Conformational changes in extracellular loop 2 during activation of the α1 glycine receptor

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The glycine receptor (GlyR) is a pentameric complex with an integral ion channel that is opened upon binding an agonist such as glycine. We have previously described specific residues within regions of the extracellular domain of the α1 GlyR that are associated with linking ligand binding to channel gating[1]. Here we describe the contribution of the extracellular loop 2 to this process. A series of substituted cysteine mutants were constructed at positions 52 to 57 (loop 2) of the α1 GlyR. The mutant and wild-type GlyRs were transiently expressed as homo-pentamers in 293 cells and by using standard patch-clamp techniques, the whole-cell dose-response curve and accessibility to covalent modification by methane thiosulfonate (MTS) reagents was determined. The E53C, T55C and D57C mutants all exhibited at least a 33-fold increase in the EC_{50} for glycine and a significant decrease in the maximum current, implicating this region in channel gating. The rate of MTS modification of the M56C receptor was 4-fold faster in the presence of glycine (open state) than in the absence (closed state), indicating that loop 2 changes conformation following ligand binding. Using the catalyst copper phenanthroline, it was possible to cross-link T55C with V280C in the M2-M3 loop in the same α1 subunit, causing a 36% reduction in maximum current. The rate of reaction for cross-linking was faster in the closed state than in the open state. The M2-M3 loop is known to be involved in allowing the channel pore to open[2]. Together, these data suggest that following ligand binding there is a conformational change in loop 2 such that it moves away from the M2-M3 loop in relative terms. This is an essential process for linking the ligand binding event to opening the channel pore.

1. Absalom et al. (2003) Journal of Biological Chemistry 278, 50151–50157.
2. Lynch et al. (2001) Journal of Neuroscience 21, 2589–2599.