiologic and pharmacologic reasons. The transport system plays a significant role in the reabsorption of peptides from the tubular filtrate. These peptides are those present in the circulation as well as those which are generated in the tubular lumen as a result of hydrolysis of larger peptides by brush border peptidases [24–26]. In addition, peptide-based formulas are being considered in place of elemental solutions containing free amino acids for parenteral nutrition [27,28]. The ability of the kidneys to reabsorb and hydrolyze these peptides may become a key determinant in the utility and efficacy of such solutions in clinical practice. Moreover, pharmacologically active peptidomimetic drugs such as aminocephalosporins are good substrates for the renal peptide transport system. Therefore, the half-life of these drugs in circulation and hence their therapeutic efficacy are influenced by the ability of the kidneys to reabsorb them and thus prevent their elimination in the urine. Successful cloning of the kidney-specific PEPT 2 cDNA makes an important contribution to a better understanding of the transport of peptides in the kidney at the molecular level.

The authors thank Ida O. Thomas for expert secretarial assistance. This work was supported by National Institutes of Health Grant DK 28389 and DK 42069.

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