Conformational changes associated with desensitisation in the ligand binding domain of the glyicne receptor

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Glycine receptor chloride channels (GlyRs) belong to the Cys-loop ligand-gated ion channel receptor family. Synaptic GlyRs are responsible for mediating inhibitory neurotransmission in the spinal cord, brainstem and retina. Agonists binding to the extracellular ligand binding domain (LBD) induce local conformational changes that are propagated to the distant activation gate in the transmembrane domain to open the channel pore. Ligand-gated channels also display a phenomenon termed desensitisation, which is the progressive fading of the ionic flux in the prolonged presence of agonist. The rates of onset and recovery from desensitisation are important parameters governing the size and decay rate of synaptic currents. Despite the physiological and pathological importance of this process, very little is known about the conformational changes mediating desensitisation in Cys-loop receptors. Here we employed voltage clamp fluorometry (VCF) in an attempt to systematically map LBD conformational changes that accompany desensitization with a view to developing a structural model of this process.

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 removed and injected with 10 ng of wildtype or mutant a1 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 10 μM sulforhodamine methanethiosulfonate (MTSR) for 25 s. 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 photomultiplier and membrane currents were recorded using conventional two-electrode voltage-clamp.

We have previously shown that the following LBD sites can be productively labelled with MTSR: A52C, Q67C, L127C, G181C, N203C, H201C, G221C, Q219C, L127C, S121C and M227C. In each case, agonist application results in non-desensitizing current and fluorescence responses. In order to induce fast current desensitization, we incorporated the intracellular A248L mutation in conjunction with each of the above mutations. Our aim as to determine whether fluorescence responses desensitized with the same time constant (τ) as the current response, or whether they remained non-desensitising. A desensitizing fluorescence response would be taken as evidence that the label was detecting a local conformational change associated with desensitization. Incorporation of the A248L mutation resulted in fast desensitizing current responses (τ < 3000ms) at all double mutant receptors studied. However, labels attached to Q67C, G181C, N203C, H201C and G221C showed non-desensitizing fluorescence responses, indicating that these residues are not involved with conformational changes mediating desensitization. The loop 2 mutant, A52C, and the pre-M1 domain mutant, M227C, are both located near the transmembrane domain interface. We found the glycine-induced fluorescence signal of both residues had the same desensitization rate as the current (A52C: τ for current = 3500 ± 200 ms and τ for fluorescence = 3150 ± 360 ms; M227C: τ for current = 3250 ± 130 ms and τ for fluorescence = 3280 ± 350 ms).

We found no evidence for desensitization-induced conformational changes in the domains that form the glycine binding site or in the inner β-sheet domain of the LBD. However, we did find evidence that labelled sites in loop 2 and the pre-M1 domain did alter conformation during receptor desensitization. These sites are located close to the interface between the LBD and the transmembrane domain. Further experiments focused on these regions should help elucidate the structural changes that mediate desensitization in this important model receptor family.