Development of an improved ivermectin-activated receptor for neuronal silencing

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Purpose: Reversible silencing of particular neuron groups should elucidate functions of neurons within a circuit and could possibly tune excess neurotransmission in disease. One method is to express an inhibitory signalling receptor and activate that receptor with a selective pharmacological agent. The α1 glycine receptor (GlyR) is a ligand-gated ion channel mediating inhibitory neurotransmission, and is also activated by the antihelminth, ivermectin. We developed a mutant GlyR that is insensitive to glycine but sensitive to ivermectin.

Methods: We used a high throughput fluorescence based assay to screen numerous mutant GlyRs expressed in HEK293 cells and found that one mutant with a single amino acid substitution was less sensitive to ivermectin than wildtype. Using site-directed mutagenesis we generated a mutant, A288G, that increased sensitivity to ivermectin. We combined this mutation with F207A, which is known to abolish glycine binding, to produce a receptor (FAAG) that is both insensitive to glycine and highly sensitive to ivermectin. We tested this new receptor by electrophysiology in HEK293 cells and cultured hippocampal neurons.

Results: In HEK293 cells, FAAG showed 50-fold lower EC50 values than WT (FAAG, 19±6 nM; WT, 1.1±0.3 μM; n=5). FAAG was only activated by high millimolar concentrations of glycine (EC50 values, FAAG, > 10 mM; WT, 39±6 μM; n=5). Cultured neurons transfected with FAAG showed chloride currents upon application of 10 and 100 nM ivermectin, while current in WT-transfected neurons were only activated by application of 1 μM ivermectin and higher.

Conclusions: FAAG appears to be a suitable pharmacological silencing receptor, as it conducts chloride current upon applications of low concentration ivermectin and is responsive to only high millimolar concentrations of glycine.