Diverse changes to $GABA_A$ receptor function by mutations that cause severe childhood epilepsies

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The γ-aminobutyric acid type A ($GABA_A$) receptor is a pentameric ligand-gated ion channel that mediates synaptic transmission in the brain. The synaptic forms of the receptor are typically composed of three α, two β and one γ subunit. Recent advances in DNA sequencing technologies have identified a considerable number of mutations within several genes that encode for $GABA_A$ receptor subunits, including the α1, β3 and γ2 subunits. These dominant mutations cause severe childhood epilepsies including Lennox-Gastaut and Dravet syndromes that are resistant to pharmacological treatment. It is unclear how these mutations affect receptor function, and whether heterozygous receptors with a single mutation are similarly affected to homozygous receptors with two mutations.

Therefore, to determine how these mutations alter the function of the receptor, we created a concatenated α1β3γ2 receptor that enabled the expression of both heterozygous and homozygous mutations in *Xenopus* oocytes and measured the responses using two-electrode voltage clamp electrophysiology. We validated wild-type receptor function by performing concentration-response curves to GABA, where the potency of GABA was similar to native receptors. Furthermore, diazepam and clobazam, both positive allosteric modulators of γ-containing $GABA_A$ receptors, enhanced GABA mediated currents.

Each subunit contains a large extracellular domain where GABA binds at the interface of α and β subunits, four transmembrane regions M1-M4 where the second, M2 lines the channel pore, two short intracellular loops M1-M2 and M2-M3 and a large intracellular M3-M4 loop. Mutations were identified in diverse regions of the receptor, and we created mutations located in a variety of regions. We chose four mutations where three (A1067T, I107T and M199I) mutations located in the extracellular domain of in the γ2 subunit did not produce currents. Alternatively, the R323Q mutation located in the M2-M3 coupling loop significantly increased the potency of the receptor to GABA.

We created four β3 mutations in both heterozygous configurations of the receptor, and in the homozygous. The D120N, T157M and Y302C heterozygous mutations all significantly increased the potency of the receptor to GABA, while receptors homozygous for these three mutations further shifted the potency of GABA and significantly reduced the efficacy of the receptors. Surprisingly, the S254F mutation located in the M1 increased the potency of the receptor to GABA on one heterozygous configuration and for homozygous receptors.

Taken together, we have demonstrated that mutations in the GABA receptor that cause severe intractable epilepsy have surprisingly diverse functional effects. Most mutations either abolish GABA-activated currents or severely impair the ability of GABA to activate the receptor. However, one mutation increased the ability of the receptor to respond to GABA. It is likely that anticonvulsants that target the GABA receptor will have different efficacies at patients with different mutations, given they lead to such diverse functional effects.