

Conformational variability of the glycine receptor M2 domain in response to activation by different agonists

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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 pore-lining M2 domains are kinked radially inwards to form a central constriction at the membrane midpoint. Current models of cys-loop receptor activation consider only structural changes associated with transitions from the resting closed to the agonist-induced open states. Little attention has been given to the possibility that different agonists and pharmacological modulators may promote different structural conformations in the pore region. Our aim was to compare the conformational changes induced by agonists, antagonists and allosteric modulators by covalently labelling two residues (α R19'C, L22'C) near the extracellular M2 boundary with a sulfhydryl-reactive fluorophore and simultaneously measuring current and fluorescence changes.

Xenopus laevis frogs were anaesthetised 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 α 1 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 μ M sulforhodamine methanethiosulfonate for 25s. Oocytes were then washed and stored in ND96 for up to 6 hrs 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. For spectral analysis a MicroSpec 2150i (Acton Research Corporation, Acton, MA) coupled to an ORCA-ER CCD camera (Hamamatsu, Hamamatsu City, Japan) replaced the photodiode detection system.

During glycine-induced activation of the homomeric α 1R19'C GlyR, fluorescence of the label attached to α R19'C increased by ~20% and the emission peak shifted to lower wavelengths, consistent with a more hydrophobic fluorophore environment. In contrast, ivermectin activated the receptors without producing a fluorescence change. Although taurine and β -alanine were weak partial agonists at the α 1R19'C GlyR, they induced large fluorescence changes. Propofol, which drastically enhanced these currents, did not induce a glycine-like blue-shift in the spectral emission peak. The inhibitors, strychnine and picrotoxin, elicited fluorescence and current changes as expected for a competitive antagonist and an open channel blocker, respectively. Glycine and taurine (or β -alanine) also produced an increase and a decrease, respectively, in the fluorescence of a label attached to the nearby L22'C residue.

Together, these results lead us to conclude that different agonists activate the GlyR by producing different conformational changes to the external region of the M2 domain. Thus, the top of M2 seems to display a conformational mobility which is not necessarily coupled to movements of the channel gate.