

A POSSIBLE EXPLANATION FOR THE PARADOX OF HYPEREXCITABILITY AND EPILEPSY IN “LOSS OF FUNCTION” VOLTAGE-GATED SODIUM CHANNEL MUTATIONS

Reduced Sodium Current in GABAergic Interneurons in a Mouse Model of Severe Myoclonic Epilepsy in Infancy. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. *Nat Neurosci* 2006;9: 1142–1149. Voltage-gated sodium channels (Na_V) are critical for initiation of action potentials. Heterozygous loss-of-function mutations in $\text{Na}_V1.1$ channels cause severe myoclonic epilepsy in infancy (SMEI). Homozygous null $\text{SCN1A}^{-/-}$ mice developed ataxia and died on postnatal day (P) 15 but could be sustained to P17.5 with manual feeding. Heterozygous $\text{SCN1A}^{+/-}$ mice had spontaneous seizures and sporadic deaths beginning after P21, with a notable dependence on genetic background. Loss of $\text{Na}_V1.1$ did not change voltage-dependent activation or inactivation of sodium channels in hippocampal neurons. The sodium current density was, however, substantially reduced in inhibitory interneurons of $\text{SCN1A}^{+/-}$ and $\text{SCN1A}^{-/-}$ mice but not in their excitatory pyramidal neurons. An immunocytochemical survey also showed a specific upregulation of $\text{Na}_V1.3$ channels in a subset of hippocampal interneurons. Our results indicate that reduced sodium currents in GABAergic inhibitory interneurons in $\text{SCN1A}^{+/-}$ heterozygotes may cause the hyperexcitability that leads to epilepsy in patients with SMEI.

COMMENTARY

Voltage-gated sodium channels (Na_V) underlie the generation and propagation of action potentials and are some of the most important proteins contributing to the excitability of neurons. These sodium channels are the primary targets of widely used and efficacious antiepileptic therapies, such as phenytoin, carbamazepine, oxcarbazepine, and lamotrigine. Therefore, it is not surprising that many of the mutations in ion channels associated with human diseases (i.e., channelopathies) involve voltage-gated sodium channels. Considering the complex physiological systems in which they function, particularly in the CNS, the precise mechanisms by which identified Na_V mutations alter neuronal excitability to produce particular disorders, including epilepsy, are not well understood.

Various genes (e.g., *SCN1A–SCN9A*) encode nine isoforms of the main pore-forming sodium channel α subunit. In addition, four genes (*SCN1B–SCN4B*) encode auxiliary β subunits that influence the biophysical properties of the channel. To date, there are nearly 20 known diseases that result from mutations in the sodium channel genes, including neuromuscular disorders and cardiac arrhythmias, with the clinical manifestations reflecting the expression pattern of the gene. For example, *SCN5A* is expressed mostly in cardiac muscle, where it is the major sodium channel of the ventricular conduction system, and mutations in this gene cause congenital long QT syndrome. Four sodium channel genes, *SCN1A*, *2A*, *3A*, and *8A* are highly expressed in the CNS, where they each exhibit a distinct subcellular localization. Accordingly, *SCN1A*, encod-

ing the sodium channel α subunit protein designated $\text{Na}_V1.1$, is expressed on the cell body of neurons throughout the brain (1), while $\text{Na}_V1.2$ appears to be localized to unmyelinated axons and dendrites (2). Since many antiepileptic drugs inhibit Na_V s, there has been speculation that differential expression of the sodium channel α and β subunits on excitatory and inhibitory neurons may lead to a preferential blockade of high-frequency excitatory neurotransmission—but this theory has yet to be substantiated. Still, the heterogeneity of sodium channel expression and localization provides a theoretical basis for understanding how mutations may cause specific diseases and for understanding the mechanisms of action by which drugs used to treat those diseases may have selective effects on subsets of neurons in the brain.

Four epilepsy syndromes, ranging from benign to severe, have been linked to mutations in sodium channels, primarily in *SCN1A*, *SCN2A*, and *SCN1B*. The most grave of these syndromes, severe myoclonic epilepsy in infancy (SMEI), is a familial syndrome characterized by febrile seizures in the first year of life with subsequent progression to medically intractable epilepsy, ataxia, and developmental delay. Most *SCN1A* mutations associated with SMEI introduce frameshifts or premature termination sequences into the coding sequence and, therefore, are predicted to produce nonfunctional channels and haploinsufficiency (3). How does loss of function of $\text{Na}_V1.1$ —one of the principal determinants of neuronal excitability—result in hyperexcitability and seizures in the brain, when it would be expected to reduce excitability? Yu and colleagues' recent work offers a possible explanation.

Yu et al. created a deletion of the *SCN1A* gene in mice and examined the phenotype of homozygous and heterozygous offspring. As expected, neurons in homozygous mice expressed no detectable $\text{Na}_V1.1$ and neurons in heterozygotes expressed

approximately one-half the amount of $\text{Na}_V1.1$ as wild-type controls. Homozygotes began having seizures on postnatal day 9, the time when $\text{Na}_V1.1$ expression normally begins, and died by postnatal day 15, often in status epilepticus. Heterozygotes, which genotypically mimic the human condition in SMEI, began having seizures after 3 weeks of life. Interestingly, this epilepsy phenotype was observed only in mice of one genetic background, but not another, suggesting that currently unknown genetic factors influence the penetrance of the *SCN1A* deletion phenotype. The mice with the epilepsy phenotype were studied further to elucidate the possible underlying mechanism for seizures.

Behavioral and EEG analysis confirmed that the heterozygous mice developed clonic and tonic-clonic seizures. Excitability at the single-cell level was examined in dissociated neurons from the hippocampi of wild-type and mutant mice. When whole-cell sodium currents from wild-type, homozygous, and heterozygous mice were recorded from hippocampal pyramidal neurons, the principal excitatory neurons of this brain region, they were virtually identical. This finding was not wholly unexpected, since it had previously been shown that $\text{Na}_V1.1$ expression in the hippocampus was relatively low compared with that of voltage-gated sodium channel 1.2 (4), suggesting that $\text{Na}_V1.2$ is the major Na_V on pyramidal neurons. However, when hippocampal inhibitory interneurons were examined in the same manner, whole-cell sodium currents were reduced by approximately 50% in the heterozygotes and by 75% in homozygotes. To understand how this selective reduction of sodium channels in interneurons affected their excitability, input-output relationships for the inhibitory neurons were determined. Increasingly depolarizing currents injected into wild-type interneurons produced a linear increase in the number of action potentials; however, this response was blunted in the heterozygotes and even more dramatically in the homozygotes. These results demonstrate that insufficient levels of $\text{Na}_V1.1$ expression, as is predicted for the human SMEI mutations, cause diminished excitability. Since this altered excitability occurred selectively in interneurons, the disease-producing *SCN1A* mutations would presumably cause hypofunction of inhibitory circuits and, consequently, hyperexcitability of neuronal networks.

The experimental data raise the question of whether, in the setting of reduced expression of a major brain sodium channel isoform, there is compensatory upregulation of other isoforms. Although no alteration of expression of $\text{Na}_V1.2$ or $\text{Na}_V1.6$ was observed in the mutant mice, expression of $\text{Na}_V1.3$, which is not normally expressed in the hippocampus, was observed on hippocampal interneurons of the heterozygous and homozygous mice. While this abnormal upregulation of another sodium channel appears to be an attempt to compensate for loss of $\text{Na}_V1.1$, it clearly is insufficient to restore normal sodium current amplitudes or excitability of these inhibitory neurons, even in the heterozygotic condition.

While SMEI is a rare condition, insights into the disorder provided by this study advance the knowledge of basic mechanisms of epilepsy. First, the Yu et al. study reveals a selective vulnerability of inhibitory versus excitatory neurons to a channelopathy involving a key protein that regulates neuronal excitability. This finding may help explain some of the phenotypic differences between mutations in other channels, such as differences in severity or in whether the mutation causes epilepsy at all. Second, the findings in this study give new promise to the idea of selective antiepilepsy therapies. For example, treatments that selectively block $\text{Na}_V1.2$ would be expected to result in relative suppression of excessive excitatory neuronal activity, at least in the hippocampus, while therapies that cause upregulation of $\text{Na}_V1.1$ or $\text{Na}_V1.3$ may enhance the excitability of inhibitory neurons.

by Gregory C. Mathews, MD, PhD

References

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