Differential movement of M4 transmembrane segments during activation of α1 and α3 glycine receptors

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The glycine receptor (GlyR) is an anion-permeable member of the pentameric ligand-gated ion channel family that mediates inhibitory neurotransmission in the spinal cord, retina and brainstem. Although α1-containing GlyRs are more widely distributed, α3-containing GlyRs are expressed in inhibitory synapses on spinal cord nociceptive neurons. Drugs that specifically enhance α3 GlyRs have emerged as potential targets for inflammatory pain. Because the neurotransmitter binding sites in the α1 and α3 GlyRs are highly conserved, there is therapeutic relevance in searching for new drug binding sites that may exhibit a greater structural diversity between these GlyR isoforms. One such site is located in the transmembrane region of the receptor formed by the outer regions of the TM1, TM3 and TM4 domains. Here, we employed voltage clamp fluorometry to test the hypothesis that the TM4 domains of the α1 and α3 GlyRs are oriented differently with respect to their TM3 domains in the closed and/or open states.

Human α1 and rat α3 GlyRs incorporating cysteine-substituted mutations at each of the final eleven C-terminal residues (K411C – Q421C) were expressed in *Xenopus* oocytes and studied using simultaneous voltage-clamp and micro-fluorometry. Oocytes were surgically removed from anaesthetized frogs by procedures approved by the University of QLD Animal Ethics Committee. All cysteines were successfully labeled with tetramethylrhodamine methanethiosulfonate (MTS-TAMRA) and all labelled α1 GlyRs produced robust fluorescence (ΔF) responses when activated by glycine. Because the labelled α1-R414C GlyR exhibited a ΔF that was opposite in sign to those of all the other tested mutants, we infer that the microenvironment of the label attached to the α1-R414C GlyR differs from that of the labels attached to the other residues. We thus hypothesised that the label attached to α1-R414C exhibits a specific, glycine-dependent interaction with another chemical group. Molecular modelling revealed W286 in TM3 as a likely interacting residue. Because the W286F mutation altered the sign of the ΔF at R414 in the α1 GlyR but not at the corresponding residue (R422) in the α3 GlyR, we infer that the α1 GlyR TM4 exerts a direct interaction with W286 whereas the corresponding α3 TM4 residue does not.

From these results, we conclude that the TM4 domains of the α1 and α3 GlyRs are orientated differently in the cell membrane relative to the rest of the receptor. Given TM4 forms part of a drug binding site, the structural differences in this site are likely to be considerable, and thus may be useful to target in the design and *in silico* screening of α3-specific modulators.