Location of an ivermectin binding site at the glycine receptor chloride channel

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Ivermectin is a semi-synthetic anthelmintic drug used widely in human medicine and veterinary practice. The biological target for ivermectin and related macrocyclic lactones is a glutamate-gated chloride channel receptor (GluClR) that is expressed in the neurons and muscle cells of nematodes and some arthropods but is absent in vertebrates. Ivermectin irreversibly activates these GluClRs at low nanomolar concentrations, thereby inhibiting neuronal activity and muscle contractility and thus inducing nematode death by flaccid paralysis. GluClRs belong to the Cys-loop ion channel receptor family that also includes nicotinic acetylcholine receptors, GABA type-A receptors and glycine receptor (GlyR) chloride channels. Although most Cys-loop receptors are sensitive to micromolar ivermectin concentrations, GluClRs and A288G mutant GlyRs are both activated by low nanomolar ivermectin concentrations. The crystal structure of the *C. elegans* α GluClR complexed with ivermectin has recently been published. Here we investigated the ivermectin binding site on the α1 GlyR using site-directed mutagenesis and patch-clamp electrophysiology.

The human α1 GlyR subunit cDNA was subcloned into the pCIS plasmid vector. Site-directed mutagenesis was performed using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA, USA) and the successful incorporation of mutations was confirmed by DNA sequencing. HEK-293 cells were transfected with wild-type or mutant GlyR cDNA and sensitivity to ivermectin was measured by recording ivermectin-mediated current magnitudes using whole cell patch clamp recording. Based on previous data from our laboratory, we hypothesized that ivermectin binds in the transmembrane domain region of the receptor. Accordingly, we performed a site-directed mutagenesis screen of all residues in the first, second and third transmembrane domains of the α1 GlyR. By doing so, we identified A288 and P230 as the two most crucial ivermectin sensitivity determinants. As these two residues face towards each other across the transmembrane subunit interface, we hypothesized ivermectin binds in the intersubunit cleft. A comparison of the actions of selamectin and ivermectin suggested the benzofuran C05-OH moiety was crucial for the binding interaction. When taken together with docking simulations, these results provide independent support for a GlyR ivermectin binding orientation similar to that seen in the GluClR crystal structure. However, whereas the crystal structure shows that ivermectin interacts with the α GluClR *via* H-bonds with L218, S260 and T285 (α GluClR numbering), our data indicate that H-bonds with residues homologous to S260 and T285 are not important for high ivermectin sensitivity or direct agonist efficacy in A288G α1 GlyRs or three other GluClRs. Our data also suggest that van der Waals interactions between the ivermectin disaccharide and GlyR M2-M3 loop residues are unimportant for high ivermectin sensitivity. Thus, although our results independently corroborate the ivermectin binding orientation as revealed by the crystal structure, they demonstrate that some of the binding interactions revealed by this structure do not pertain to other highly ivermectin-sensitive Cys-loop receptors. Understanding the mechanisms of ivermectin binding will be crucial for designing new drugs as anthelmintics and as therapies for a wide range of human neurological disorders.