ESSENTIAL MOLECULAR FEATURES OF SUBSTRATES VIA HUMAN SODIUM-COUPLED MONOCARBOXYLATE TRANSPORTER (hSMCT)

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hSMCT (SLC5A8) has been shown to act as an electrogenic and Na⁺-coupled transporter for a variety of monocarboxylates. hSMCT is expressed abundantly in colon, ileum, kidney and thyroid gland, and play an important role in the active transport of a variety of endogenous monocarboxylates. On the basis of the broad substrate-specificity of hSMCT, the therapeutic drugs with monocarboxylate might interact with hSMCT, indicating that it is very important to investigate the essential molecular features of substrates determining the affinity and capacity via hSMCT. In the present study, functional characteristics and substrate-specificity of the heterologously expressed hSMCT in Xenopus laevis oocytes were determined by electrophysiological techniques and uptake studies. On the basis of the present data regarding the structural requirements of transportable substrates, it is proposed that the substrate recognition site of the hSMCT-transporter involves at least three domains: One is for the negatively charged carboxyl group and the most crucial domain (P₁). The second binding domain (P₂) is in the vicinity of P₁ domain, and very tight and hydrophilic. The third domain (P₃) is located at the opposite side of P₁ domain, and hydrophobic and relatively wide, explaining that hSMCT can tolerate a diversity of substrates. The findings provide the frameworks not only for a design of selective substrates/inhibitors via hSMCT, but also a prediction of therapeutic drug-drug interaction via hSMCT.

MOLECULAR IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF A pH-SENSITIVE FOLATE TRANSPORTER IN HUMAN INTESTINE

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It is well known that the intestinal absorption of folate is mediated by a specific transport system that functions optimally at acidic pH. However, the molecular mechanism involved has not been clarified yet. While there is a suggestion that reduced folate carrier 1, which functions optimally at around neutral pH when cloned, may functionally modulated in the intestine, the presence of an unidentified transporter has also been suggested. To resolve this disputed issue, we report here the identification of a human intestinal cDNA encoding a transporter protein responsible for intestinal folate transport. The cloned transporter could, when expressed in HEK293 cells, mediate the transport of folate at an acidic extracellular pH of 5.5 in a manner independent of Na⁺ and insensitive to membrane potential. But its transport activity was absent at near neutral pH. Thus, this transporter was found to have the character of a pH-sensitive folate transporter (PSFT) with acidic pH optimum for its operation. Folate transport mediated by our cloned PSFT was saturable with a Kₘ of 0.60 µM, and extensively inhibited by reduced folates, such as 5-methyltetrahydrofolate, as well as by methotrexate. Probenecid, BSP and DIDS were also found to be potent inhibitors of the PSFT. These results suggest that the functional features of the PSFT are consistent with those of the intestinal folate transport system reported for intestinal brush border membrane vesicles and Caco-2 cells. In addition, PSFT activity in Caco-2 cells was significantly reduced by the RNA interference of the PSFT gene. Thus, it is likely that the PSFT is the long sought intestinal folate transporter, which also plays an important role in the absorption of methotrexate, an antifolate drug.