A novel startle mutation of the α1 glycine receptor subunit reveals the role of W170 in receptor function

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Startle disease is a neurological disorder that results in neonatal hypertonia and an exaggerated response to unexpected auditory, visual and tactile stimuli. This disorder results from a deficiency in glycinergic neurotransmission - largely as a consequence of glycine receptor (GlyR) mutation. The novel startle mutation α1 (W170S) (Al-Futaisi *et al*., 2012) is located on β strand 8 in the extracellular domain, based upon homology models using the glutamate gated (hyperlink) and $GABA_A$ (Miller and Aricescu, 2014) chloride channel structures as templates. This places α1 (W170) adjacent to the ligand binding site but facing towards the hydrophobic core of the subunit. In addition, W170 appears to be conserved across all subunits of pentameric ligand gated anion receptors of multiple species including both vertebrates and invertebrates.

Whole-cell patch clamp recordings of HEK293 cells expressing homomeric α1 (W170S) GlyRs indicated a 4-fold increase in glycine EC_{50} (117 ± 1.1 μM, n = 7) in comparison with wild-type GlyRs (30.1 ± 1.1 μM, n = 5). Substitution with alanine increased the glycine EC_{50} further (240 ± 1.1 μM, n = 6), as did substitution with other large hydrophobic residues (W170F, 175 ± 1.2 μM, n = 5 and W170Y, 1185 ± 1.2 μM, n = 5), compared to wild-type (P < 0.05). In the presence of the partial agonist β-alanine, α1 (W170S) GlyR currents were not significantly changed (EC_{50} 77 ± 1.3 μM, n = 5) from wild-type (68 ± 1.2 μM, n = 5, P > 0.05). Single channel recordings in the presence of glycine did not reveal any significant changes to the open time distributions of α1 (W170S) compared to wild-type. Long-range mutant cycle analysis (Gleitsman *et al*., 2009) of heteromeric α1 (W170A) β GlyR (EC_{50} 191 ± 1.3 μM, n = 4) and the reporter gating mutation α1 (L9'S) β (1.4 ± 1.3 μM, n = 4), demonstrated a coupling coefficient of 2.4 when compared to wild-type heteromeric α1β (105 ± 1.3 μM, n = 4) and α1 (W170A-L9'S) β (7.0 ± 1.1 μM, n = 4) GlyRs. The coupling coefficient indicates that W170 is likely to be involved in the process of receptor gating.

Investigations of our homology models indicate that W170 forms a H-bond between the nitrogen on the indol ring and the backbone of α1 (R96), which is not present with substitutions to F and Y. This H-bond links β strands 8 and 9, which are continuous with highly mobile ligand binding loops F and C, respectively. Both the location and functional effects of mutation suggests that W170 stabilises a conformation following ligand binding that is required for the full agonist activity of glycine. We hypothesize that the partial agonist β-alanine is unable to promote this particular conformation. In addition, it seems plausible that this function could be preserved in other pentameric ligand gated anion channels including GABA and glutamate gated chloride receptors, since the W residue is conserved at homologous positions in these receptor subunits.

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