## Role of charged residues in coupling ligand binding and channel activation in the extracellular domain of the glycine receptor

*N.L. Absalom[1], T.M. Lewis[2], W. Kaplan[3], K.D. Pierce[1] and P.R. Schofield[1], [1]Neurobiology Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, [2]Department of Physiology and Pharmacology, School of Medicine, University of New South Wales, NSW 2052 and [3]Peter Wills Bioinformatics Centre, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia.*

The glycine receptor is a member of the ligand gated ion channel receptor superfamily that mediates fast synaptic transmission in the brainstem and spinal cord. Following ligand binding, the receptor undergoes a conformational change that is conveyed to the transmembrane regions of the receptor resulting in the opening of the channel pore. Using the acetylcholine binding protein structure as a template, we modelled the extracellular domain of the glycine receptor α-1 subunit and identified the location of charged residues within loops 2 and 7 (the conserved Cys-loop). These loops have been postulated to interact with the M2-M3 linker region between the transmembrane domains 2 and 3 as part of the receptor activation mechanism. Charged residues were substituted with cysteine, resulting in a shift in the concentration-response curves to the right in each case. Covalent modification with 2-trimethylammonioethyl methanthiosulfonate was demonstrated only for K143C, which was more accessible in the open state than the closed state, and resulted in a shift in the EC50 towards wild-type values. Charge reversal mutations (E53K, D57K and D148K) also impaired channel activation, as inferred from increases in EC50 values and the conversion of taurine from an agonist to an antagonist in E53K and D57K. Thus, each of the residues E53, D57, K143 and D148 are implicated in channel gating. However, the double reverse charge mutations E53K:K276E, D57K:K276E and D148K:K276E did not restore glycine receptor function. These results indicate that loops 2 and 7 in the extracellular domain play an important role in the mechanism of activation of the glycine receptor.