Characterisation of ASCT-mediated transport at low pH

A.J. Scopelliti, R.M. Ryan and R.J. Vandenberg, Department of Pharmacology, Blackburn Building D06, The University of Sydney, NSW 2006, Australia.

The alanine, serine and cysteine transporters (ASCT1 and 2) are electroneutral exchangers. They belong to the Solute Carrier Family 1, along with human and prokaryotic glutamate transporters (Excitatory Amino Acid Transporters - EAATs and Glt_{Ph} respectively). Neutral amino acid exchange is coupled to only one Na^+ ion, as opposed to the three Na^+, one H^+ ion and K^+ coupling required for acidic amino acid transport by the EAATs. Although amino acid exchange by ASCT is not H^+-coupled, its substrate specificity is pH-dependent. Cysteate is a substrate at pH5.5, but not at neutral pH. Conversely, alanine and serine are substrates at neutral pH, but are lesser substrates at pH 5.5. We initiated a study of ASCT1 to provide more detailed comparisons with the well characterized EAATs so as to provide a better understanding of the molecular basis for similarities and differences between ASCT and the EAATs. ASCT1 was expressed in *Xenopus laevis* oocytes and the two electrode voltage clamp technique was used to characterize the pH dependence of amino acid exchange. In addition to coupled ion-substrate fluxes, ASCT1 also supports an uncoupled anion current, which is similar to that of the EAATs. As coupled amino acid-ion exchange is electroneutral, we monitored the generation of the uncoupled anion current as a measure of substrate flux. Using this method alanine and serine generate large uncoupled anion currents. We demonstrate that the acidic amino acids L-aspartate and L-cysteate are also substrates at pH5.5, but L-glutamate and D-aspartate are not substrates. While L-alanine and L-serine exchange at neutral pH can only be supported by Na^+, we demonstrate that L-aspartate and L-cysteate exchange at low pH can be supported by a variety of cations, including Li^+, K^+ and Cs^+. To determine the molecular basis for these changes in substrate selectivity and cation coupling, we created and tested a range of mutations in the transport domain of ASCT1. Residues that have previously been implicated in substrate and/or cation coupling in the EAATs and are also different between the EAATs and ASCT1 were targeted for further investigation. One of these mutations, A382T, in transmembrane domain 7 was found to relax the substrate specificity towards acidic amino acids. This mutant allowed the transport of L-aspartate at pH7.5, although produced much smaller currents than L-alanine or L-serine. At pH5.5, L-aspartate and L-cysteate mediated currents were 3-4fold larger than those mediated by neutral amino acids, indicating a much larger anion flux when L-aspartate or L-cysteate are bound. Furthermore, D-aspartate, but not L-glutamate generated anion fluxes. Exploiting the apparent variation in transport mechanisms between acidic and neutral amino acids in ASCT may aid in furthering the understanding of transport mechanisms in the EAATs.