Concatenated $GABA_A$ receptors reveal diverse molecular phenotype of epilepsy-causing mutations

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Recent advances in whole genome sequence have enabled the identification of de novo mutations that cause a range of childhood epilepsies. Multiple mutations have been discovered in genes that encode for subunits of the γ-aminobutyric acid receptor type A ($GABA_A$), specifically GABRA1, GABRB3 and GABRG2 that encode the α1, β3 and γ2 subunits respectively. These mutations are dominant and will express both wild-type and mutant subunits, resulting in a mixture of receptors being expressed at the cell surface.

To determine the consequences on receptor function, we created a concatenated γ2-β3-α1-β3-α1 receptor and expressed it in Xenopus oocytes. The receptor responded to GABA at a similar concentration range to receptors created from free subunits and was positively modulated by the benzodiazepine clobazam. We then introduced the γ2(R323Q), β3(E77K), β3(D120N), β3(T157M), β3(S254F) and β3(Y302C) mutations in either heterozygous or homozygous configurations. We measured the change in function by constructing concentration-response curves to GABA and estimating the maximum open probability (Est Po) by applying GABA, etomidate and diazepam.

The potency of GABA at the γ2(R323Q) mutation was decreased while the maximum Est Po was unchanged. Similarly, when the β3(D120N) and β3(T157M) mutations were introduced at either location the GABA potency was decreased, however when the mutations were introduced at both locations the activation by GABA was completely abolished. The β3(Y302C) mutation at either location decreased the maximum Est Po and reduced the potency of GABA, while mutations at both locations shifted the concentration-response curve to the right and further reduced the maximum Est Po.

The β3(E77K) and β3(S254F) receptors displayed a very different phenotype. Introducing the β3(E77K) mutation to either location increased the potency of GABA and was defined as a gain-of-function mutation. Introducing the β3(S254F) at different locations either reduced or increased the GABA potency depending on the location of the mutation.

In most cases, introducing the epilepsy-causing mutations impaired GABA function, and the introduction of a mutation at one location caused an intermediate phenotype compared to the introduction of two mutations. However, there were exceptions to this, where the mutation altered the function of the receptor differently depending on the location, or shifted the concentration-response curve to the left.