Brush-border peptidases alter the kinetic properties of neutral amino acid transporters B0AT1 and B0AT3

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Protein digestion, absorption and re-absorption of the resulting amino acids are fundamental functions of the small intestine and kidneys. Until recently, the classic view of protein digestion was that no direct interaction between the brush-border peptidases and amino acid transporters occurred. However, the discovery that that the carboxy-peptidase Angiotensin Converting Enzyme 2 (ACE2) is required for the trafficking of the neutral amino acid transporter B0AT1 to the cell surface *in vitro* and *in vivo* in the intestine has led to a re-evaluation of this traditional view. These discoveries raise the possibility that other brush border peptidases may play a functional role in neutral amino acid transport in the small intestine and/or kidney. It is now known that transporters other than B0AT1, especially B0AT3 (XT2), require ACE2 for trafficking to the cell surface.

Here, we show that the brush-border peptidases Aminopeptidase N (APN) and ACE2 regulate the kinetic properties of the neutral amino acid transporters B0AT1 and B0AT3, respectively. In both cases this regulation involves an increase in substrate affinity of the transporters. Co-expression of APN with B0AT1 resulted in a 2-fold increase in substrate affinity of the transporter for its neutral amino acid substrates. Co-expression of B0AT3 with ACE2 resulted in a 6-fold increase in the substrate affinity of B0AT3 for its main substrates alanine and glycine. Co-immunoprecipitation of B0AT1 and APN reveals that the two proteins form a complex in the apical membrane of the small intestine as do B0AT3 and ACE2 in the kidney. This suggests the formation of brush-border metabolons in both these organs. Site-directed mutagenesis and functional assays studies of APN suggests that the peptidase increases the substrate affinity of B0AT1 by creating a change in the local concentration of substrate that the transporter "sees" in the vicinity of the plasma membrane.