The glycine receptor – a new therapeutic target for chronic inflammatory pain

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Glycine receptor (GlyR) chloride channels are members of the pentameric ligand-gated ion channel family that mediates fast neurotransmission in the brain. Although GlyRs are best known for mediating inhibitory neurotransmission in reflex circuits of the spinal cord, they also mediate inhibitory neurotransmission onto spinal nociceptive neurons in superficial laminae of the spinal cord dorsal horn. GlyR α3 subunits, which are otherwise sparsely distributed, are abundantly expressed in these synapses. Chronic inflammatory pain sensitization is caused by a prostaglandin E2 (PGE2)-mediated activation of protein kinase A (PKA), which in turn phosphorylates α3 GlyRs at S346, leading to a diminution of glycinergic synaptic current magnitude. This disinhibits spinal nociceptive sensory neurons resulting in chronic inflammatory pain sensitization. Due to their sparse distribution outside the spinal cord dorsal horn, α3 GlyRs have emerged as preferred therapeutic targets for chronic pain, and agents that selectively potentiate α3 GlyRs have been shown to exhibit analgesic efficacy in animal models of chronic inflammatory pain. Here we sought to compare glycine-induced conformational changes in α1 and α3 GlyRs to identify structural differences that might be exploited in the design of α3-specific therapeutics.

GlyRs comprising mutated α1 or α3 subunits 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 Queensland Animal Ethics Committee.

Using voltage-clamp fluorometry, we showed that glycine-mediated conformational changes in the extracellular M2-M3 channel gating domain were indeed significantly different between α1 and α3 GlyRs. By using a chimeric approach, we found that structural variations in their respective large intracellular M3-M4 domain were responsible for this difference. This prompted us to test the hypothesis that phosphorylation of S346 (in the M3-M4 domain of the α3 GlyR) might also induce extracellular conformation changes. Surprisingly, phosphorylation modified glycine-induced structural changes in both the M2-M3 loop and the glycine-binding site of α3 GlyRs. As an independent way of confirming this finding, we showed that the potency of β-carboline antagonists that bind in the α3 GlyR glycine binding site, is enhanced by phosphorylation.

These results are important for two reasons. First, they provide the first direct evidence for phosphorylation producing extracellular conformational changes in any ligand-gated ion channel family member, and thus suggest new loci for investigating how phosphorylation modulates structure and function in this receptor family. Second, by demonstrating that inflammatory pain sensitization confers a unique conformational change in the α3 GlyR glycine-binding site, they raise the possibility of developing novel analgesic drugs that selectively target disease-affected GlyRs.