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Tissue Distribution of a Peptide Transporter mRNA in Sheep, Dairy Cows, Pigs, and Chickens¹

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ABSTRACT: A 446-bp cDNA fragment encoding a peptide transport protein was cloned from sheep omasum and used as a probe to study the distribution of the peptide transport protein mRNA in various tissues of sheep, dairy cows, pigs, and chickens. Because the predicted amino acid sequence of this fragment was 85.8, 90.5, and 90.5% identical to rabbit, human, and rat intestinal peptide transporter (PepT1), respectively, it is believed that this cloned fragment represents PepT1 from sheep. In sheep (n = 5) and lactating Holstein cows (n = 3), hybridization was observed with mRNA from the omasum, rumen, duodenum, jejunum, and ileum. The estimated size of mRNA was 2.8 kb. No hybridization was observed with mRNA from the abomasum, cecum, colon, liver, kidney, and semitendinosus and longissimus muscles of either species or the mammary gland of the dairy cows. In pigs (n = 6), the probe hybridized with

mRNA from the duodenum, jejunum, and ileum. There was no hybridization with mRNA from the stomach, large intestine, liver, kidney, and semitendinosus and longissimus muscles. Two bands, 3.5 and 2.9 kb, were observed with northern blot analysis, indicating two RNA transcripts that may result from alternative mRNA processing. In White Leghorns (n = 15) and broilers (n = 20), the strongest hybridization was found in the duodenum, but the jejunum and ileum showed faint bands. The size of mRNA in chickens was 1.9 kb. Other tissues, including the crop, proventriculus, gizzard, ceca, liver, kidney, and muscles showed no hybridization to the probe. In conclusion, mRNA for PepT1 is present in the small intestine of all animals examined and the omasal and ruminal epithelium of sheep and dairy cows. The size of the mRNA varied among species.

Key Words: Peptides, Tissues, Messenger RNA, Intestines

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Introduction

Existence of a transport process for intact peptides through mammalian intestinal and renal epithelial membranes has been recognized for several years, and the importance of peptide transport has been well documented (Matthews, 1991; Leibach and Ganapathy, 1996). Only recently has the presence of peptide transporters been identified in mammals (Fei et al., 1994; Liang et al., 1995; Miyamoto et al., 1996). The identified mammalian peptide transporters are divided into two major groups, PepT1 and PepT2. The

predicted protein structure of these peptide transporters has 12 transmembrane domains and a very large extracellular loop between transmembrane domains IX and X (Fei et al., 1994; Meredith and Boyd, 1995).

The amino acid sequences of the 12 transmembrane domains are highly conserved, but the sequences of the extracellular loops are less conserved (Daniel, 1996). These peptide transporters appear to be able to transport most di- and tripeptide substrates, including some peptidomimetics (Amasheh et al., 1997; Steel et al., 1997). The PepT1 is mainly expressed in small intestine, and PepT2 is mainly expressed in kidney (Fei et al., 1994; Liang et al., 1995; Miyamoto et al., 1996; Saito et al., 1996). Our studies indicate that a peptide transporter(s) is present in forestomach of sheep (Matthews et al., 1996; Pan et al., 1997). Several cultured cell lines and mammary tissue explants have the ability to utilize exogenous methionine-containing peptides as sources for their methionine needs (Pan et al., 1996; Wang et al., 1996; Pan and Webb, 1998).

Therefore, our goal was to test the hypothesis that a peptide transporter(s) also exists in tissues of com-

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monly raised farm animals. The objectives of the present study were to verify the existence of the peptide transporter mRNA in the sheep forestomach and to survey the distribution of the peptide transporter mRNA in other tissues in sheep, dairy cows, pigs, and chickens.

Materials and Methods

Animals and Tissue Preparation. The protocols followed were reviewed and approved by the Virginia Tech Animal Care Committee. A total of five yearling crossbred sheep, three primiparous lactating Holstein cows, six crossbred pigs (8 wk of age), one group of broilers ($n = 20$, 5 wk of age), and two groups of White Leghorns ($n = 7$ and $n = 8$, 14 wk of age) were used in the experiment. The sheep (average BW 62.8 kg) were stunned with a captive bolt pistol and killed by exsanguination. The rumen and omasum were removed and washed with ice-cold .9% saline. Ruminal epithelium was peeled from underlying and connective tissues. Individual omasal plies were removed. The fundic region of the abomasum and intestinal sections, including the duodenum, jejunum, ileum, cecum, and colon, were washed with ice-cold .9% saline, and the epithelium was scraped using a glass slide. The duodenal tissue collected was the first 1 m of small intestine beginning at the pyloric sphincter. The ileal tissue was the 1 m distal portion of the small intestine that ended at the ileocecolic junction. Approximately 1 m from the middle of the small intestine was taken as the jejunal tissue. The colon and cecum were taken starting from the ileocecolic junction. Samples of liver, kidney, and semitendinosus and longissimus muscle were also removed. All of the tissues and scrapings were quickly frozen in liquid N_2 and later stored at $-80^\circ C$. The dairy cows (average 382 d in milk and 31.9 kg/d of milk yield) were killed following the same procedure used with the sheep. Stomach, intestinal sections, liver, kidney, and semitendinosus and longissimus muscles were collected and stored as described for the sheep. In addition, mammary tissue from the right rear quarter was collected and stored. The pigs (average BW 18.0 kg) were killed by i.v. injection of sodium pentobarbital. The stomach and intestinal sections, including the duodenum, jejunum, ileum, cecum, and colon, were collected and processed as described for the sheep. Likewise, liver, kidney, and semitendinosus and longissimus muscles were quickly removed and sampled. Broilers (average BW 1.63 kg) and White Leghorns (average BW 1.96 kg) were killed by cervical dislocation. The gastrointestinal tract, liver, kidney, and samples of fibularis longus and pectoralis muscles were quickly removed. Gastrointestinal segments were cleaned by rinsing with ice-cold .9% saline. The epithelial lining was peeled off from the crop, proventriculus, and gizzard and saved. The proximal enlarged loop of the small intestine was

taken as the duodenum. The rest of the small intestine was divided by Meckel's diverticulum into upper and lower portions and corresponded to the jejunum and ileum. The ceca were also sampled. The epithelium from the intestinal segments was scraped using a glass slide. All the collected tissues and scrapings were quickly frozen in liquid N_2 and stored at $-80^\circ C$. Prior to the extraction of total RNA, composites of individual tissues were formed. Tissues from the 20 broilers were combined to make one group. The tissues from the White Leghorns were combined to produce two samples; one contained tissues from seven and the other contained tissues from eight birds.

Preparation of Total RNA and Poly(A)⁺ RNA. Total RNA was extracted from the tissues using the method described by Puissant and Houdebine (1990) and modified by Matthews et al. (1996) and Pan et al. (1997). Total RNA was dissolved in diethyl pyrocarbonate (DEPC)-treated water. The absorbance was measured at 260 and 280 nm to determine RNA content and purity. The integrity of total RNA was checked indirectly by examining the integrity of ribosomal RNA after RNA electrophoresis and ethidium bromide staining.

Poly(A)⁺ RNA was isolated from total RNA using oligo(dT) cellulose (Sambrook et al., 1989). Approximately 150 mg of oligo(dT) cellulose was used to make one column, with a volume of approximately 1 mL. The column was regenerated by washing with .1 *N* NaOH and then neutralized to less than pH 8.0 with column loading buffer containing 20 mM Tris-HCl (pH 7.6), 1 mM EDTA, .5 *M* NaCl, and .1% *N*-laurylsarcosinate. Typically, 4 to 5 mg of total RNA was loaded per column. After washing with column loading buffer to eliminate nonspecific binding, the column was eluted with 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA. The poly(A)⁺ RNA was precipitated with .1 volume of 3 *M* sodium acetate (pH 5.0) and 1 volume of isopropanol at $-20^\circ C$ overnight. The precipitate was centrifuged at $12,000 \times g$ for 20 min, and the poly(A)⁺ RNA was recovered by dissolving the pellet in DEPC-treated water and stored at $-80^\circ C$ for future use.

Cloning of a Partial cDNA Encoding Ovine PepT1. Primers (IDT, Coraville, IA) for reverse transcription coupled polymerase chain reaction (RT-PCR) were designed based on a comparison of published nucleic acid sequences of rabbit, human, and rat PepT1. The following primer for RT was used: 5' TTA GCC CAG TCC AGC CAG AG 3' (nt 808–827 in rabbit PepT1). The two primers for PCR were as follows: 5' TGG CTG GG(G/A) AAG TTC AAG AC 3' (nt 259–278 in rabbit PepT1) and 5' CTT CTT GTA CAT (C/T)CC ACT GC 3' (nt 686–705 in rabbit PepT1). A 446-bp fragment was obtained from sheep omasal epithelial total RNA by RT-PCR using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Life Technologies, Gaithersburg, MD) and *Taq* DNA polymerase (Promega, Madison, WI). The PCR was performed for

35 cycles of 94°C for 1.5 min, 57°C for 2 min, and 72°C for 1.5 min. The fragment was cloned into plasmid pTZ18R (Pharmacia Biotech, Piscataway, NJ) and sequenced with the dideoxy chain-termination method using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, OH). The confirmed fragment (446 bp) was used as a probe for northern blot analysis.

Northern Blot Analysis. Ten micrograms of poly(A)⁺ RNA from each tissue was electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde and stained with ethidium bromide. The RNA was transferred to a nylon membrane, and blots were hybridized and washed at medium stringency for chickens and high stringency for other animals according to the manufacturer's protocol (MSI, Westboro, MA). The probe was the sheep PepT1 cDNA fragment purified by Glassmilk (Bio101, La Jolla, CA) and labeled with [α -³²P]dATP (ICN Pharmaceuticals, Costa Mesa, CA) by nick translation. The blots were then exposed to Kodak XAR-5 film with intensifying screens at -80°C.

The size of the mRNA bands was determined relative to an RNA marker (.24 to 9.5 kb; Life Technologies). The bands on northern blots were quantified using a laser densitometer (Molecular Dynamics, Mitsubishi, Japan). Arbitrary densitometric units (ADU) were determined for each band. Because the northern blots were conducted for every tissue of a given animal on the same blot, a relative comparison of mRNA abundance among tissues could be determined. The relative densitometric unit (RDU) for the jejunum of a given animal was set as 1.0, and RDU of other tissues of the same animal were calculated by comparison of the ADU with the ADU for the jejunum.

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GG CTG GGG AAG TTC AAG ACG ATC GTG TCG CTG TCC ATC GTC TAC ACC ATT
   L  G  K  F  K  T  I  V  S  L  S  I  V  Y  T  I
GGG CAG GTA CTC ATC GCT GTG AGC TCA ATT AAT GAC CTC ACT GAC TTC AAC
 G  Q  V  V  I  A  V  S  S  I  N  D  L  T  D  F  N
CAT  GAT GGA ACC CCA AAC AAT ATT TCT GTG CAC GTG GCG CTC TCC ATG ATT
 H  D  G  T  P  N  N  I  S  V  H  V  A  L  S  M  I
GGC CTG GTC CTG ATC GCT CTG GGT ACC GGA GGG ATA AAG CCT TGC GTG TCT
 G  L  V  L  I  A  L  G  T  G  G  I  K  P  C  V  S
GCA TTT GGC GGA GAT CAG TTT GAA GAG GGC CAG GAA AAG CAA AGG AAC AGA
 A  F  G  G  D  Q  F  E  E  G  Q  E  K  Q  R  N  R
TTT TTT TCC ATC TTT TAT TTG GCC ATT AAT GCT GGA AGT TTG CTT TCT ACT
 F  F  S  I  F  Y  L  A  I  N  A  G  S  L  L  S  T
ATC ATC ACC CCC ATG CTC AGA GTT CAG GTA TGC GGA ATT CAC AGT AAG CAA
 I  I  T  P  M  L  R  V  Q  V  C  G  I  H  S  K  Q
GCT TGT TAC CCC CTG GCC TTT GGG GTT CCT GCT GCA CTC ATG GCT GTA TCT
 A  C  Y  P  L  A  F  G  V  P  A  A  L  M  A  V  S
CTG ATC GTG TTT GTC ATT GGC AGT GGA ATG TAC AAG AAG
 L  I  V  F  V  I  G  S  G  M  Y  K  K

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Figure 1. Nucleic acid and amino acid sequences of the cDNA fragment (446 bp) cloned from sheep omasal epithelium.

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Sheep  LGKFKTIVSLSIVYTIGQVVIAVSSINDLTDFNHGDT
Rabbit -----W-----A-TSL--V-E--N-----
Human  -----A-TS-----H-----
Rat    -----A--S-----HD---S

Sheep  PNNISVDVALSMIGLVLIALGTGGIKPCVSAFGGDQF
Rabbit -DSL-P---VC---L-----
Human  -DSL-P---V--L--A-----
Rat    ---LPL-----A-----

Sheep  EEGQEKQRNRF-FSIFYLAINAGSLLSTIITPMLRVQV
Rabbit -----V---Q
Human  -----Q
Rat    -----I---Q

Sheep  CGIHSKQACYPLA-FGVPAALMAVSLIVFVIGSGMYKK
Rabbit ---V-----I--I-----I-----
Human  -----A--L-----
Rat    ---Q-----A--L-----

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Figure 2. Comparison of the amino acid sequence of the sheep cDNA fragment of peptide transporter with other published sequences (rabbit, human, and rat PepT1). Dashes indicate the same amino acid as sheep. Underlined sequences indicate transmembrane domains.

Results

Cloning of a Partial cDNA Encoding a Sheep Peptide Transporter. Based on our previous studies, a peptide transport protein(s) capable of transporting peptides up to four amino acids in length is present in sheep omasal epithelium (Matthews et al., 1996; Pan et al., 1997). Therefore, sheep omasal epithelium mRNA was used as the template for RT-PCR to develop a probe for northern blot analysis. The published sequences of rabbit, human, and rat PepT1 (Fei et al., 1994; Liang et al., 1995; Miyamoto et al., 1996) were used to design oligonucleotides to a highly homologous region (from transmembrane domain III to VI). After RT-PCR amplification, a 446-bp fragment from sheep omasal epithelium was cloned and sequenced (Figure 1). The predicted amino acid sequence of this fragment was 85.8, 90.5, and 90.5% identical to rabbit, human, and rat PepT1, respectively (Figure 2). The fragment has low similarity to any known PepT2 sequences (data not shown). Therefore, the cloned fragment likely represents sheep PepT1 and is suitable for use as a probe to detect PepT1 mRNA.

Tissue Distribution of Peptide Transporter mRNA. For sheep and dairy cows, mRNA from the rumen, omasum, and small intestine showed positive hybridization in northern blot analysis (Figures 3 and 4). The size of the mRNA that hybridized to our probe was 2.8 kb for the sheep and dairy cows. From the summary of the northern blot analysis for the five sheep and three cows tested, the pattern of distribution seemed to be consistent within the same species, although the relative abundance varied among animals (Table 1). Among these tissues, the abundance of PepT1 was higher in the jejunum and ileum

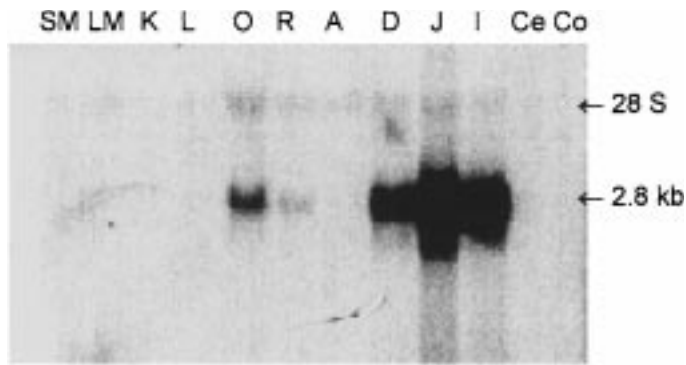


Figure 3. Tissue distribution of peptide transporter mRNA in sheep. SM = semitendinosus muscle; LM = longissimus muscle; K = kidney; L = liver; O = omasum; R = rumen; A = abomasum; D = duodenum; J = jejunum; I = ileum; Ce = cecum; and Co = colon. The 28 S indicates position of 28 S ribosomal RNA.

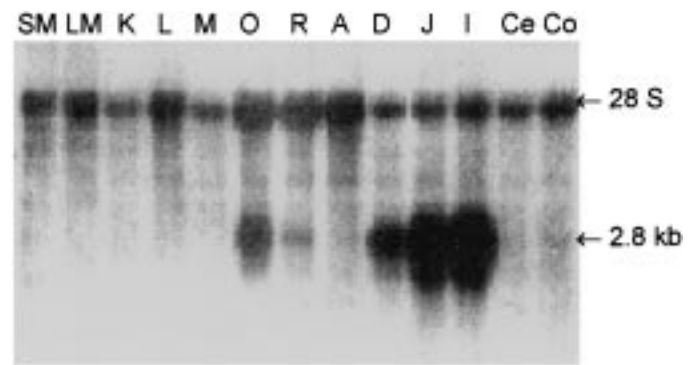


Figure 4. Tissue distribution of the peptide transporter mRNA in dairy cows. SM = semitendinosus muscle; LM = longissimus muscle; K = kidney; L = liver; M = mammary gland; O = omasum; R = rumen; A = abomasum; D = duodenum; J = jejunum; I = ileum; Ce = cecum; and Co = colon. The 28 S indicates position of 28 S ribosomal RNA.

than in the omasum and duodenum. The mRNA from the rumen showed only minimal hybridization, indicating that PepT1 was present in low abundance. PepT1 was not detectable in the abomasum, liver, kidney, cecum, colon, longissimus and semitendinosus muscles, or mammary gland in dairy cows. Hybridization of the probe to 28 S ribosomal RNA in some tissues was likely the result of nonspecific hybridization.

In pigs, PepT1 mRNA was found only in the small intestine (Figure 5). Interestingly, there were two bands, 2.9 and 3.5 kb, found in the northern blots of swine small intestine. With the exception of one pig, the relative distribution of PepT1 mRNA among the intestinal segments was remarkably consistent among pigs (Table 2). Abundance of PepT1 mRNA in five of the six pigs was greatest in the jejunum, followed by the duodenum. One pig showed the highest PepT1 mRNA abundance in the duodenum. The PepT1 abundance was lowest in the ileum of all pigs.

In chickens, only a mRNA of 1.9 kb from the small intestinal region showed hybridization with our sheep PepT1 probe (Figure 6). Abundance of PepT1 mRNA was greatest in the duodenum, and it was approximately 33 and 25% in jejunum and ileum, respectively, of that in the duodenum (Table 3). No PepT1 mRNA was detected in the preintestinal regions of the gastrointestinal tract, including the crop, proventriculus, and gizzard, as well as the ceca, liver, kidney, or fibularis longus and pectoralis muscles.

Discussion

The recent cloning and characterization of intestinal (PepT1) and renal (PepT2) peptide transporters from different species has provided strong evidence of the importance of the peptide transport system to

Table 1. Densitometric analysis of northern blots from sheep and dairy cows

Animal	Tissue (RDU) ^a												
	SM ^b	LM	K	L	M	O	R	A	D	J	I	Ce	Co
Sheep 1	— ^c	—	—	—	—	—	—	—	.35	1.0	1.96	—	—
Sheep 2	—	—	—	—	—	.26	.42	—	.29	1.0	8.60	—	—
Sheep 3	—	—	—	—	—	.17	.07	—	.22	1.0	.81	—	—
Sheep 4	—	—	—	—	—	.72	.14	—	.34	1.0	18.96	—	—
Sheep 5	—	—	—	—	—	.06	.02	—	.22	1.0	.52	—	—
Cow 1	—	—	—	—	—	.02	.02	—	.21	1.0	.69	—	—
Cow 2	—	—	—	—	—	2.47	1.17	—	.71	1.0	6.18	—	—
Cow 3	—	—	—	—	—	.35	.17	—	.28	1.0	1.18	—	—

^aRelative densitometric units (RDU) for the jejunum was set as 1.0, and RDU for other tissues were calculated by comparing the arbitrary densitometric units (ADU) to the ADU for the jejunum for a given animal.

^bSM = semitendinosus muscle; LM = longissimus muscle; K = kidney; L = liver; M = mammary gland; O = omasum; R = rumen; A = abomasum; D = duodenum; J = jejunum; I = ileum; Ce = cecum; Co = colon.

^cThe tissues showing no hybridization are indicated with dashes.

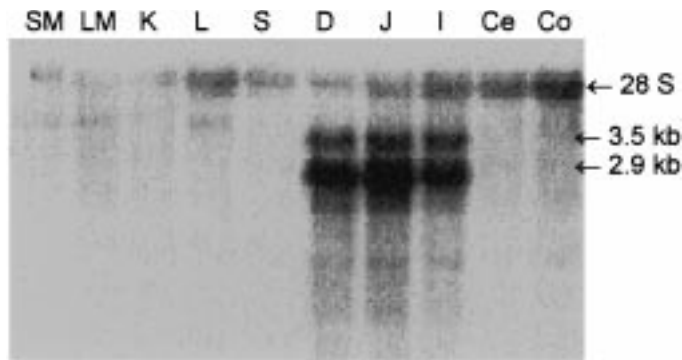


Figure 5. Tissue distribution of peptide transporter mRNA in pigs. SM = semitendinosus muscle; LM = longissimus muscle; K = kidney; L = liver; S = stomach; D = duodenum; J = jejunum; I = ileum; Ce = cecum; and Co = colon. The 28 S indicates position of 28 S ribosomal RNA.

mammalian species. The unique structural and functional features shared by these peptide transport proteins have prompted considerable interest in examining these systems.

In the present experiments, a 2.8-kb mRNA from the small intestine of sheep and dairy cows, 3.5- and 2.9-kb mRNA from swine small intestine, and a 1.9-kb mRNA from chicken small intestine hybridized to our ovine PepT1 probe in northern blots. Because our sheep PepT1 cDNA probe has low sequence similarity to PepT2 and did not hybridize to mRNA from kidney, we conclude that the probe detected only PepT1 mRNA in these species.

The sizes of the sheep, dairy cow, and one of the pig mRNA (2.8 to 2.9 kb) were comparable to the sizes of rabbit (2.9 kb; Fei, et al., 1994) and rat (2.9 kb; Miyamoto et al., 1996) PepT1 mRNA. The other pig PepT1 mRNA was 3.5 kb, which is comparable to the size of human PepT1 mRNA (3.3 kb; Liang et al.,

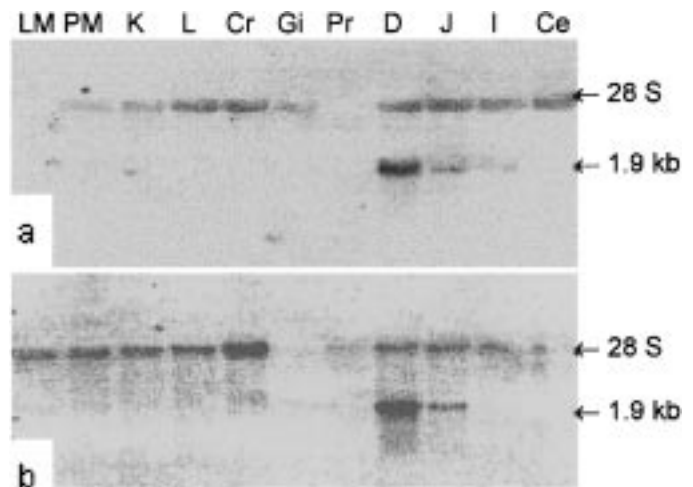


Figure 6. Tissue distribution of peptide transporter mRNA in chickens. LM = fibularis longus muscle; PM = pectoralis muscle; K = kidney; L = liver; Cr = crop; Gi = gizzard; Pr = proventriculus; D = duodenum; J = jejunum; I = ileum; and Ce = cecum. (a) Northern blot results from broilers; (b) northern blot results from White Leghorns. The 28 S indicates position of 28 S ribosomal RNA.

1995). The two sizes of mRNA transcripts detected in pigs may be the result of alternative RNA splicing, or there may be two mRNA that are translated into two peptide transporter proteins. Chicken PepT1 mRNA (1.9 kb) was significantly smaller than the mammalian PepT1 mRNA. This probably indicates that the chicken PepT1 protein in the small intestine is quite small. It may be comparable in size to the recently cloned rat brain peptide transporter (PHT1), which contains only 572 amino acid residues (Yamashita et al., 1997). This much smaller protein lacks the large extracellular loop predicted for the other peptide transporters reported. The lack of the extracellular

Table 2. Densitometric analysis of northern blots from pigs

Animal	Tissue (RDU) ^a									
	SM ^b	LM	K	L	S	D	J	I	Ce	Co
Pig 1	— ^c	—	—	—	—	.69 ^d	1.0	.42	—	—
Pig 2	—	—	—	—	—	2.90	1.0	.34	—	—
Pig 3	—	—	—	—	—	.65	1.0	.34	—	—
Pig 4	—	—	—	—	—	.62	1.0	.34	—	—
Pig 5	—	—	—	—	—	.54	1.0	.51	—	—
Pig 6	—	—	—	—	—	.54	1.0	.32	—	—

^aRelative densitometric units (RDU) for the jejunum was set as 1.0, and RDU for other tissues were calculated by normalizing the arbitrary densitometric units (ADU) to the ADU for the jejunum for a given animal.

^bSM = semitendinosus muscle; LM = longissimus muscle; K = kidney; L = liver; S = stomach; D = duodenum; J = jejunum; I = ileum; Ce = cecum; Co = colon.

^cThe tissues showing no hybridization are indicated with dashes.

^dThe 2.9-kb bands were scanned with the densitometer, and the ADU and corresponding RDU were calculated.

Table 3. Densitometric analysis of northern blots from chickens

Animal ^b	Tissue (ADU) ^a										
	LM ^c	PM	K	L	Cr	Gi	Pr	D	J	I	Ce
Broilers	— ^d	—	—	—	—	—	—	2.28	1.0	.60	—
Leghorns 1	—	—	—	—	—	—	—	3.98	1.0	.64	—
Leghorns 2	—	—	—	—	—	—	—	3.12	1.0	.94	—

^aRelative densitometric units (RDU) for the jejunum was set as 1.0, and RDU for other tissues were calculated by normalizing the arbitrary densitometric units (ADU) to the ADU for jejunum for a given animal.

^bChickens were tested as groups. The group of broilers contained 20 broilers. White Leghorns were divided into two groups. Group 1 contained seven White Leghorns and Group 2 contained eight.

^cLM = fibularis longus muscle; PM = pectoralis muscle; K = kidney; L = liver; Cr = crop; Gi = gizzard; Pr = proventriculus; D = duodenum; J = jejunum; I = ileum; Ce = cecum.

^dThe tissues showing no hybridization are indicated with dashes.

loop seems to have no effect on the absorptive function of the protein. It will be interesting to further investigate the protein encoded by the 1.9-kb mRNA.

The distribution of PepT1 mRNA along the small intestine was not the same among species. The duodenum, jejunum, and jejunum and ileum seemed to be the primary sites of PepT1 mRNA abundance in chickens, pigs, and ruminants, respectively. Assuming that the relative presence of mRNA is indicative of expression of the transport protein, we conclude that the extent of peptide transport is also variable along the length of the small intestine. The differences among species may reflect the site of protein digestion and the availability of peptide substrates. The major site of amino acid absorption is recognized to be the jejunum in laboratory animals such as rats (Baker and George, 1971) and the ileum in sheep (Phillips et al., 1976, 1979) and cattle (Wilson and Webb, 1990).

In addition to the intestine, the rumen and omasum are sites of considerable absorptive activity in ruminants. Our experiments have provided evidence that intact dipeptides can be transferred across the omasal and ruminal epithelial tissues (Matthews and Webb, 1995). Expression of peptide transport capability by *Xenopus laevis* oocytes injected with mRNA from sheep omasal epithelium provided further evidence that peptide transport occurs in the ruminant stomach (Matthews et al., 1996; Pan et al., 1997). Results from the present study indicate that the ruminal and omasal epithelia of sheep and dairy cows have a 2.8-kb mRNA that encodes for PepT1. The hybridization of the probe with the mRNA from the omasal epithelium was much stronger than with mRNA from the ruminal epithelia in sheep and dairy cows. Matthews et al. (1996) also reported higher absorptive activity of substrate peptides in the omasum than in the rumen. Both of these areas of the ruminant stomach are recognized for their capabilities to absorb large quantities of VFA. Prior to the results of research from our laboratory indicating that peptides may be absorbed from the rumen and omasum, ammonia was the only end product of protein digestion recognized as being absorbed to any significant extent from the ruminant stomach.

Reports from others have shown the presence of mRNA for PepT1 at very low levels in the liver and kidney of the rabbit and human (Fei et al., 1994; Liang et al., 1995). Miyamoto et al. (1996) also reported detectable bands of mRNA from kidney in the rat, but they did not detect any hybridization to their probe with liver mRNA. In the present study, we were unable to detect any hybridization on northern blots to our probe with mRNA in the liver or kidney in any of the animals we examined. That our observations differed from previous reports could be due to the different probe we used. Our probe was only a partial cDNA to PepT1. Lower expression of the peptide transporter mRNA in the liver and kidney may not have been detectable in our system due to reduced sensitivity of the partial cDNA probe. Alternatively, our results may indicate a different pattern of expression for the peptide transporter mRNA in these commonly raised farm animals. Saito et al. (1995) reported their observations on the tissue distribution of PepT1 in the rat. In their northern blot analysis using the full-length rat PepT1 cDNA as the probe, the 2.9-kb mRNA was detected only in the small intestine, as in our studies.

In previous studies from our laboratory, MAC-T and C₂C₁₂ (Pan et al., 1996), ovine myogenic satellite cells (Pan and Webb, 1998), and cultured mouse mammary explants (Wang et al., 1996) were studied for their ability to utilize exogenous methionine-containing peptides as methionine sources. The results showed that all of these cells and the explants had the ability to utilize exogenous methionine-containing peptides as sources of methionine for protein synthesis. With the cultured cells and explants, there was evidence that transport of the peptide into the cells was occurring, indicating the possible presence of one or more peptide transport proteins. In the present study, when we screened for the presence of PepT1 mRNA in muscle and mammary gland tissues, we did not detect any in these tissues. One explanation for these results may be that the methionine-containing peptides previously observed to be utilized by cultured cells and explants enter cells via mechanisms other

than peptide transporters. Also, there may be a peptide transporter(s) present other than the one that will hybridize with our PepT1 probe. The PepT1 probe likely did not allow us the ability to detect the existence of all peptide transporter mRNA that have important physiological functions. To date, no detectable PepT1 mRNA in skeletal muscle has been reported in any animal examined (Fei et al., 1994; Liang et al., 1995; Miyamoto et al., 1996). The present study provides the only examination of the mammary gland of which we are aware.

In conclusion, a cDNA probe for the peptide transport protein PepT1 was developed from sheep omasal epithelium. The PepT1 mRNA was detected in the small intestinal region in all the animals examined and in omasal and ruminal epithelium of sheep and dairy cows. The size of the mRNA varied among species. It was 2.8 kb in sheep and dairy cows; two bands, 3.5 and 2.9 kb, were detected in pigs; and the mRNA in chickens was only 1.9 kb. The existence of mRNA transcript(s) is strong evidence for the presence of a peptide transporter protein(s). Thus, peptide absorption from the gastrointestinal tract seems to be a physiologically relevant process in all of the species examined.

Implications

The present study provides information on the general tissue distribution of the mRNA of a peptide transporter in several farm animals. These results will contribute to the cloning and further characterization of the peptide transporter(s) in these animals. The application of molecular techniques to the study of mechanisms of the peptide transport system in these farm animals will reveal the nutritional significance of this biological process. The knowledge gained will lead to better management of the nutritional needs of these animals and minimization of environmental contamination by wasted nitrogen.

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