Modulation of GlyT2 by endogenous lipid based compounds occurs through interactions with EL3

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Glycine is an inhibitory neurotransmitter in the spinal cord. At inhibitory synapses glycine activates the strychnine-sensitive glycine receptor and its concentrations are regulated by a combination of the GlyT1 and GlyT2 transporters. Increasing inhibitory glycinergic transmission has been demonstrated to have analgesic effects. In a wide range of animal models selective inhibitors of GlyT2 have analgesic effects (Dohi *et al.*, 2009). Our lab has identified a series of endogenous lipid based compounds that are selective inhibitors of GlyT2 with little or no activity at the closely related glycine transporter GlyT1. The most potent of these inhibitors is oleoyl-L-carnitine (IC50=0.34μM) (Carland *et al.*, 2012). Oleoyl-L-carnitine exhibits a slow washout, the duration of which can be shortened through application of cyclodextrin. This has led to the hypothesis that oleoyl-L-carnitine is interacting with GlyT2 *via* a site that is exposed to the lipid membrane.

The crystal structure of a prokaryotic homologue of GlyT2, LeuT, has revealed that extracellular loop 3 (EL3) is both exposed to the lipid membrane and undergoes significant conformational changes that are required for transport (Krishnamurthy *et al.*, 2012). Extracellular loop 3 of GlyT2 contains 26 residues, with 11 of these residues different between GlyT1 and GlyT2. We have initiated a study of the roles of residue differences between GlyT1 and GlyT2 and also the roles of charged residues in GlyT2 as potential binding sites for oleoyl-L-carnitine. The mutant E459AK460A was generated and expressed in *Xenopus laevis* oocytes and the electrogenic transport properties were compared to wild type GlyT2 using the two electrode voltage clamp technique. The removal of these charged residues did not change the affinity of GlyT2 for glycine and did not have any significant effect on oleoyl-L-carnitine inhibition of GlyT2. Further mutants W451M, K457Q and T462L have been constructed and will be tested for oleoyl-L-carnitine inhibition of glycine transport.

Carland JE, Mansfield RE, Ryan RM, Vandenberg RJ (2012) Oleoyl-L-carnitine inhibits glycine transport by GlyT2. *British Journal of Pharmacology* doi: 10.1111/j.1476-5381.2012.02213.x.

Dohi T, Morita K, Kitayama T, Motoyama N, Morioka N (2009) Glycine transporter inhibitors as a novel drug discovery strategy for neuropathic pain. *Pharmacology & Therapeutics* **123:** 54-79.

Krishnamurthy H, Gouaux E (2012) X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. *Nature* **481:** 469-474.