Ivermectin interacts with an intersubunit transmembrane domain of the glycine receptor

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Ivermectin is a widely-used anti-parasitic drug that is effective against nematodes and insects. It paralyses and starves nematodes by activating inhibitory currents at glutamate-gated chloride channels (GluCl). It also activates other members of the Cys-loop ligand-gated ion channel superfamily including the human glycine receptors (GlyR). The location of the ivermectin binding site on these receptors is not known. Homomeric and heteromeric Cys-loop receptors are formed by five subunits that each contain a large N-terminal ligand-binding domain and four membrane-spanning helices (M1-M4). We recently showed that ivermectin sensitivity at GluCls and GlyRs depends on the amino acid identity at a particular location in the third transmembrane (M3) domain (GlyR Ala288). We hypothesized that tryptophan substitution of residues vicinal to Ala288 might also impair activation by ivermectin and provide a structural basis for understanding the binding interaction between ivermectin and GlyR.

We used site-directed mutagenesis to generate several GlyRs containing a bulky tryptophan residue in a domain formed by M3 (including Ala288) from one subunit and M1 from an adjacent subunit, according to the high-resolution structures of analogous proteins. HEK-293 cells were transfected with wild-type (WT) or mutant GlyR DNA and sensitivity to ivermectin was measured by recording ivermectin-mediated current magnitudes using whole cell patch clamp recording. Several mutants showed 2-4-fold shifts in EC_{50} values for activation by ivermectin. However, the M1 mutant Pro230Trp showed a 100-fold increase in ivermectin EC_{50} value ($p<0.001$, $n=4$). M3 Leu291Trp and M1 Leu233Trp were not activated by ivermectin at all ($n=8$). Furthermore, at both of these mutants, ivermectin irreversibly inhibited responses to glycine, with 30 μM ivermectin causing 100% inhibition of responses to saturating glycine concentrations ($n=5$). Ala288Trp was poorly expressed but showed small responses to glycine and no responses to ivermectin.

Ala288, which is located in M3, is directly opposite Pro230, located in M1, in our model of the intersubunit transmembrane domain, and substitution of either residue for bulky amino acids decreases sensitivity to activation by ivermectin. M3 Leu291 is directly opposite M1 Leu233 in this domain, and mutation of either of these leucine residues for tryptophan converts ivermectin from an agonist to a ligand that antagonises activation by glycine. Since mutations in the upper part of this domain reduce the sensitivity to activation by ivermectin and mutations in the lower part reverse the effects of ivermectin, we conclude that while this domain might contribute to an ivermectin binding site, it certainly forms a crucial part in the agonist transduction pathway. This result provides an important insight into the binding and gating mechanisms of this important anti-parasitic drug.