Pyramidal Neuron Axon Initial Segment Dysregulation in Nav β1 Subunit Epilepsy: A Tip of the Iceberg?

Axon Initial Segment Dysfunction in a Mouse Model of Genetic Epilepsy With Febrile Seizures Plus.

Wimmer VC, Reid CA, Mitchell S, Richards KL, Scaf BB, Leaw BT, Hill EL, Royeck M, Horstmann MT, Cromer BA, Davies PJ, Xu R, Lerche H, Berkovic SF, Beck H, Petrou S. *J Clin Invest* 2010;120:2661–2671.

Febrile seizures are a common childhood seizure disorder and a defining feature of genetic epilepsy with febrile seizures plus (GEFS+), a syndrome frequently associated with Na+ channel mutations. Here, we describe the creation of a knockin mouse heterozygous for the C121W mutation of the β1 Na+ channel accessory subunit seen in patients with GEFS+. Heterozygous mice with increased core temperature displayed behavioral arrest and were more susceptible to thermal challenge than wild-type mice. Wild-type β1 was most concentrated in the membrane of axon initial segments (AIS) of pyramidal neurons, while the β1(C121W) mutant subunit was excluded from AIS membranes. In addition, AIS function, an indicator of neuronal excitability, was substantially enhanced in hippocampal pyramidal neurons of the heterozygous mouse specifically at higher temperatures. Computational modeling predicted that this enhanced excitability was caused by hyperpolarized voltage activation of AIS Na+ channels. This heat-sensitive increased neuronal excitability presumably contributed to the heightened thermal seizure susceptibility and epileptiform discharges seen in patients and mice with β1(C121W) subunits. We therefore conclude that Na+ channel β1 subunits modulate AIS excitability and that epilepsy can arise if this modulation is impaired.

Commentary

Voltage-gated sodium channels (Nav) are composed of multi-subunit protein complexes, and their density becomes greatest at the axon initial segment where action potentials initiate. Mutations of the Nav β1 subunit (encoded by the *SCN1B* gene) are associated with genetic (generalized) epilepsy with febrile seizures (FS) plus (GEFS+) in a subset of patients with GEFS+. Wimmer et al. have now reported a new mouse model of human familial epilepsy resulting from a GEFS+ epilepsy-associated mutation (C121W, adjacent to an Ig-like extracellular loop) and reconstitute febrile seizure susceptibility (1). Of interest, an adjacent β1-subunit mutation, R125C, was recently found to be homozygous in a patient with Dravet syndrome (severe myoclonic epilepsy of infancy, SMEI). As the vast majority of SMEI patients display haploid insufficiency of α subunit Nav1.1, and many GEFS+ patients display α-subunit Nav1.1 mutations, Occam's razor or the law of parsimony would suggest that all Nav mutations, whether of the Nav1.1 α or even the β1 subunit, should generate epilepsy through a common mechanism (2). This common mechanism was discovered during studies of mice with an α-subunit Nav1.1 haploid insufficiency; GABAergic inhibitory, but not glutamatergic pyramidal, neurons in the hippocampus are uniquely dependent on Nav1.1 and therefore display impaired Nav current and a failure to sustain high action potential firing rates even when missing half of the normal number of channels (3, 4).

Occam's razor might in this case be a Gillette twin blade, since β1-subunit knockout mice failed to identify defects in Nav currents of hippocampal GABAergic neurons, suggesting that α- (Nav1.1) and β1-subunit mutations might cause GEFS+ and SMEI through distinct mechanisms (5). Patino et al. (5) focused on GABAergic neurons from the hippocampal CA3 region, where β1-subunit defects did lead to a loss of Nav1.1 protein staining and a compensatory increase of Nav1.3 (6). They found that the sodium currents of GABAergic neurons from CA3 were unaltered while pyramidal neurons displayed an increased peak voltage and amplitude of sodium action potentials, suggesting a possible increased excitability of pyramidal neurons rather than a decreased excitability of GABAergic neurons.

Wimmer et al. extended this finding, reporting that mice engineered with the Nav β1-subunit mutation C121W displayed increases in subiculum pyramidal neuron excitability (1). Intriguingly, they also provided evidence for a temperature-sensitive increase of AIS excitability.

While this new finding should be factored into the potential circuit changes that might contribute to seizure susceptibility in these patients, one must also consider other still unexplored explanations for the seizure propensity of these mice and the patients they model.

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First, while the evidence for temperature-sensitive enhancement of the AIS excitability is intriguing, is it really the cellular correlate of febrile seizures? SMEI mouse models with Nav1.1 haploid insufficiency and reduced excitability of GABAergic neurons also show temperature-sensitive seizures (7) and Nav1.1 mutations underlie GEFS+. Although not yet tested, Nav1.1 haploid insufficiency is unlikely to produce this same temperature-sensitive increase of AIS excitability in pyramidal neurons; hence arguing for a different mechanism for fever-induced seizures in Nav1.1 epilepsy (e.g., respiratory alkalosis [8]).

Second, β1-subunit defects were shown very clearly to reduce Nav1.1 surface expression (4); a parameter not quantified in the Wimmer et al. (1) study. Since the Nav1.1 α subunit is mutated to produce SMEI and GEFS+ (like β1 subunit; for example, see Patino et al. [5]) and is necessary to sustain high action potential firing rates in GABAergic neurons (3, 4), future studies of this new β1 mutant mouse model should rule out defects in GABAergic neuron excitability as contributing to seizure susceptibility. As the change in GABAergic neuron excitability could be remote from the somatic compartment (e.g., axon; [6]), it is important to assess this using alternate means such as measuring the frequency of IPSC currents in target pyramidal neurons during both spontaneous and induced activity of the network.

Third, interestingly, the β1 subunit was recently shown to regulate axonal development (9, 10), and β1-subunit knockouts increase the number of degenerating axons (6). These changes might also contribute to seizure susceptibility.

Fourth, β1-subunit loss disturbs axon internode structure and decreases axon conduction velocities (6). Such a defect, if found in the C121W mutant, could, in principle, directly impair GABAergic neuron axonal transmission or indirectly impair feed-forward/feedback excitatory input to GABAergic neurons to reduce network inhibition.

Overall, this recent study establishes a new and very exciting mouse model of a genetic human epilepsy disorder allowing devoted basic scientists the opportunity to dig deeper into epilepsy pathophysiology and hard working translational scientists the opportunity to pursue preclinical drug testing with the hope of discovering new cures for this scourge of humankind.

by Matthew Anderson, MD, PhD

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