Delineating the gating pathway in the α1 glycine receptor ligand-binding domain

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Glycine receptor chloride channels (GlyRs) mediate inhibitory neurotransmission in the central nervous system. They are members of the pentameric Cys-loop ligand-gated ion channel family. Individual subunits are each composed of a large N-terminal extracellular ligand-binding domain and 4 transmembrane α-helices (M1-M4). The ligand-binding domain of Cys-loop receptors is comprised of an inner and an outer β-sheet. Although glycine induces global changes in the structure of this domain, little is known about which conformational changes are crucial for the gating of these receptors. Recent studies on other Cys-loop family members have demonstrated an important role of the inner β-sheet during channel activation. Here, we used voltage-clamp fluorometry to address the question of whether the outer β-sheet and the domain immediately preceding the M1 domain (the pre-M1 domain) also form an essential element of the α1 GlyR activation pathway. The voltage-clamp fluorometry technique involves introducing a cystine residue into a domain of interest, specifically labelling it with a sulfhydryl-reactive fluorophore and simultaneously measuring current and fluorescence changes. This technique allows us to monitor movements in domains distant from the channel gate. *Xenopus laevis* frogs were anaesthetized in 1g/l ethyl-m-aminobenzoate according to procedures approved by the University of Queensland Animal Ethics Committee. Stage VI oocytes were then removed and injected with 10 ng of wildtype or mutant α1 GlyR mRNA into the cytosol and incubated for 3-10 days at 18°C. For labelling, oocytes were placed into ice-cold ND96 saline solution containing 10μM sulforhodamine methanethiosulfonate for 25s. Oocytes were then washed and stored in ND96 for up to 6 h before recording. For recording, oocytes were placed on the stage of an inverted fluorescence microscope. Fluorescence signals were recorded by a photodiode and membrane currents were recorded using conventional two-electrode voltage-clamp. We labelled multiple residues in three agonist-binding domains (loop E, loop F and loop C) and in the pre-M1 domain with environmentally sensitive fluorophores to follow structural rearrangements during activation. Our results confirmed that current and fluorescence changes in loop E in the inner β-sheet are closely correlated, implying a role for this domain in channel gating. In contrast, labelled residues in the outer β-sheet (loop F and loop C) and the pre-M1 domain exhibited fluorescence responses only at high glycine concentrations. Additionally, fluorescence responses in loop F and loop C did not discriminate between agonist (glycine) and antagonist (strychnine) binding. These results imply a gating pathway in the α1 GlyR ligand-binding domain that involves loop E in the inner β-sheet. We propose that structural rearrangements in the outer β-sheet are not involved with gating but maybe associated with locking the ligand into the binding site at high concentrations. The results presented here and elsewhere strongly suggest that loop E in the inner sheet is one of the main structural elements triggering channel opening. It is thus tempting to speculate that the local agonist-mediated conformational change at the binding site is forwarded to the transmembrane domains *via* the conserved Cys-loop (or the β6–β7 loop) which is directly connected to loop E. As the Cys-loop lies in close physical proximity to the M2-M3 linker, a gating pathway connecting binding site and channel gate *via* loop E, Cys-loop, M2-M3 linker and finally the M2 helix containing the channel gate, appears a likely scenario.