Loop 9 residues adjacent to loop 2 are involved in glycine receptor activation

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Inter-subunit interactions within the extracellular domain of Cys-loop receptors, such as the glycine receptor (GlyR), are important for receptor activation properties. Our GlyR homology model predicts inter-subunit contacts between loop 9 and loop 2 of adjacent subunits. Our aim was to investigate the role of this interaction. In particular, residues L184 and Q186 (loop 9) are predicted to be within 4-5&#197 of T55 and M56 (loop 2). Cysteine residues were introduced at these positions alone and in pairs. The observed glycine EC_{50} was only modestly increased in M56C, L184C and Q186C mutants compared to wild-type (*n*=4-6), but was increased by 29-fold in T55C. The availability of cysteines for covalent modification was investigated using MTSES or MTSET. Changes in current evoked by EC_{30} and I_{max} glycine concentrations were monitored. MTSET application had no effect on M56C and Q186C, but increased the current response to an EC_{30} concentration in T55C (*n*=4). In contrast, the current response of L184C to EC_{30} and I_{max} concentrations was decreased (*n*=4). MTSES application had no effect on T55C and Q186C, but increased the EC_{30} current and decreased the I_{max} response of M56C and L184C (*n*=4). No glycine-evoked currents were detected for the double mutants T55C/Q186C and M56C/L184C (*n*=4). These results suggest either Q186C is not accessible to MTS reagents or the covalent modification has no functional effects. L184C is covalently modified and demonstrates differential effects of charge. Our results confirm the role of loop 2 in gating and suggest a role for loop 9 in GlyR gating.