The Regionalization of PepT1, NBAT and EAAC1 Transporters in the Small Intestine of Rats Are Unchanged from Birth to Adulthood


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ABSTRACT The ontogenetic development of PepT1, NBAT and EAAC1 along the vertical and horizontal axes of the rat small intestine was evaluated using semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry. The proximodistal profiles of mRNA levels showed that PepT1 was evenly distributed, whereas NBAT had greater expression in the proximal part, and EAAC1 in the distal part. These regionalizations were the same from postnatal days 4 to 50. PepT1 and NBAT proteins were detected in the microvilli of enterocytes along the length of the villi. NBAT was also found in the cytoplasm. Surprisingly, EAAC1 was located exclusively in the microvilli of enterocytes in the crypt and the bases of the villi. These protein expression patterns were similar in all parts of the small intestine (proximal, median and distal), at all ages. We conclude that the expression of PepT1, NBAT or EAAC1 are differently regulated according to both the horizontal and vertical axes.


KEY WORDS:  ● ontogeny ● amino acid transporters ● small intestine ● rats

The small intestine of animals from altricial species such as rat, mouse or rabbit is not mature at the time of birth. In rats, morphogenesis is not complete until wk 3 after birth, when profound changes occur, including increases in intestinal weight, villous and crypt height, cell migration rate, RNA and DNA content (1), and the adaptation of intestinal enzymatic activity (2). Although the consequences of postnatal development of the small intestine on nutrient absorption are now recognized (3), little is known about the ontogenesis of amino acid and peptide transport and transporters. The aim of this study was to describe the postnatal maturation patterns of three amino acid and peptide transporters in rat small intestine, namely PepT1, NBAT and EAAC1. EAAC1 is responsible for the uptake of glutamate, a major metabolic fuel of the enterocyte through the Na+-dependent transport system X_{A,C^-} (4). NBAT represents the heavy chain of a heterodimeric transporter involved in the heteroechange of cationic and neutral amino acids corresponding to the b0,+ trans- port system (5). PepT1 was identified as the protein-mediating H+-peptide cotransport in the brush border membrane of intestinal epithelial cells (6). The expression of mRNA coding for these transporters was measured, and the distribution of these proteins along the crypt-villus axis was studied from birth to adulthood. Because the intestinal absorption of nutrients is known to vary along the proximodistal axis, we considered the evolution of these transporters in the proximal, median and distal parts of the small intestine.

MATERIALS AND METHODS

Maintenance and dissection of animals. Outbred female Sprague-Dawley rats with litters of 11 pups were used throughout this study (Iffa Credo, L’Arbresle, France) according to the guidelines of the French National Animal Care Committee. The litter was kept with the dam until postnatal day 50 (d 50) and began to nibble solid food at d 21 (maintenance rat diet A04, U.A.R, France: 12:1 kJ/g; 83.9% cereal and cereal products, 4.1% vitamin and mineral mixture, 4% animal proteins and 8% vegetable proteins). Rats were killed by chloroform asphyxiation in random order without regard to sex on d 4, 8, 10, 12, 21, 30 and 50. The small intestine was rapidly removed and divided into three segments of equal length, i.e., proximal, median and distal parts. At the distal part of each segment, a portion of 0.5 cm was removed for immunohistochemistry.

RNA analysis. Because it was not possible to scrape the small intestine, the entire mucosa was used for all experiments. Intestinal segments were finely crushed in liquid nitrogen and resuspended in a denaturing solution (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7.0, 5 g/L sarcosyl) for mRNA extraction according to Chomczynski et al. (7). After RNA integrity had been confirmed by ethidium bromide staining, first strand cDNAs were synthesized from 2 μg of total RNA using oligo(dT)12–18 as primers in the presence of MML-V reverse transcriptase (RT)1 (Invitrogen, Cergy Pontoise, France), for 1 h at 37°C; 5 μL of each RT product was used for polymerase chain reaction (PCR) (50 μL final reaction volume) with primers chosen from the conserved part of the coding regions for EAAC1 (forward primer (P1): 5’-GGGAAGATCATAGAAGTT-3’, reverse primer (P2): 5’-TGAACCGCTTCCAGGAGC-CAGT-3’), NBAT (P1: 5’-CCTCAGGGAGATCTCTTCTTG-3’, P2: 5’-TGCGACGCTGGATTTCCATTACAC-3’) and PepT1 (P1: 5’-GGACTGCGCTAAAGAGAAATACG-3’, P2: 5’-TTACGCTTCCAGGAGC-CAGT-3’). Amplification was performed using a MJ Re-

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Abbreviations used: P1, forward primer; P2, reverse primer; PCR, polymerase chain reaction; RT, reverse transcriptase.
search PTC-200 Thermocycler (Merck-Eurolab, Fontenay sous Bois, France) for 35 cycles, which consisted of denaturation (95°C, 45 s), annealing (56°C, 45 s) and extension (72°C, 1 min) with Taq DNA Polymerase (Invitrogen, Cergy Pontoise, France). To enable semiquantitative analysis, the housekeeping gene β-actin was also amplified (P1: 5’-TGGATACCTGGCATCCATGAAA-3’, P2: 5’-TAAAACGCAGCTTGACTAACGTCCG-3’) (8). β-Actin (473 bp) and EAAC1 (539 bp), NBAT (542 bp) or PepT1 (640 bp) PCR products were separated by electrophoresis through a 2% agarose gel, stained with ethidium bromide and quantified using a digital imaging system (Alpha Innotech Corporation, San Leandro, CA). All PCR products were verified by sequencing (Genset, Paris, France).

**Immunohistochemistry.** Fragments collected at the distal part of each proximal, median and distal segment of freshly dissected small intestine were fixed in alcohol formalin acetic acid fixative (ethyl alcohol/formaldehyde/glacial acetic acid/water, 75/2/5/8, v/v/v/v) for 4 h at room temperature. Paraffin sections (4 μm) were then autoclaved at 120°C for 40 min in target retrieval buffer (10 mmol/L citrate buffer, pH 6) for demasking, then rinsed with 150 mmol/L, pH 7.2 PBS. Affinity-purified rabbit anti-rat PepT1 and anti-rat NBAT antibodies were produced against two synthetic peptides, VCKENYPSLEPSQTNM for PepT1 (9) and NS-DYHTVNVQDK for NBAT (10) (Agro Bio, La Ferte St Aubin, France). For NBAT detection, sections were incubated for 24 h at room temperature with anti-rat NBAT antibodies (5 mg/L). For NBAT detection, sections were incubated for 24 h at room temperature with anti-rat PepT1 antibodies (5 mg/L). All sections were then incubated with a goat anti-rabbit immunoglobulin G coupled with a dextran-peroxidase complex (EnVision Kit, DAKO, Trappes, France). After endogenous peroxidases were blocked, the sections were incubated overnight either at 4°C with rabbit anti-rat EAAC1 antibodies (10 mg/L; EAAC11A, Alpha Diagnostic International, San Antonio, TX) (11) or at room temperature with anti-rat PepT1 antibodies (5 mg/L). All sections were then incubated with a goat anti-rabbit immunoglobulin G coupled with a dextran-peroxidase complex (EnVision Kit, DAKO, Trappes, France). Detection was performed using 3,3′-diaminobenzidine tetrahydrochloride. Rabbit preimmune serum was used instead of the specific antibodies as a negative control.

**Statistical analyses.** Data are given as means ± SD. Comparisons between the different days and intestinal segments were performed using two-way ANOVA and Fisher’s test (StatView). Differences with P < 0.05 were considered significant.

**RESULTS**

Quantification of PepT1, NBAT and EAAC1 mRNA along the rat small intestine from postnatal d 4 to 50. PepT1, NBAT and EAAC1 mRNA were detected all along the small intestine, from d 4 to 50 (Fig. 1). Each of these transporters showed a specific pattern of distribution. PepT1 mRNA was evenly distributed from the proximal to the distal small intestine. The level of NBAT mRNA showed an oral to aboral gradient with a higher expression in the proximal than in the median small intestine on d 4 and 10 (P < 0.05). The NBAT mRNA level tended to be higher (P < 0.06) in the proximal than in the distal small intestine on d 4, 10 and 21. In comparison, EAAC1 mRNA exhibited an inverse gradient, with higher expression in the distal small intestine compared with the proximal on d 10 (P < 0.05) and with the median on d 12 and 30 (P < 0.05). The absence of significant gradient at the other ages may have resulted from the high standard deviations related to the outbred characteristic of the rat strain. PepT1, NBAT and EAAC1 distribution patterns remained the same from birth to adulthood.

Next, we examined the changes in mRNA expressions of these transporters during development. PepT1 mRNA levels decreased on d 50 compared with d 21 in the proximal and median parts of the small intestine (P < 0.05) and remained constant in the distal part. No variation in the level of NBAT mRNA was observed from d 4 to 50. In contrast, the level of EAAC1 mRNA increased from d 4 to 21 all along the small intestine (P < 0.05). This level was lower on d 4 and 8 in the proximal and distal parts, on d 10 in the proximal part, and on d 12 in the median and distal part compared with d21 (P < 0.05). Differences relative to d 30 were similar (Fig. 1).

**Immunohistochemical localization of PEPT1, NBAT and EAAC1 proteins along the crypt-villus axis of rat small intestine.** Staining for PEPT1 was detected from d 4 to 50 on the brush border of epithelial cells in the villi (Fig. 2) with a gradient from the crypt-villus junction toward the tips of villi. No labeling was observed in crypt cells. NBAT immunoreactivity was observed in villi from base to tip from d 4 to 50 (Fig. 2). Staining was observed mainly in the brush border membrane of epithelial cells, but a light staining was also detected in their cytoplasm. No NBAT immunoreactivity was seen in crypt cells. EAAC1 immunoreactivity was restricted to the brush border membrane of epithelial cells located at the intervillous space at d 4 and of those lining the crypt on d 21 and 50 (Fig. 2). No labeling was observed in the upper two thirds of the villi between d 4 and 50. No difference in the distribu-
necessarily correlate with that of the functional b0,+ thus, the expression of NBAT described in this study may not parallel those of its substrates. However, it may thus correlate with the expression of PepT1.

In conclusion, our results provide the first demonstration that EAAC1 and NBAT expression is strong during the first days after birth. The regionalization of PepT1, NBAT and EAAC1 mRNA along the entire small intestine and the localization of corresponding proteins along the crypt-villus axis are the same in newborn and adult rats. Conversely, PepT1 and EAAC1 mRNA levels vary as a function of age.

**LITERATURE CITED**