Mapping the chloride permeation pathway of a human glutamate transporter

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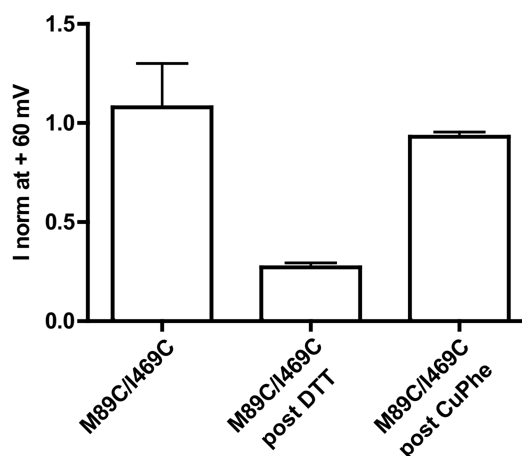
The concentration of glutamate within a glutamatergic synapse is tightly regulated by excitatory amino acid transporters (EAATs). EAATs function not only as glutamate transporters, but also as substrate activated chloride (Cl^-) channels. Several crystal structures of the EAAT homologue, GltPh, at different stages of the transport cycle have been solved (Boudker *et al.*, 2007; Reyes *et al.*, 2009; Verdon *et al.*, 2012; Yernool *et al.*, 2004). In the most recent structure to be solved (Verdon *et al.*, 2012) a small cavity lined by polarizable residues, several of which have been implicated in Cl^- permeation (Ryan *et al.*, 2004), has been identified. We hypothesize that throughout the transport cycle this cavity opens up to form the Cl^- channel. In this study, site directed mutagenesis of EAAT1 and electrophysiology have been utilized to determine if this cavity forms part of the Cl^- permeation pathway. Additionally, double cysteine mutants were generated in a cys-less EAAT1 background and analysed in an attempt to trap the protein in a purely Cl^- conducting state to assist with further structural studies of GltPh. When WT and mutant EAAT1 transporters are expressed in *Xenopus laevis* oocytes, the current observed at +60 mV is primarily attributed to Cl^- conductance. For this reason, current at +60 mV is indicative of Cl^- channel function. When residues T396, S366 and P392 are mutated to valine, alterations in Cl^- channel function occur without effecting glutamate transport (See table for K0.5 values and currents at +60 mV) thus indicating a role for these residues in the formation of the Cl^- permeation pathway.

	$K_{0.5}$ L-Glutamate (μM)	normalised I at +60 mV
EAAT1 WT	28 ±2	1.8 ± 0.1
T396V	40 ± 2	0.59 ± 0.01
P392V	18 ± 1	4.1 ± 0.3
S366V	15 ± 2	2.9 ± 0.5



After treatment of the double cysteine mutant M89C/I469C with the reducing agent dithiothreitol (DTT), the current at +60 mV decreases. Subsequent treatment with the oxidizing reagent copper phenanthroline (CuPhe) leads to a recovery of the current at +60 mV (see Figure). These results suggest that these two cysteine residues can form a spontaneous crosslink and trap the transporter in a Cl^- conducting state.

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