## Signal transduction at the subunit interfaces of the human glycine receptor

*T.M. Lewis, M. Drwal, D. Tam, L.S. Walton and M.J. Windley, Department of Physiology, School of Medical Sciences, UNSW, NSW 2052, Australia.*

The family of pentameric ligand gated ion channels (pLGICs) contribute to fast synaptic signalling in both vertebrates and invertebrates. This includes the receptors to the known neurotransmitters acetylcholine, GABA, serotonin, glycine, histidine, glutamate and zinc. How these receptors are able to respond to the binding of neurotransmitter and transduce this into the opening of the integral channel pore appears to be a complex interaction of molecular events both within and between subunits. Our work has contributed to the understanding of these molecular interactions and conformational changes in pLGIC by using the glycine receptor as a model system to test predictions from published crystal structures and molecular dynamics simulations. Initially this was informed by the crystal structures of the acetylcholine binding protein, a homologue of the extracellular domain of the nicotinic acetylcholine receptor (Brejc *et al.*, 2001). This identified structures at the interface between the extracellular and transmembrane domains of the receptor that were predicted to connect ligand binding events to opening the channel pore. One example is the loop between beta strands 1 and 2 (β1-β2 loop) of the extracellular domain. Mutations in this loop impair receptor function and the rate of covalent modification with substituted cysteine residues demonstrated a ligand-dependent conformational change (Cederholm *et al.*, 2010). These results were consistent with the β1-β2 loop contributing to the signal transduction pathway.

The crystal structures of prokaryotic ligand-gated ion channels from *Erwinia chrysanthemi* (ELIC) and *Gloeobacter violaceus* (GLIC) provided the first full-length receptor structures (Hilf & Dutzler, 2008; Bocquet *et al.*, 2009; Hilf & Dutzler, 2009). Comparison of the ELIC structure in a closed pore conformation with the GLIC in an apparently open pore conformation, suggested a ‘quaternary twist’ of the receptor complex and rotation of each individual subunit (Bocquet *et al.*, 2009). Thus, subunits rotate past each other with the transition between the resting and activated states. We investigated the possible contribution of charged residues at the inter-subunit interface that may form electrostatic interactions to stabilise the resting and activated states. Individual mutations to alanine impaired receptor function (an increase in the $EC_{50}$ value for glycine) in many, but not all instances. One specific electrostatic interaction was identified between E110 and K116. Mutant-cycle analysis of these residues with E110K and K116E mutations indicated weak electrostatic coupling between the residues, stabilising the closed state. Other charged residues do not demonstrate unambiguous electrostatic pairing. Thus, it may be a network of electrostatic interactions at the inter-subunit interface that is important to stabilise the resting and actived states, rather than individual interactions.

Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux J-P, Delarue M & Corringer P-J. (2009). *Nature* **457,** 111-114

Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB & Sixma TK. (2001). *Nature* **411,** 269-276.

Cederholm JME, Absalom NL, Sugiharto S, Griffith R, Schofield PR & Lewis TM. (2010). *Journal of Neurochemistry* **115,** 1245-1255.

Hilf RJC & Dutzler R. (2008). *Nature* **452,** 375-379.

Hilf RJC & Dutzler R. (2009). *Nature* **457,** 115-118.

---

Research supported by NHMRC project grant 1023202.