Effects of new human startle disease mutations on glycine receptor function and structure

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Startle disease, or hyperekplexia, is a rare neurological disorder characterized by exaggerated startle responses. It is mainly caused by hereditary mutations in the human glycine receptor (hGlyR). hGlyRs, which conduct chloride ions, play a major role in inhibitory neurotransmission and are widely distributed in the central nervous system. Most synaptic hGlyRs consist of two α1 and three β subunits. Mutations in the genes that code for hGlyR subunits can cause changes in expression efficiency and function, thereby disrupting inhibitory neurotransmission, leading to startle disease. In this study, we describe the functional characterization of 17 novel hGlyR α1 and β mutations discovered on the basis of genetic and clinical analyses of hyperekplexia index cases.

Expression constructs for wild type and mutated α1 and β hGlyRs were transfected into HEK AD293 cells to quantify the functional properties of both homomeric and heteromeric hGlyRs. This was done *via* a fluorescence flux assay to average effects over large cell numbers and *via* patch-clamp electrophysiology for higher resolution analysis from individual cells. Additionally, the cellular localization of the mutated subunits was determined using immunofluorescence and a biotinylation assay. For voltage-clamp fluorometry experiments, hGlyR RNA was injected into frog oocytes.

We found that startle disease mutations in the hGlyR α1 mainly affected the functional properties of the channels. The mutations produced a variety of effects including spontaneous channel activity and fast desensitization. In contrast, mutations in the β subunit invariably affected the trafficking of functional receptors to the cell surface. The molecular basis of the functional defects was further characterized for some mutated subunits. Of particular note, the V280M mutation located in the TM2-TM3 loop caused spontaneous, side-chain volume dependent channel openings. Our data suggest that perturbations at the 280 position affected conformational changes at the extracellular end of TM2 and in the TM2-TM3 loop - both channel segments which are important in signal transduction.

This study establishes the hGlyR β subunit as the 3rd major gene for startle disease. Moreover, by identifying new mutations that disrupt hGlyR function in unexpected ways, the results of this study provide important new insights into the structure and function of this important Cys-loop receptor model system.