INTRODUCTION

The transport of amino acids is mediated by membrane-bound transporters of free amino acids or small peptides. The proton-coupled oligopeptide transporter family includes peptide transporters 1 and 2 (PepT1 and PepT2) and peptide/histidine transporter 1 (PHT1) are all members of the proton-coupled oligopeptide transporter family, which are important for the transport of amino acids in peptide form. The PepT1 acts as a low-affinity/high-capacity transporter and PepT2 as a high-affinity/low-capacity transporter for di- and tripeptides. The PHT1 transports di- and tri-peptides as well as histidine. The objective of this study was to profile PepT1, PepT2, and PHT1 mRNA expression in the proventriculus, duodenum, jejunum, ileum, ceca, large intestine, brain, heart, bursa of Fabricius, lung, kidney, and liver in layer chicks on embryonic days 18 and 20 and days 1, 3, 7, 10, and 14 posthatch. Absolute quantification real-time PCR was used to measure gene expression. Expression of PepT1 was greatest in the duodenum, jejunum, and ileum. Expression of PepT1 increased in the duodenum, jejunum, and ileum from late embryonic stages to posthatch and in the large intestine from late embryonic stages to day 10 posthatch. In the ceca, PepT1 expression increased from embryonic day 20 to day 1 posthatch and then decreased. Expression of PepT2 was greatest in the brain and kidney. Expression of PepT2 increased from day 10 to 14 in the bursa of Fabricius and decreased in the proventriculus, duodenum, jejunum, and liver from late embryonic stages to posthatch. In the small intestine and liver, PepT2 may function to transport di- and tri-peptides during embryogenesis. The PHT1 was expressed in all tissues analyzed. Expression of PHT1 increased in the jejunum, large intestine, brain, and liver posthatch and decreased in the proventriculus from embryonic stages to posthatch. A tissue × age interaction was observed for all genes. The uptake of peptides in the developing chick is regulated by peptide transporters that are expressed in a tissue- and development-specific manner.

Key words: chicken, peptide transport, PepT1, PepT2, PHT1

ABSTRACT Peptide transporters 1 and 2 (PepT1 and PepT2) and peptide/histidine transporter 1 (PHT1) are all members of the proton-coupled oligopeptide transporter family, which are important for the transport of amino acids in peptide form. The PepT1 acts as a low-affinity/high-capacity transporter and PepT2 as a high-affinity/low-capacity transporter for di- and tripeptides. The PHT1 transports di- and tri-peptides as well as histidine. The objective of this study was to profile PepT1, PepT2, and PHT1 mRNA expression in the proventriculus, duodenum, jejunum, ileum, ceca, large intestine, brain, heart, bursa of Fabricius, lung, kidney, and liver in layer chicks on embryonic days 18 and 20 and days 1, 3, 7, 10, and 14 posthatch. Absolute quantification real-time PCR was used to measure gene expression. Expression of PepT1 was greatest in the duodenum, jejunum, and ileum. Expression of PepT1 increased in the duodenum, jejunum, and ileum from late embryonic stages to posthatch and in the large intestine from late embryonic stages to day 10 posthatch. In the ceca, PepT1 expression increased from embryonic day 20 to day 1 posthatch and then decreased. Expression of PepT2 was greatest in the brain and kidney. Expression of PepT2 increased from day 10 to 14 in the bursa of Fabricius and decreased in the proventriculus, duodenum, jejunum, and liver from late embryonic stages to posthatch. In the small intestine and liver, PepT2 may function to transport di- and tri-peptides during embryogenesis. The PHT1 was expressed in all tissues analyzed. Expression of PHT1 increased in the jejunum, large intestine, brain, and liver posthatch and decreased in the proventriculus from embryonic stages to posthatch. A tissue × age interaction was observed for all genes. The uptake of peptides in the developing chick is regulated by peptide transporters that are expressed in a tissue- and development-specific manner.

Key words: chicken, peptide transport, PepT1, PepT2, PHT1

INTRODUCTION

The transport of amino acids is mediated by membrane-bound transporters of free amino acids or small peptides. The proton-coupled oligopeptide transporter family includes peptide transporters 1 and 2 (PepT1 and PepT2) and peptide/histidine transporters 1 and 2 (PHT1 and PHT2; Botka et al., 2000). The PepT1 (SLC15A1), PepT2 (SLC15A2), PHT2 (SLC15A3), and PHT1 (SLC15A4) are members of the solute carrier family (Daniel and Kottra, 2004). Members of this family transport their various substrates in a species-, tissue-, and development-specific manner.

The PepT1 is a low-affinity, high-capacity transporter and the main mechanism by which di- and tripeptides produced by luminal digestion are absorbed in the small intestine. Net substrate transport by PepT1 is electrogenic, driven by the inside-negative membrane potential of the cell, and dependent on extracellular pH (Fei et al., 1994). In addition, PepT1 transports peptidomimetics such as β-lactam antibiotics (e.g., penicillin and cephalosporin), angiotensin-converting enzyme inhibitors, aminopeptidase inhibitors, and ester prodrugs (reviewed in Rubio-Aliaga and Daniel, 2008). The PepT1 is predominantly expressed in the epithelial mucosa of the rat small intestine, particularly in the villus present on the apical membrane of epithelial cells (Freeman et al., 1995). As luminal contents move down the small intestine, the concentration of di- and tri-peptides decreases due to absorption and levels of PepT1 protein in the small intestine decrease in the distal tract (Chen et al., 1999; Jappar et al., 2010). In the kidney, PepT1 has only been observed in the apical membrane of S1 and proximal S2 segments of the proximal tubule within the rat nephron. The PepT1 was not expressed in any other part of the nephron (Smith et al., 1998). In the adult broiler chicken, PepT1 was expressed in the small intestine, with low levels in the kidney and liver (Chen et al., 1999, 2002).
The PepT2 is a low-capacity, high-affinity transporter that shares 50% amino acid identity and 70% similarity with the PepT1 gene (Liu et al., 1995). The PepT2 transports many of the same substrates as PepT1 in an electrogenic manner; however, PepT2 exhibits a 40 times greater substrate affinity than PepT1 for various peptides (Boll et al., 1996; Terada et al., 2000). The PepT2 is mainly expressed in the kidney and brain with some expression in other tissues such as the enteric nervous system, lung, mammary gland, and spleen (reviewed in Rubio-Aliaga and Daniel, 2008). In the kidney, PepT2 is expressed in the apical membrane of the nephron, specifically in the distal straight part of S2 and S3 segments (Shen et al., 1999).

The PHT1 and PHT2 are structurally similar to PepT1 and PepT2 and transport free histidine and certain di- and tri-peptides (Yamashita et al., 1997; Sakata et al., 2001; Daniel and Kottra, 2004), but their functions are less well defined. The PHT1 is expressed in the immune and nervous systems as well as the gastrointestinal tract and thus may serve as a transporter of peptides/histidine across the cell membrane (Yamashita et al., 1997; Herrera-Ruiz et al., 2001; Bhargawaj et al., 2006). Expression of PHT1 was mainly in the lymphatic system with abundant expression in the lung, spleen, and thymus (Sakata et al., 2001). Both PHT1 and PHT2 appear to serve a dual function as a transporter of peptides and histidine from inside the endosome/lysosome to the cytosol. In a mouse macrophage cell line and transfected cell lines, immunohistochemical analysis showed that PHT1 and PHT2 protein was found on the late endosomes/lysosomes and not on the cell surface (Sakata et al., 2001; Sasawatari et al., 2011).

In the chicken, PepT1 has been extensively studied in intestine but not in other tissues, while PepT2 and PHT1 have not been characterized. A search of the chicken genome did not reveal a PHT2 gene homolog. The objective of this study was to perform a comprehensive analysis of PepT1, PepT2, and PHT1 mRNA expression among a wide array of tissues in both pre- and posthatch chicks.

**MATERIALS AND METHODS**

**Birds and Tissue Collection**

Fertile eggs were obtained from Leghorn chickens (S37 generation of P. Siegel’s high antibody selected line, Zhao et al., 2012). After hatch, chicks were housed in groups of 5 to 6 chicks per cage. On sampling days, chicks were randomly selected from all hatched chicks. All animal procedures were approved by the Institutional Animal Care and Use Committee at Virginia Tech. Samples were collected from 5 chicks killed by cervical dislocation on embryonic d18 (e18) and 20 (e20) and d1, 3, 7, 10, and 14 posthatch. Brain, heart, proventriculus, duodenum, jejunum, ileum, ceca, large intestine, bursa, kidney, liver, and lung samples were collected from each bird. Bursa and ceca were not collected on e18, whole intestine (as opposed to duodenum, jejunum, and ileum segments) was collected at e18, and lungs were not collected on e18 and e20. All samples were stored at −80°C until use.

**Total RNA Extraction**

The RNA was extracted from tissue samples using Tri Reagent according to the Isolation of RNA protocol (Molecular Research Center Inc., Cincinnati, OH). Tissues were homogenized using an IKA Ultra-Turrax T25 basic homogenizer and chloroform was used in the phase separation step. The RNA was suspended in 100 μL of DEPC-treated water (0.1%, Sigma-Aldrich, St. Louis, MO) and incubated for 10 min at 58°C. Initial concentration was determined using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Concentration was confirmed and purity analyzed using Agilent RNA 6000 Nano Chips on an Agilent 2100 Bioanalyzer (Agilent, Foster City, CA) following the manufacturer’s protocol. Each sample had a RNA Integrity Number greater than 6.5. All extracted RNA samples were stored at −80°C.

**Absolute Quantification Real-Time PCR**

The absolute quantification real-time PCR assay was used to measure PepT1, PepT2, and PHT1 mRNA. The methods for generating standard curves, reverse transcription, and real-time PCR have been described in Gilbert et al. (2007). Briefly, for each gene, plasmids containing partial chicken PepT1, PepT2, or PHT1 cDNA were linearized and used for in vitro transcription (MEGAscript T7 or SP6 in vitro transcription kit, Ambion, Austin, TX). Primers used for cloning chicken PepT1 have been reported in Gilbert et al. (2007), and primers used for cloning chicken PepT2 and PHT1 are shown in Table 1. The resulting cRNA was quantified using a RiboGreen assay (Molecular Probes). A dilution series of 10^10 to 10^4 cRNA molecules per microliter was created for each gene. The dilution series for each gene was reverse transcribed using the high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). All cDNA samples and standards were diluted 1:30 before real-time PCR analysis. Two microliters of diluted cDNA were added to each well of a 96-well plate with 23 μL of real-time PCR master mix, which contained 12.5 μL 2× SYBR Green Master Mix (Applied Biosystems), 0.5 μL forward primer (5 μM), 0.5 μL reverse primer (5 μM), and 9.5 μL of DEPC water. Real-time PCR primers used for cloning PepT1 have been reported in Gilbert et al. (2007) and real-time PCR primers used for cloning PepT2 and PHT1 are shown in Table 1. Samples were gently mixed, and the plate was loaded into an Applied Biosystems 7300 Real-Time PCR instrument. The following PCR reaction was run: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.
Statistical Analysis

All data were analyzed by ANOVA using JMP Statistical Discovery Software from SAS (SAS Institute Inc., Cary, NC). Each gene was analyzed separately. For each gene, the model included the main effects of tissue and age, the interaction of age \times tissue, and bird number nested within age. Significant effects and interactions \((P < 0.05)\) were further evaluated with Tukey’s test for pairwise comparisons.

RESULTS

Expression of PepT1 mRNA was found to be the greatest within the gastrointestinal tract with very low expression in the proventriculus, brain, heart, bursa of Fabricius, lung, kidney, and liver (Figure 1). A tissue \times age interaction was observed for PepT1 \((P < 0.0001)\). In the duodenum, expression of PepT1 increased from e18 to d 1 \((495 to 14,500 \text{ molecules of PepT1 per ng of total RNA}, P = 0.021)\), with no difference in expression after d 1. Within the jejunum, peak expression of PepT1 was observed at d 1 with 33,000 molecules of PepT1 per ng of total RNA and was greater than all other time points \((P < 0.001)\). Within the ileum, expression of PepT1 increased from e20 to d 1 \((820 to 15,400 \text{ molecules of PepT1 per ng of total RNA}, P = 0.026)\). Expression was not different from d 1 through d 14. Within the ceca, PepT1 expression increased from e20 to d 1 \((500 to 1,700 \text{ molecules of PepT1 per ng of total RNA}, P = 0.032)\). After d 1, PepT1 expression declined to d 7 \((P = 0.023)\) and then remained constant. In the large intestine, expression of PepT1 increased from d 3 to 10 \((660 to 3,400 \text{ molecules of PepT1 per ng of total RNA}, P = 0.020)\).

Greatest expression of PepT2 mRNA was observed in the kidney and brain (Figure 2). A tissue \times age interaction was observed \((P < 0.0001)\). Within the kidney, expression of PepT2 increased from e20 to d 10 \((1,200 to 5,600 \text{ molecules of PepT2 per ng of total RNA}, P = 0.012)\). Expression then decreased from d 10 to 14 \((1,300 \text{ molecules of PepT2 per ng of total RNA}, P = 0.014)\). Expression in the brain was the greatest, but did not change from e18 to d 14 (Figure 2).

Although expression of PepT2 was lower in the gastrointestinal tract, lung, heart, bursa of Fabricius, and liver, there were some significant developmental changes (Figure 2). Within the proventriculus, expression of PepT2 decreased from e18 to d 14 \((60 to 5 \text{ molecules of PepT2 per ng of total RNA}, P = 0.015)\). In the small intestine, expression was greatest at e18 with an average of 200 molecules of PepT2 per ng of total RNA and declined in both the duodenum at e20 to 30 molecules of PepT2 per ng of total RNA \((P = 0.001)\) and in the jejunum at d 3 to 20 molecules of PepT2 per ng of total RNA \((P = 0.009)\). In the ileum, expression of PepT2 was the greatest at d 1 \((480 \text{ molecules of PepT2 per ng of total RNA})\) compared with all time points except d 14 \((P < 0.04)\). In the liver, expression of PepT2 decreased from e18 to d 1 \((630 to 15 \text{ molecules of PepT2 per ng of total RNA}, P = 0.004)\). Expression of PepT2 remained constant over time in the heart, ceca, large intestine, and lung. Expression within the bursa of Fabricius increased from d 10 to 14 \((15 to 85 \text{ molecules of PepT2 per ng of total RNA}, P < 0.001)\).

Expression of PHT1 mRNA was observed in all tissues examined at varying levels. A tissue \times age interaction was observed \((P = 0.0002)\). Some tissues showed developmental specific changes (proventriculus, duodenum, jejunum, large intestine, and brain), whereas other tissues (ceca, ileum, heart, bursa of Fabricius, lung, and kidney) showed constant expression over time (Figure 3).

Expression in the proventriculus and duodenum declined with age. In the proventriculus, expression of PHT1 declined from e20 to d 7 \((480 to 100 \text{ molecules of PHT1 per ng of total RNA}, P = 0.010)\). Lowest expression of PHT1 was observed at d 14 when compared with e18 \((P = 0.009)\) and e20 \((P = 0.001)\). In the duodenum, expression declined from e18 to d 7 \((4.450 in whole small intestine to 1,300 \text{ molecules of PHT1 per ng of total RNA}, P = 0.046)\).

Expression in the jejunum, large intestine, brain, and liver increased with age. Expression in the jejunum increased from d 3 to 14 \((1,500 to 9,000 \text{ molecules of PHT1 per ng of total RNA}, P = 0.010)\). In the large intestine, expression increased from d 3 to 10 \((3,000 to 9,000 \text{ molecules of PHT1 per ng of total RNA}, P = 0.030)\). Expression within the brain was constant from e18 to d 10 with an average of 5,100 molecules of PHT1 per ng of total RNA, then increased on d 14 \((21,000 \text{ molecules of PHT1 per ng of total RNA}, P = 0.0013)\).

### Table 1. The PCR and cloning primers for peptide transporter 2 (PepT2) and peptide/histidine transporter 1 (PHT1)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PepT2</td>
<td>Forward real-time primer</td>
<td>TGACTGGCCATCGGAAACA</td>
</tr>
<tr>
<td></td>
<td>Reverse real-time primer</td>
<td>ACCCGTGTCACCATTCTAACCT</td>
</tr>
<tr>
<td></td>
<td>Forward cloning primer</td>
<td>GCTCCATCGATTCCAAAG</td>
</tr>
<tr>
<td></td>
<td>Reverse cloning primer</td>
<td>TGGATCTGGCTGATCAAAAC</td>
</tr>
<tr>
<td>PHT1</td>
<td>Forward real-time primer</td>
<td>AGGCGCAGGGAGTCTCTCA</td>
</tr>
<tr>
<td></td>
<td>Reverse real-time primer</td>
<td>TGCACCTTACCATCCTCAAACA</td>
</tr>
<tr>
<td></td>
<td>Forward cloning primer</td>
<td>AAGCCTCAGTGAGTGCAGTGCG</td>
</tr>
<tr>
<td></td>
<td>Reverse cloning primer</td>
<td>AGGCTGCTGGAAACGATGA</td>
</tr>
</tbody>
</table>

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Expression within the liver remained constant from e18 to d 1 then increased from d 1 to 10 (1,500 to 5,000 molecules of PHT1 per ng of total RNA, \( P = 0.011 \)).

**DISCUSSION**

The PepT1 and PepT2 are mainly present on the apical membrane of epithelial cells, where they function to uptake di- and tri-peptides in the intestine, kidney, and brain. The functional role of PHT1 is less clear because it is more uniformly expressed. Expression of these peptide transporters has been extensively studied in various tissues in mammals (human, rat, mouse, rabbit, reviewed in Rubio-Aliaga and Daniel, 2008), but only PepT1 has been characterized in birds.

Expression of PepT1 in mammals and birds is mainly localized to the gastrointestinal tract. The PepT1 was found in this study to be expressed the greatest within small intestinal tissue. Chen et al. (2002) found similar results in broiler chickens with the strongest mRNA hybridization in the duodenum, jejunum, and ileum, less hybridization in the kidney and cecum, and no hybridization in the liver, crop, or proventriculus. Li et al. (2008) observed similar expression patterns in male Aviagen commercial broilers, with all small intestinal segments increasing in PepT1 mRNA expression from e18 to d 14, although an extreme peak in jejunum PepT1 at d 3 was not observed. Gilbert et al. (2007) also observed an increase of PepT1 mRNA from c20 to d 14 within all segments of Aviagen commercial broilers and greatest quantities of PepT1 mRNA in the duodenum when compared with the ileum, with jejunum quantities being intermediate.

The high level of PepT1 expression in the intestine suggests that it plays an important role in amino acid uptake. Interestingly, homozygous *PepT1* null mice were healthy and physically similar in BW and development compared with wild-type mice (Hu et al., 2008). This brings into question the importance of PepT1 in the mouse because no upregulation of other peptide or amino acid transporters was observed to compensate even though increased plasma amino acid levels were observed (Nässl et al., 2011). In the chicken, Gilbert et al. (2007) found high levels of expression of amino acid transporters in the distal segments of the small intestine. If these amino acid transporters function at the same rate regardless of PepT1 expression, perhaps PepT1 expression in the small intestine is not essential, but when PepT1 is expressed, protein-containing diets are used more efficiently. Homozygous *PepT1* null chickens would clarify the importance of PepT1 for amino acid assimilation in the chicken.
Although expression of PepT1 in the large intestine and ceca are not significantly greater than the rest of the tissues sampled, the low level expression in these tissues may be biologically significant. Expression in the latter portions of the gastrointestinal tract would be advantageous to salvage all possible di- and tri-peptides before they are excreted.

Increase of PepT1 expression from pre- to posthatch is likely due to preparation for consumption of exogenous feed by birds posthatch. This pattern has been seen for chickens (Gilbert et al., 2007; Li et al., 2008; Speier et al., 2012), turkeys (Van et al., 2005; de Oliveira et al., 2009), and pigeons (Dong et al., 2012). After hatch, the chick transitions from utilizing the yolk, which is mostly fat, to carbohydrate- and protein-based feed. During late embryogenesis, enterocytes, which line the small intestine, are immature. After hatch, enterocytes mature and villi elongate to increase surface area for absorption (Geyra et al., 2001; Uni et al., 2003). Greater expression of PepT1 may occur to efficiently use the increased peptide load from exogenous feed consumed by the chick. Expression of PepT1 may also increase simply due to the structural changes in the small intestine from embryonic stages to posthatch, as enterocyte numbers increase with maturity. Thus, expression of PepT1 in a tissue sample may change due to an increased number of enterocytes rather than an increase in PepT1 per enterocyte.

In mammals, PepT2 is expressed the greatest in the kidney and brain. The PepT2 was observed widely distributed throughout the rat brain by Berger and Hediger (1999), particularly in astrocytes, subependymal cells, and ependymal cells. Expression of PepT2 in the mammalian brain aids in the homeostasis of neuropeptides at the blood-cerebrospinal fluid barrier (Teuscher at al., 2001). Shen et al. (1999) observed PepT2 in the rat kidney, specifically situated on the apical membrane in the S2 and S3 segments of the nephron. The PepT2, in conjunction with PepT1, which is expressed in the S1 segment, coordinately act to reabsorb peptides. Despite the high level of expression in kidney and brain, mice lacking the PepT2 gene did not show obvious phenotypic abnormalities but did show reduced peptide transport in the brain and kidney (Rubio-Aliaga et al., 2003; Shen et al., 2003). In this study, the greatest expression of PepT2 was observed in the brain with expression in the kidney less than the brain but greater than all other tissues examined. These high expression levels may indicate a role for PepT2 in the homeostasis of neuropeptides.
levels are consistent with mammalian distribution of PepT2 in the kidney and brain. Although PepT2 would be expected to serve the same functional role in the chicken kidney and brain, further research needs to be conducted to confirm cellular distribution and function.

Expression of PepT2 increased in the bursa of Fabricius at d 14. Rapid bursal growth occurs from hatch to 4 wk of age (Whittow, 2000), and PepT2 may provide peptides to the developing tissue. Many small peptides have been observed that can modulate the immune system and have been used in vaccines and as treatments for immune deficiencies and cancer (St. Georgiev, 1990). The PepT2 may transport these small peptides in the chicken shortly after hatch. Further investigation into the substrates of PepT2 and spatial distribution of PepT2 mRNA in the bursa will help characterize the role of PepT2 in bursal function.

One interesting finding is the expression of PepT2 in the gastrointestinal tract and liver during late embryogenesis, which suggests that PepT2 may be serving as an embryonic peptide transporter. In the case of the gastrointestinal tract, PepT2 may function as an important peptide transporter before the induction of PepT1 expression. During late embryogenesis, chicks swallow some of the amniotic fluid, which enters the embryonic intestine (Moran, 2007). The PepT2 may play an important role at this time in the absorption of peptides from the amniotic fluid.

The PHT1 may play a dual role as a transporter of peptides/histidine across the cell membrane and from the endosome/lysosome to the cytosol (Sasawatari et al., 2011). In mammals, expression of PHT1 has not been as extensively characterized as PepT1 and PepT2 and has not been previously reported in the chicken. In this study, PHT1 was more uniformly expressed in many tissues compared with PepT1 and PepT2 and was observed in all tissues analyzed. Expression of PHT1 has been found in immune cells, such as dendritic cells, activated macrophages and B-cells (Sasawatari et al., 2011), supporting the wide range of distribution found in mammals. Herrera-Ruiz et al. (2001) observed PHT1 mRNA by reverse-transcription PCR in rat stomach, small intestine, ceca, and colon and in human heart, kidney, leukocytes, liver, lung, ovary, pancreas, placenta, prostate, skeletal muscle, small intestine, spleen, testis, and thymus. Bhardwaj et al. (2006) also found expression of PHT1 in the human gastrointestinal tract. The broad distribution of PHT1 expression observed in mammals is similar to the broad distribution observed in chickens found in this study. Expression of PHT1

Figure 3. Expression of peptide/histidine transporter 1 (PHT1) in developing chick tissues. Five birds were sampled at embryonic d 18 (e18), embryonic d 20 (e20), d 1, 3, 7, 10, and 14 posthatch. Proventriculus, duodenum, jejunum, ileum, ceca, large intestine, whole brain, heart, bursa of Fabricius (bursa), lung, kidney, and liver samples were collected from each bird at every day sampled, except whole intestine was collected at e18, lungs were not collected on e18 and e20, and only 4 samples of bursa of Fabricius and ceca were collected on e18. The RNA was extracted from each sample and analyzed for PHT1 mRNA expression by absolute quantification real-time PCR. A tissue × age interaction was observed (P = 0.0002).
increased in liver, brain, large intestine, and jejunum posthatch, suggesting an increase in expression due to maturation of the tissue.

The subcellular localization of PHT1 remains to be clearly defined. Bhardwaj et al. (2006) reported that PHT1 is located at the villus epithelium in the intestinal epithelium and cells transiently transfected with PHT1 can transport histidine and carnosine. These results suggest that PHT1 may be localized to the cell surface of intestinal cells, which with PepT1 may mediate the uptake of peptides from the intestinal lumen. In other tissues, PHT1 may be more important in the transport of peptides/histidine from the lysosome to the cytosol. Agu et al. (2011) investigated the expression of PHT1, PepT1, and PepT2 in human nasal epithelium and found that PepT1 and PepT2 protein were present on the brush border membrane but PHT1 was localized to the cell nucleus and not the cell surface. Further investigation into the cellular localization of PHT1 within multiple tissues is needed to clarify the functional role of PHT1.

In conclusion, the expression of PepT1, PepT2, and PHT1 in tissues of chickens is similar to mammals. The PepT1 is highly expressed in the small intestine. The PepT2 is predominantly expressed in the brain and kidney, and expressed in the gastrointestinal tract, liver, and lung during late embryogenesis, suggesting that it may serve as an embryonic peptide transporter. In contrast, PHT1 expression is widely distributed throughout the chick. Peptide transport in the chick is mediated by the expression of PepT1, PepT2, and PHT1 in a tissue- and development-specific manner.

REFERENCES


