Heads and tails: lipid inhibitors of glycine transport

S. Mostyn,[1] J.E. Carland,[1] T. Rawling,[2] R.M. Ryan[1] and R.J. Vandenberg,[1] [1]Discipline of Pharmacology, School of Medical Sciences, University of Sydney, NSW 2006, Australia and [2]Faculty of Science, University of Technology, Ultimo, NSW 2007, Australia.

The glycine transporter, GlyT2, is the target for a number of drugs that can alleviate neuropathic and inflammatory pain. We have identified a series of lipids that inhibit GlyT2 and also show promise as leads for the development of a novel class of analgesics. Three lipids that show high affinity and efficacy are N-Arachidonyl-Glycine (NAGly), N-Oleoyl-Glycine (NOGly) and Oleoyl-L-Carnitine (OLCarn). All three lipids are non-competitive inhibitors and are unlikely to bind at the substrate binding site. We have used site-directed mutagenesis to identify potential lipid binding sites and to understand allosteric communication between the lipid binding site and inhibition of transport. Mutations in four distinction regions of the protein influence the potency and extent of inhibition. Extracellular loop 4 undergoes considerable conformational changes in the transport cycle, and mutations in the loop disrupt inhibition by all three lipids. This site is unlikely to form a lipid binding site but it may influence the conformational changes of the protein. We reasoned that transmembrane domains that flank EL4 may also influence the conformational changes required for lipid inhibition. Mutations on the membrane-exposed external surface of TM8 differentially reduce the potency of the three lipids and suggest potential head and tail group interactions of the lipids with the transporter. Mutations of additional residues in TM8 that face the opposite direction and point towards the substrate binding site also reduce the potency of lipid inhibition. Finally, S479 forms part of the substrate binding site of GlyT2 and the S479G mutation relaxes the substrate selectivity to allow sarcosine to be transported, and also reduces the potency of all three lipids to inhibit glycine transport. No other mutations in the substrate binding site, that alter substrate selectivity, have any effects on lipid inhibition. We propose a model where the lipids bind to the external membrane-exposed surface of the transporter, and alter the conformational changes in extracellular loop 4 and also the substrate binding site to disrupt glycine transport. We are currently using this information to develop novel lipid-based inhibitors that may have theraputic value in treating chronic pain.