A molecular determinant of tropisetron inhibition of the glycine receptor Cl^- channel

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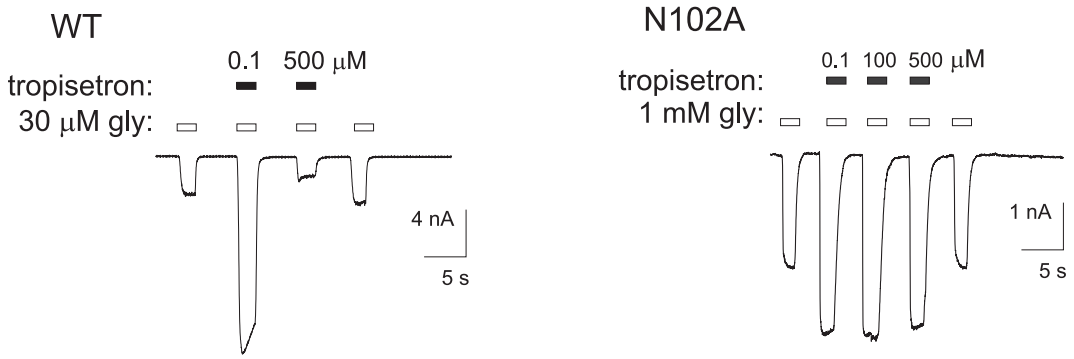
Tropisetron, an antagonist of the 5-HT3 receptor cation channel, is used clinically as an anti-emetic drug. It also has potent effects on the structurally related glycine receptor Cl^- channel (GlyR). At low (sub-micromolar) concentrations, tropisetron potentiates the GlyR and at higher concentrations it produces inhibition (Supplisson & Chesnoy-Marchais, 2000). Since prostaglandins increase the transmission of pain impulses to the brain *via* downregulation of spinal glycinergic neurotransmission, the GlyR has emerged as a novel target for therapies directed at neuropathic pain (Harvey *et al.*, 2004). As a potentiating agent, tropisetron is a lead compound for the development of novel analgesic therapeutics directed at the GlyR. However, the locations of the inhibitory and potentiating tropisetron binding sites on the GlyR are unknown. This study sought to identify the tropisetron inhibitory binding site on homomeric α1 and heteromeric α1β GlyRs.

HEK293 cells were transfected with WT and mutant GlyR cDNA using the calcium phosphate precipitation protocol. When co-transfecting α1 and β subunits, their respective cDNAs were combined in a ratio of 1:10. The transfection solution was removed after 24h and glycine-gated currents were recorded using whole-cell patch clamp techniques over the following 24-72 h. Heteromeric GlyRs were identified by GFP fluorescence coupled to β subunit expression and by their reduced sensitivity of heteromeric GlyRs to picrotoxin.

We first confirmed that sub-micromolar concentrations of tropisetron elicited potentiation and that concentrations above 100 μM inhibited the WT α1 GlyR (Figure, left panel). We then used 500 μM tropisetron to screen a large number of mutant GlyRs in which various known ligand binding sites were abolished. We investigated the principal ligand-binding domain A (*via* mutations I93A, A101H/C, N102A/C/D/Q), domain B (F159A, Y161C) and domain C (K200A, H201A, Y202F, N203A). We also serially eliminated the zinc binding sites (H107N, H109N) and the alcohol binding site (S267C). The four N102 mutations were the only tested mutations that abolished inhibition and in each case this was achieved without affecting tropisetron potentiation (Figure, right panel). When the N102Q mutant α1 subunit was co-expressed with the WT β subunit, tropisetron inhibition returned to near normal potency. N125 in the β subunit residue corresponds to N102 in the α1 subunit. When the N102Q mutant α1 subunit was co-expressed with the N125D mutant β subunit, tropisetron inhibition was also normal.



We conclude that N102 in the α1 subunit is a specific determinant of tropisetron inhibition. Its location in the agonist binding pocket implies that it may be a tropisetron binding site. Our results indicate that β subunits also contain tropisetron inhibitory sites. However, the location of the β subunit site does not correspond to its location in the α1 subunit.

Supplisson, S. & Chesnoy-Marchais, D. (2000) *Molecular Pharmacology*, 58, 763-777.

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