

(WHAT TO DO) WHEN EPILEPSY GENE MUTATIONS STOP MAKING SENSE

An Epilepsy Mutation in the Sodium Channel *SCN1A* That Decreases Channel Excitability. Barela AJ, Waddy SP, Lickfett JG, Hunter J, Anido A, Helmers SL, Goldin AL, Escayg A. *J Neurosci* 2006;26:2714–2723. Mutations in three voltage-gated sodium channel genes *SCN1A*, *SCN2A*, and *SCN1B* and two GABA_A receptor subunit genes *GABRG2* and *GABRD* have been identified in families with generalized epilepsy with febrile seizures plus (GEFS⁺). A novel mutation, R859C, in the Na_v1.1 sodium channel was identified in a four-generation, 33-member white family with a clinical presentation consistent with GEFS⁺⁺. The mutation neutralizes a positively charged arginine in the domain 2 S4 voltage sensor of the Na_v1.1 channel subunit. This residue is conserved in mammalian sodium channels as well as in sodium channels from lower organisms. When the mutation was placed in the rat Na_v1.1 channel and expressed in *Xenopus* oocytes, the mutant channel displayed a positive shift in the voltage dependence of sodium channel activation, slower recovery from slow inactivation, and lower levels of current compared with the wild-type channel. Computational analysis suggests that neurons expressing the mutant channel have higher thresholds for firing a single action potential and for firing multiple action potentials, along with decreased repetitive firing. Therefore, this mutation should lead to decreased neuronal excitability, in contrast to most previous GEFS⁺ sodium channel mutations, which have changes predicted to increase neuronal firing.

Sodium Channel Dysfunction in Intractable Childhood Epilepsy with Generalized Tonic–Clonic Seizures Rhodes TH, Vanoye CG, Ohmori I, Ogiwara I, Yamakawa K, George AL Jr. *J Physiol* 2005;569(Pt 2):433–445. Mutations in *SCN1A*, the gene encoding the brain voltage-gated sodium channel₁ subunit (Na_v1.1), are associated with genetic forms of epilepsy, including generalized epilepsy with febrile seizures plus (GEFS⁺ type 2), severe myoclonic epilepsy of infancy (SMEI), and related conditions. Several missense *SCN1A* mutations have been identified in probands affected by the syndrome of intractable childhood epilepsy with generalized tonic–clonic seizures (ICEGTC), which bears similarity to SMEI. To test whether ICEGTC arises from molecular mechanisms similar to those involved in SMEI, we characterized eight ICEGTC missense mutations by whole-cell patch clamp recording of recombinant human *SCN1A* heterologously expressed in cultured mammalian cells. Two mutations (G979R and T1709I) were nonfunctional. The remaining alleles (T808S, V983A, N1011I, V1611F, P1632S, and F1808L) exhibited measurable sodium current, but had heterogeneous biophysical phenotypes. Mutant channels exhibited lower (V983A, N1011I, and F1808L), greater (T808S), or similar (V1611F and P1632S) peak sodium current densities compared with wild-type (WT)-*SCN1A*. Three mutations (V1611F, P1632S, and F1808L) displayed hyperpolarized conductance–voltage relationships, while V983A exhibited a strong depolarizing shift in the voltage dependence of activation. All mutants except T808S had hyperpolarized shifts in the voltage dependence of steady-state channel availability. Three mutants (V1611F, P1632S, and F1808L) exhibited persistent sodium current ranging from 1–3% of peak current amplitude that was significantly greater than WT-*SCN1A*. Several mutants had impaired slow inactivation, with V983A showing the most prominent effect. Finally, all of the functional alleles exhibited reduced use-dependent channel inhibition. In summary, *SCN1A* mutations associated with ICEGTC result in a wide spectrum of biophysical defects, including mild-to-moderate gating impairments, shifted voltage dependence, and reduced use dependence. The constellation of biophysical abnormalities for some mutants is distinct from those previously observed for GEFS⁺ and SMEI, suggesting possible, but complex, genotype–phenotype correlations.

Single-Channel Properties of Human Nav1.1 and Mechanism of Channel Dysfunction in *SCN1A*-Associated Epilepsy. Vanoye CG, Lossin C, Rhodes TH, George AL Jr. *J Gen Physiol* 2006;127:1–14. Mutations in genes encoding neuronal voltage-gated sodium channel subunits have been linked to inherited forms of epilepsy. The majority of mutations (>100) associated with generalized epilepsy with febrile seizures plus (GEFS⁺) and severe myoclonic epilepsy of infancy (SMEI) occur in *SCN1A* encoding the Na_v1.1 neuronal sodium channel subunit. Previous studies demonstrated functional heterogeneity among mutant *SCN1A* channels, revealing a complex relationship between clinical and biophysical phenotypes. To further understand the mechanisms responsible for mutant *SCN1A* behavior, we performed a comprehensive analysis of the single-channel properties of heterologously expressed recombinant WT-*SCN1A* channels. Based on these data, we then determined the mechanisms for dysfunction of two GEFS⁺-associated mutations (R1648H, R1657C) both affecting the S4 segment of domain 4. WT-*SCN1A* has a slope conductance

(17 pS) similar to channels found in native mammalian neurons. The mean open time is 0.3 ms in the -30 to -10 mV range. The R1648H mutant, previously shown to display persistent sodium current in whole-cell recordings, exhibited similar slope conductance but had an increased probability of late reopening and a subfraction of channels with prolonged open times. We did not observe bursting behavior and found no evidence for a gating mode shift to explain the increased persistent current caused by R1648H. Cells expressing R1657C exhibited conductance, open probability, mean open time, and latency to first opening similar to WT channels but reduced whole-cell current density, suggesting decreased number of functional channels at the plasma membrane. In summary, our findings define single-channel properties for WT-*SCN1A*, detail the functional phenotypes for two human epilepsy-associated sodium channel mutants, and clarify the mechanism for increased persistent sodium current induced by the R1648H allele.

COMMENTARY

Many topics are rendered less intriguing by involuntary, early exposure. It is possible that for many neuroscientists the sodium channel could be included in this category. Qualitative treatments of Hodgkin and Huxley analysis of the sodium currents underlying the action potential in squid giant axon are found in introductory texts and universally taught, just following explanations of the Nernst equation and the resting membrane potential (1). Although useful for introducing many essential concepts, this early positioning forces shortcuts and simplifications. Information on the s_4 positive charge-bearing helical voltage sensor, voltage-dependent opening (activation), and closing (deactivation) is taught, as is how the channels rapidly enter an inactivated state that prevents reopening. Indeed, this process of inactivation and the associated refractory period ensures the unidirectional flow of the nerve impulse. Pedagogy that emphasizes the reliable, uniform, all-or-none aspects of sodium channel function during nondecremental propagation of action potentials leaves them seeming a little bland. The new studies reviewed here describe sodium channels that behave in unexpected and perplexing ways. Although confusing, these findings hold the potential for provoking broader interest in these seemingly well-known channels.

Over the past 15 years, mutations in genes encoding several skeletal muscle, cardiac, and nerve sodium channel subunits have been identified in patients with disorders characterized by paroxysmal hyperexcitability, including forms of periodic paralysis, hereditary ventricular arrhythmia, and epilepsy (2). Electrophysiological analysis in heterologous cells has revealed that many of the disease-provoking mutant channels increase channel openings, sometimes by enhanced activation but most commonly by causing abnormally delayed and/or incomplete inactivation. These observations fit with the view that sodium channels in mammalian excitable cells, as in squid axon, must function in a uniform way and that even slight excesses above the normal activity level could lead to symptomatic hyperexcitability (3).

As the number of known epilepsy-linked sodium channel mutations has grown and the spectrum of associated syndromes

has broadened (see Stafstrom Basic Review in this issue), it has become clear that the model of how channel dysfunction can lead to epilepsy is oversimplified. Alekov et al. showed that an epilepsy mutation could be associated with enhanced inactivation predicted to result in a decrease in sodium channel currents (4). Most dramatically, a large number of frame-shift mutations expected to result in truncated, nonconducting channel proteins have been found in cases of severe myoclonic epilepsy of infancy (SMEI) (2). How can mutations that increase and decrease the activity in the same channel lead to epilepsy syndromes of variable but overlapping severity, from mild (simple febrile seizures) to catastrophic (SMEI)?

The current papers highlight this nettlesome issue through rigorous biophysical study of additional mutations in *SCN1A*, encoding the channel subunit $Na_v1.1$. The R859C mutant channels described by Barela and Waddy et al. require greater membrane depolarization for activation than the wild type, a change predicted to reduce currents in vivo. The eight mutants analyzed by Rhodes et al. include two that fail to form functional channels; the others exhibit quite heterogeneous changes in properties. Vanoye et al. use elegant single-channel recordings to define the kinetic changes underlying the behavior of two mutations involving neighboring residues on the same transmembrane segment of $Na_v1.1$. Consistent with previous whole-cell patch-clamp studies of the mutations, one increases openings, reflecting a defect in inactivation gating, while the other shows normal opening and closing kinetics but a lowered total number of functional numbers.

Although reductive approaches of this kind are invaluable, the contribution of an ion channel to behavior can only be discerned once its functional profile is understood at several levels—molecular, subcellular, cellular, and neuronal network. Given its importance, it is surprising how little is known about the cell biology of neuronal $Na_v1.1$. Unlike in skeletal muscle and heart where a single type of sodium channel predominates, in brain, individual neurons simultaneously express multiple varieties of sodium channels (5). The classic role of initiation and propagation of action potentials in axons is mainly the responsibility of $Na_v1.6$ (and in some instances $Na_v1.2$), channels that so far only rarely have been implicated in human

epilepsy (6). $\text{Na}_v1.1$ (encoded by *SCN1A*, for which over 100 human epilepsy mutations are known) appears to be expressed at low-to-moderate densities but not typically on axons. Instead, $\text{Na}_v1.1$ appears to contribute to the excitability of neuronal somata and dendrites, helping to shape excitatory postsynaptic potentials and supporting the backpropagation of action potentials into dendrites; however, the specific in vivo functional profile of $\text{Na}_v1.1$ needs to be far better understood.

Much can be learned by combining molecular, cell biological, and electrophysiological approaches to analyze function of mutant neuronal channels in vivo. For example, one of the mysteries regarding benign familial neonatal seizures (BFNS) has been that the mutations in *KCNQ2* and *KCNQ3* potassium channel subunits, which cause the disorder, often have very little effect on channel function when expressed in cell lines or *Xenopus* oocytes (7). New work suggests that these channels have previously unsuspected roles on axons and that some of the BFNS mutants are transported quite inefficiently to their proper axonal targets (8,9). A way in which a sodium channel loss-of-function mutation could lead to hyperexcitability in a neuronal circuit is illuminated by other recent studies involving $\text{Na}_v1.1$ knockout mice (10). Hippocampal inhibitory neurons from the mutants (but not excitatory pyramidal cells) show a dramatic reduction in detectable sodium channel current, suggesting that seizures in these mice could result from a loss of inhibition in cortical circuits. The inhibitory neurons also show a remarkable compensatory increase in expression of $\text{Na}_v1.3$, a sodium channel isoform with biophysical properties quite different from the missing $\text{Na}_v1.1$ channels. Further analysis of these mutant mice and of mice bearing missense mutations associated with human epilepsy may answer the unsettled questions about $\text{Na}_v1.1$ raised by the current papers. Along the way, investigators will likely have to discard the simplified notion that the function of sodium channels in the neurons is restricted to faithfully “reporting out” the decisions made by synapses—

instead, these channels may well be found in the thick of the action.

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