

HOW A SODIUM CHANNEL MUTATION CAUSES EPILEPSY

Functional and Biochemical Analysis of a Sodium Channel β_1 -Subunit Mutation Responsible for Generalized Epilepsy with Febrile Seizures Plus Type 1

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Generalized epilepsy with febrile seizures plus type 1 is an inherited human epileptic syndrome, associated with a cysteine-to-tryptophan (C121W) mutation in the extracellular immunoglobulin domain of the auxiliary β_1 subunit of the voltage-gated sodium channel. The mutation disrupts β_1 function, but how this leads to epilepsy is not understood. In this study, we make several observations that may be relevant for understanding why this β_1 mutation results in seizures. First, with electrophysiological recordings from mammalian cell lines, coexpressing sodium channel α subunits and either wild-type β_1 or C121W β_1 , we show that loss of β_1 functional modulation, caused by the C121W mutation, leads to increased sodium channel availability at hyperpolarized membrane potentials and reduced sodium channel rundown during high-frequency channel activity, compared with channels coexpressed with wild-type β_1 . In contrast, neither wild-type β_1 nor C121W β_1 significantly affected sodium current time course or the voltage dependence of channel activation. We also show, by using a *Drosophila* S2 cell adhesion assay, that the C121W mutation disrupts β_1 - β_1 homophilic cell adhesion, suggesting that the mutation may alter the ability of β_1 to mediate protein-protein interactions critical for sodium channel localization. Finally, we demonstrate that neither functional modulation nor cell adhesion mediated by wild-type β_1 is occluded by coexpression of C121W β_1 , arguing against the idea that the mutant β_1 acts as a dominant-negative subunit. Together, these data suggest that C121W β_1 causes subtle effects on channel function and subcellular distribution that bias neurons toward hyperexcitability and epileptogenesis.

COMMENTARY

The first human mutation identified in the voltage-gated sodium ion channel linked with inherited epilepsy in humans was actually not in the pore-forming channel protein itself, but in a gene encoding the β_1 subunit, a smaller auxiliary protein that accompanies the channel to the membrane, where it regulates the pore behavior in response to voltage. The human mutation changes a single amino acid in an extracellular, “Velcro”-like loop of the protein possibly related to cell adhesion. What could be more tempting to a channelologist than to discover the reason that this unfolded protein loop leads to epilepsy?

The first step is to understand what the mutation could do to boost the sodium current of a “model” neuron. It does not increase the number of channels, because an assay for surface expression revealed that both mutant and wild-type subunits were equally effective in assisting membrane incorporation of the channel. Nevertheless, in *Xenopus* oocytes, one difference was clear: it prevented rapid inactivation of current flow through the pore, thus prolonging the time course of sodium entry. This agrees with the general view that excess depolarization due to a persistent sodium current is epileptogenic. In mammalian cells derived from a Chinese hamster ovary line, however, the authors show that coexpressing the mutant C121W1 β_1 subunit with either of two different sodium channel pore subtypes (there are actually 10 different such subunits in the nervous system) did not prolong the current, but instead increased the amplitude of sodium current evoked at hyperpolarized potentials, and also reduced the spike “run-down” normally observed at high firing frequency (because of a summing failure of recovery after each opening); both behaviors might enhance neuronal excitability. Because the mutant subunit did not alter either the voltage dependence of activation or duration of the current in this cell system, the results suggest a mechanism different from that originally proposed.

The sodium channel is responsible not only for the upstroke and duration of the action potential, but by virtue of its position and density, also determines where on the cell impulses originate, as well as the conduction velocity, branch block, and repetitive firing patterns. In this regard, a second, non-pore-related function of the β_1 subunit potentially re-

sides in the immunoglobulin-like fold of the protein that is exposed to the extracellular space. This loop might play a role as a tether affecting channel localization in the membrane, or recognizing signals on another cell. With an aggregation assay in cultured *Drosophila* cells, the authors indeed showed a defect in the adhesive function postulated for this extracellular loop. Finally, because the human pedigree described with this mutation showed a dominant mode of inheritance, the authors examined whether a single defective copy of this gene might actively block one or the other functions of the unaffected copy (a dominant negative interaction); however, they found no evidence for this in either assay, indicating that the phenotype is likely to arise from a simple deficit of wild-type subunits.

These results bring us closer to understanding the kinds of potential defects that might appear in neurons of $\beta 1$ chan-

nelopathy patients bearing the C121W1 mutation, although clearly the network excitability changes induced by altered sodium channel function in the developing brain are far more complex, and raise additional questions. Should not this channel defect increase the excitability of inhibitory interneurons as well as excitatory cells? How can a single channelopathy give rise to so many different seizure phenotypes, a defining feature of the pedigree in which it was originally isolated? Perhaps not surprisingly, even once the genes for the best-understood molecules controlling neuronal excitability are linked to epilepsy, we continue to puzzle over its origin. One almost wonders whether the term “idiopathic” remains an apt descriptor of these epilepsies even after the gene has been identified.

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