

WHEN IS HOT NOT SO HOT? FEVER REDUCES BRAIN INHIBITION

Why Does Fever Trigger Febrile Seizures? GABA_A Receptor γ 2 Subunit Mutations Associated with Idiopathic Generalized Epilepsies Have Temperature-Dependent Trafficking Deficiencies

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With a worldwide incidence as high as 6.7% of children, febrile seizures are one of the most common reasons for seeking pediatric care, but the mechanisms underlying generation of febrile seizures are poorly understood. Febrile seizures have been suspected to have a genetic basis, and recently, mutations in GABA_A receptor and sodium channel genes have been identified that are associated with febrile seizures and generalized seizures with febrile seizures plus pedigrees. Pentameric GABA_A receptors mediate the majority of fast synaptic inhibition in the brain and are composed of combinations of α (1–6), β (1–3), and γ (1–3) subunits. In $\alpha\beta\gamma$ 2 GABA_A receptors, the γ 2 subunit is critical for receptor trafficking, clustering, and synaptic maintenance, and mutations in the γ 2 subunit have been monogenically associated with autosomal dominant transmission of febrile seizures. Here, we report that whereas trafficking of wild-type α 1 β 2 γ 2

receptors was slightly temperature dependent, trafficking of mutant α 1 β 2 γ 2 receptors containing γ 2 subunit mutations [γ 2(R43Q), γ 2(K289M), and γ 2(Q351X)] associated with febrile seizures was highly temperature dependent. In contrast, trafficking of mutant α 1 β 2 γ 2 receptors containing an α 1 subunit mutation [α 1(A322D)] not associated with febrile seizures was not highly temperature dependent. Brief increases in temperature from 37 to 40°C rapidly (<10 min) impaired trafficking and/or accelerated endocytosis of heterozygous mutant α 1 β 2 γ 2 receptors containing γ 2 subunit mutations associated with febrile seizures but not of wild-type α 1 β 2 γ 2 receptors or heterozygous mutant α 1(A322D) β 2 γ 2 receptors, suggesting that febrile seizures may be produced by a temperature-induced dynamic reduction of susceptible mutant surface GABA_A receptors in response to fever.

COMMENTARY

Genes involved in human epilepsies recently have been identified, primarily coding for proteins involved in neurotransmission, such as receptors and ion channels (1). Some types of epilepsy, usually the more rare forms, show simple inheritance, but most syndromes demonstrate complex genetics. Genes for epilepsy, however, have been identified with some frequency in recent years and afford great hope for new understanding of mechanisms and potential therapeutics.

Identification of an epilepsy gene does not mean that there is automatically an explanation of how the mutation causes seizures. Most of the mutations are missense point mutations, with a modest change in protein activity. Some mutations involve frame shifts, deletions, or truncations, leading to more severely damaged proteins and sometimes lack of protein. Whether the disorder is dominant or recessive also influences the extent of malfunction of heterozygous mutations, as some impaired but also some normal protein is present. Even with

channelopathies, identified in numerous human diseases, mutations can affect phenotype in multiple manners at the protein level. For example, in nicotinic acetylcholine receptors, implicated in autosomal dominant nocturnal frontal lobe epilepsy, mutations can alter function by affecting the agonist binding site, the ion channel, the allosteric coupling between the two, the subunit interfaces that affect heteropentamer assembly, as well as intracellular domains involved in protein–protein interactions needed for intracellular trafficking and cross-talk with other signaling systems, including other receptors, second messengers, and/or protein phosphorylation (2). These functions must be assessed in vitro to pinpoint possible epileptogenic consequences of each mutation. With most point mutations, more than one alteration in protein function typically occurs. However, some investigators are unaware of this fact and have studied only one change in protein activity rather than addressing the multiple effects on the phenotype that actually occur, which has led to minor controversies between investigators. One intriguing question is how any of the identified mutations might cause febrile seizures. Kang et al. have a viable theory.

Prominent among the ion channels with mutations producing epilepsy are the GABA_A receptor genes, a family of

19 subunits arranged as heteropentamers (like nicotinic acetylcholine receptors), but with a chloride channel mediating inhibition, instead of a cation channel mediating excitation (3). As the major inhibitory neurotransmitter, GABA has long been implicated in seizures: reduced GABA or GABA function produces seizures and many anticonvulsants act by enhancing GABA-mediated inhibition (3,4).

Mutations in the GABA_A receptor $\alpha 1$ subunit and the $\gamma 2$ subunit, both on chromosome 5, as well as in the less common δ subunit on chromosome 1 have been associated with human epilepsy (3). Another GABA_A receptor subunit that produces epilepsy, when totally knocked out in the mouse, is the $\beta 3$ subunit found on human chromosome 15 (4). In the case of $\beta 3$ subunit, the mice have numerous neurodevelopmental problems, show generalized abnormal EEG, myoclonic jerks, and absence-like behaviors that progress to tonic-clonic seizures and become more severe with age.

The mutations in GABA_A receptor associated with human epilepsy occur at numerous places on the different subunits (3). The phenotypes include autosomal generalized epilepsy with febrile seizures plus (GEFS+) on $\gamma 2$ (K289M) (5), with a second $\gamma 2$ mutation (Q351X) also showing GEFS+ (6). Another mutation in $\gamma 2$ (R43Q) was found in a family with childhood absence and febrile seizures (7). An important $\alpha 1$ mutation (A322D) is associated with a type of autosomal dominant juvenile myoclonic epilepsy (8). This mutation is in the transmembrane helix 3. The heterozygote shows the phenotype, possibly reflecting a dominant-negative interaction of the mutant protein, with normal protein, leading to a significant reduction in the amount of cell surface functional receptor. Some investigators speculate that reduced surface expression can be equally or more important in producing impaired function than the altered channel properties of receptors expressed at the surface with one or even two copies of the point-mutated subunit (3).

Studies describing how the various $\gamma 2$ subunit mutations (Figure 1) can produce a seizure phenotype reveal that the three mutations—R43Q in the extracellular domain; K289M in the extracellular loop between transmembrane helix 2 (M2) and helix 3 (M3); and Q351X in the intracellular domain—all demonstrate decreased inhibitory currents in heterologous cell expression systems (3). Q351X shows truncation, no functional channels, and no protein trafficking to the cell surface. K289M displays impaired ligand gating, less current, and accelerated deactivation. R43Q exhibits reduced inhibitory current, but investigators now agree that the primary defect involves reduced cell-surface trafficking. The $\gamma 2$ subunit is actually the most abundant GABA_A receptor subunit; it is required for synaptic localization and exhibits other unique trafficking properties, both intracellular and at the cell membrane (4). Lack of the $\gamma 2$ subunit in mice (i.e., knockout) leads to early lethality associ-

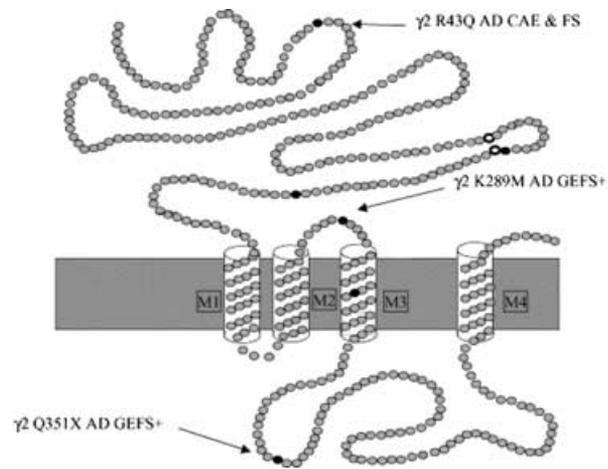


FIGURE 1. Febrile seizure mutations in GABA_A receptor $\gamma 2$ subunit. The illustration shows a schematic subunit sequence with inferred membrane topology. The three mutations in the $\gamma 2$ subunit discussed by Kang et al. are highlighted. The N-terminal half is extracellular, M1-M4 represent the transmembrane helices 1-4; M2 is the ion channel wall. Between M1 and M2 and between M3 and M4 are intracellular spaces. The loop between M2 and M3 is extracellular.

ated with abnormal synaptic inhibition and pleiotropic CNS abnormalities (9).

Since the three $\gamma 2$ mutations all exhibited febrile seizures, Kang et al. asked how three disparate types of detailed functional alterations might have a common temperature sensitivity and found just such a property. Noting that many phenotypes caused by point mutations of membrane proteins show reduced cell-surface expression of the protein, the authors found that all three mutations studied in vitro exhibit within minutes at 40°C a temperature-dependent reduction in cell-surface trafficking and suggested that the finding resulted from the abnormal folding of the protein at 40°C instead of 37°C. The investigators present strong evidence for a temperature-dependent trafficking defect and the hypothesis of a febrile seizure mechanism is intuitively sound, however the theory is still unproven. The GABA_A receptor mutation $\alpha 1$ A322D, which produces epilepsy but not febrile seizures, did not exhibit temperature-dependent trafficking deficiency. Two mutations in the δ subunit also show susceptibility to febrile seizures but their temperature dependence has not been reported.

What functions of the brain might be sensitive to such a relatively small increase in temperature as occurs with fever? There are many possibilities. Temperature-sensitive (missense) mutations are common in microbial and yeast genetics, with the protein being denatured at normal or slightly higher growth temperatures, but not at lower, permissive temperatures. Consider the temperature coefficient Q10 (fold-change in rate for a 10° change in temperature) for biochemical phenomena (10).

A chemical reaction, that is, a bond breaking or formation (e.g., synthesis of GABA), would have a Q10 of about 2. In contrast, opening a channel, like a GABA_A receptor, involving a simple protein conformational change, would likely have a Q10 less than 2. Likewise, protein folding is a temperature-sensitive process, but the Q10 is not high. However, with a cascade of complex chemical reactions—such as intracellular protein trafficking and membrane reactions—the Q10 would likely be 2 or larger. Thus, trafficking is very temperature-dependent: receptor function will increase with temperature as a result of elevated numbers of functional receptors, up to a point at which the mutated proteins are denatured. Changing GABA_A receptor content at the cell surface is a feasible possibility for the temperature-sensitive events occurring with fever and is supported by the evidence of significant change in the relevant temperature range. A precedent, supporting this theory, is the temperature dependence of a clinically important genetic disease, cystic fibrosis. In one type of point mutation in the cystic fibrosis transmembrane-conductance regulator (CFTR) (11), the deficit in function was demonstrated to involve temperature-dependent intracellular retention and degradation of the protein. Thus, the findings of Kang et al. provide an intriguing new idea: heat is good for chemical reactions. But, as the saying goes, you can get too much of a good thing.

by Richard W. Olsen, PhD

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