

ANOTHER “TONIC” IN THE REALM OF EPILEPSY

GABRD Encoding a Protein for Extra- or Perisynaptic GABA_A Receptors Is a Susceptibility Locus for Generalized Epilepsies

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A major challenge in understanding complex idiopathic generalized epilepsies has been the characterization of their underlying molecular genetic basis. Here we report that genetic variation within the *GABRD* gene, which encodes the γ -aminobutyric acid (GABA)_A receptor δ subunit, affects GABA current amplitude consistent with a model of polygenic susceptibility to epilepsy in humans. We have found a *GABRD* Glu177Ala variant that is heterozygously associated with generalized epilepsy with febrile seizures plus. We also report an Arg220His allele in *GABRD* that is present in the general population. Compared with wild-type receptors, $\alpha 1\beta 2\delta$ GABA_A receptors containing δ Glu177Ala or Arg220His have decreased GABA_A receptor current amplitudes. As GABA_A receptors mediate neuronal inhibition, the reduced receptor current associated with both variants is likely to be associated with increased neuronal excitability. Because δ subunit-containing receptors localize to extra- or perisynaptic membranes and are thought to be involved in tonic inhibition, our results suggest that alteration of this process may contribute to the common generalized epilepsies.

receptors. These receptors are pentameric hetero-oligomers assembled from seven different subunit classes, some of which have multiple members: $\alpha(1-6)$, $\beta(1-3)$, $\gamma(1-3)$, δ , ϵ , θ , and π (1). In theory, a bewildering array of various heteropentameric combinations could assemble from all of the subunits and their splice variants, but most GABA_A-receptor subtypes found in the brain appear to be assemblies of a limited number (a few dozen at most) of well-defined subunit combinations (1,2). GABA_A receptors at synapses usually contain γ subunits, in particular γ_2 subunits, which are required for benzodiazepine sensitivity (1). This phasic (synaptic) GABAergic transmission is produced by high concentrations of GABA (0.3 to 1 mM) that are short lived in the cleft (<1 msec) (3,4). Depending on the synapse, the GABA concentration transient may or may not saturate the GABA_A receptors present on the postsynaptic side. Saturated or not with GABA, the activation of synaptic GABA_A receptors produces an inhibitory postsynaptic current shaped by the properties and number of receptors and by the magnitude and duration of the GABA transient. Epilepsy researchers knew how important GABA_A receptors were in epilepsies, but the genetic proof had to wait until 2001 when, first, mutations in the γ_2 subunit were shown to be associated with childhood absence epilepsy, febrile seizures, and generalized epilepsy with febrile seizures plus (GEFS+), and, second, a mutation in the α_1 subunit was discovered in a family with autosomal dominant juvenile myoclonic epilepsy (5,6). These mutations are expected to change the effectiveness of synaptic (phasic) inhibition, and studies are well under way to characterize just how synaptic inhibition is affected by the altered subunits.

Lately, another type of inhibition, tonic inhibition, has received much attention (7). The word “tonic” refers to the mode of activation of the GABA_A receptors underlying this type of inhibition. Specific receptors located just outside the perimeter of GABA synapses (perisynaptic) or far away from the synapses (extrasynaptic) are activated by the micromolar levels of GABA constantly present in the extracellular space. If these receptors are nondesensitizing and have a high affinity for GABA, they will generate a constant-current flow through the membrane, thus the term “tonic” inhibition. The magnitude of this perpetual conductance can effectively alter the input/output gain control of the neuron (7). The magnitude of the tonic current is not large, a few tens of pA, but when time averaged, it carries more than 75% of the charge through GABA_A receptors,

COMMENTARY

Epileptologists know about “tonic” from the tonic seizures common to the Lennox–Gastaut syndrome. But from now on, epileptologists will need to become familiar with another use of “tonic” in relation to epilepsies: *tonic inhibition*. Just like synaptic (phasic) inhibition in the brain, tonic inhibition also is mediated by γ -aminobutyric acid (GABA)_A

even when the synaptic (phasic) currents bombard the cells with high frequencies (7).

Thus far, two classes of GABA_A-receptor combinations have been shown to mediate tonic inhibition. The δ subunit-containing GABA_A receptors are localized peri- or extrasynaptically, have a high affinity for GABA, and little desensitization. These receptors have been shown to underlie tonic inhibition in cerebellar and dentate gyrus granule cells (8). In CA1 pyramidal cells, where δ subunits are scarce, the γ subunit-containing $\alpha_5\beta_3\gamma_3$ receptors seem to underlie tonic inhibition (9). It is not known how many other GABA_A receptor combinations satisfy the criteria to be mediators of tonic inhibition, but the δ subunit-containing receptors are clearly at the forefront of tonic inhibitory activity in the brain.

This finding of an inexorable link of δ subunits to tonic inhibition is why the study by Dibbens et al., describing epilepsy-associated mutations in the δ subunit encoding the *GABRD* gene, should be exciting for researchers who think GABAergic inhibition is important in this disorder. Thus only a very few epileptologists should be left unexcited! Moreover, the study may be the first step toward unveiling complex, multiple gene traits associated with epilepsies.

Some of the mutations described in the article by Dibbens et al. disrupt the function of the receptors underlying tonic inhibition. Two mutations (Glu177Ala and Arg220Cys) in the extracellular N-terminal domain of δ subunits were found to be heterozygously associated with GEFS+ in unrelated patients. None of these mutations were found in the population at large. When expressed in human embryonic kidney (HEK293T) cells, the Glu177Ala mutated δ subunits together with α_1 and β_2 subunits had the usual high GABA affinity but much less GABA efficacy. The receptor expression might have been low, or the coupling of GABA binding to channel gating might have been impaired. Interestingly, the Arg220Cys mutation did not seem to have an effect on the receptors. However, the $\alpha_1\beta_2\delta$ subunit combinations used in the study comprise only a small portion of the δ subunit-containing GABA_A receptors found in the forebrain. Most δ subunits assemble with α_4 subunits (or α_6 subunits in the cerebellum) to form functional receptors, and it would not be surprising to find subunit-specific differences in the behavior of the mutated receptors. It also must be pointed out that the experiments in this study mostly used high (1 mM) GABA concentrations to activate the mutated receptors. Other differences may become obvious when using low GABA concentrations (in the μ M range) that these receptors are likely to experience in the brain.

One of the most intriguing findings of the study is that another mutation of Arg220 to His, found in 4.2% of the general population, also significantly reduced GABA currents in the expression system. This mutation occurred with roughly

the same frequency in patients with febrile seizures (4.5%), GEFS+ (3.1%), idiopathic generalized epilepsy (8.3%), and juvenile myoclonic epilepsy (3.7%), leading the authors to propose that this mutation could be a susceptibility allele responsible for the complex epilepsies when combined with other, yet to be identified, susceptibility alleles. It is anticipated that some of the other key susceptibility alleles will be identified in the near future leading to a better understanding of the etiologies of complex epilepsies. However, considering that δ subunit-containing GABA_A receptors are the targets of important endogenous modulators, such as neuroactive steroids (8) and much abused exogenous modulators, such as ethanol (10), the fact that 4.2% of us are walking around with a mutation affecting the tonic inhibition in our brain should spark even more interest in the study of this form of inhibition.

Editor's note: Please see article by Richardson pages 239–242 which highlights the same topic.

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