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DRAVET SYNDROME Insights into pathophysiology and therapy from a mouse model of Dravet syndrome

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Summary

Mutations in voltage-gated sodium channels are associated with epilepsy syndromes with a wide range of severity. Complete loss of function in the $Na_v1.1$ channel encoded by the *SCN1A* gene is associated with severe myoclonic epilepsy in infancy (SMEI), a devastating infantile-onset epilepsy with ataxia, cognitive dysfunction, and febrile and afebrile seizures resistant to current medications. Genetic mouse models of SMEI have been created that strikingly recapitulate the SMEI phenotype including age and temperature dependence of seizures and ataxia. Loss-of-function in $Na_v1.1$ channels results in severely impaired sodium current and action potential firing in hippocampal γ-aminobutyric acid (GABA)ergic interneurons without detectable changes in excitatory pyramidal neurons. The resulting imbalance between excitation and inhibition likely contributes to hyperexcitability and seizures. Reduced sodium current and action potential firing in cerebellar Purkinje neurons likely contributes to comorbid ataxia. A mechanistic understanding of hyperexcitability, seizures, and comorbidities such as ataxia has led to novel strategies for treatment.

Keywords

Severe myoclonic epilepsy in infancy; SCN1A; Febrile seizures; Ataxia; Mouse genetic model

Much of our current knowledge of the pathophysiology of epilepsy comes from the use of animal models, typically models of acute, provoked seizures. Recently, mouse genetic models have been created based upon known human epilepsy-associated mutations for multiple diseases including SMEI (severe myoclonic epilepsy in infancy, Dravet syndrome). These models recapitulate many aspects of the human disease and have provided important insights into cellular and molecular pathophysiology underlying seizures and comorbidities. An important goal is to use the new understanding of syndrome-specific pathophysiology in these genetic models to identify novel treatment strategies.

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Disclosure

The authors declare no conflicts of interest.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

A Mouse Genetic Model of SMEI

Voltage-gated Na^+ channels in the brain are complexes of an α subunit containing the voltage sensor and ion-conducting pore, in association with auxiliary β subunits ($\beta1$–$\beta4$), which modify the kinetics and voltage dependence of gating and serve as cell adhesion molecules (Catterall, 2000). Four functional voltage-gated sodium channel α subunits are expressed in adult mammalian brain: $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, and $Na_V1.6$ channel subtypes, encoded by the *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* genes, respectively. Several mutations in Na_V channel genes have been associated with epilepsy, but mutations in *SCN1A* are the most common. More than 300 mutations in the exons of the *SCN1A* gene, which codes for the α subunit of the $Na_v1.1$ sodium channel, have been identified in patients with SMEI, accounting for approximately 70% of cases. The majority of these are truncation or deletion mutations predicted to cause a loss of function. Additional affected individuals may have undetected mutations in the noncoding sequences of *SCN1A* that impair gene expression. To understand the mechanistic basis for hyperexcitability, seizures, and comorbidities in SMEI, animal models, called mouse SMEI (mSMEI) for convenience, were generated by targeted deletion of a major exon in the *Scn1a* gene in mouse (Yu et al., 2006) and knockin of a stop codon in the *Scn1a* gene in mouse (Ogiwara et al., 2007). These mice are viable, fertile, and display key phenotypic features of SMEI including ataxia, and spontaneous and thermally induced seizures beginning on postnatal day 20 (P20).

Heterozygous Deletion of *Scn1a* Is Sufficient to Produce Phenotypic Features of SMEI

SMEI is defined by age-dependent seizures of multiple types frequently provoked by elevated body temperature. Children with SMEI are normal at birth but subsequently, around the age of 9 months, develop progressively worsening febrile and afebrile seizures, including tonic–clonic, myoclonic, and absence forms, that are refractory to current therapies (Oguni et al., 2005). Interictal electroencephalography (EEG) is typically normal early in SMEI, but multifocal epileptic activities develop later (Dravet et al., 2005). Severe developmental and psychomotor delay, ataxia, spasticity, sleep disorders, cognitive impairments, and often premature deaths follow.

Studies in a mouse model of SMEI (mSMEI) demonstrated a similar striking temperature- and age-dependence of the onset and progression of generalized and myoclonic seizures (Oakley et al., 2009). P17–18 mSMEI did not have thermally induced seizures, but nearly all P20–22 and P30–46 mSMEI had myoclonic seizures followed by generalized seizures with elevated core body temperature. Spontaneous seizures were only observed in mSMEI older than P22, indicating that mSMEI had become susceptible to thermally induced seizures before spontaneous seizures. Interictal spike activity was seen at normal body temperature in most P30–46 mSMEI but not in P20–22 or P17–18 mice, suggesting that interictal epileptic activity correlates with seizure susceptibility. These results provide evidence that heterozygous deletion of *Scn1a* is sufficient to produce the key epileptic phenotypic features of SMEI.

Ataxia, spasticity, and failure of motor coordination contribute substantially to the developmental delay and functional impairments of SMEI patients and are major determinants of their poor quality of life, burden of care, and premature deaths (Dravet et al., 2005). Behavioral studies in mSMEI indicated severe motor deficits in homozygous $Na_V1.1$ knockout mice, including irregularity of stride length during locomotion, impaired motor reflexes in grasping, and mild tremor in limbs when immobile, consistent with cerebellar dysfunction (Yu et al., 2006; Kalume et al., 2007). A milder impairment of normal gait was

observed in the heterozygotes after P21 (Kalume et al., 2007). These studies provide evidence that heterozygous deletion of *Scn1a* is sufficient to produce ataxia.

What Causes Hyperexcitability, Seizures, and Comorbidities in SMEI?

It is unclear why haploinsufficiency of a sodium channel critical for initiation of action potentials should cause hyperexcitability and seizures. In fact, numerous antiepileptic medications work by blocking sodium channels (i.e., phenytoin, lamotrigine, and carbamazepine). To address this question, whole-cell sodium current was studied in hippocampal neurons in wild type mice and mSMEI. Voltage-dependent activation or inactivation of sodium channels was unchanged (Yu et al., 2006). However, the sodium current density was substantially reduced in inhibitory interneurons in the hippocampus of $Na_V1.1$ (+/−) and $Na_V1.1$ (−/−) mice, but not in their excitatorypyramidal neurons. This reduction in sodium current caused a loss of sustained high-frequency firing of action potentials in hippocampal and cortical interneurons (Yu et al., 2006; Ogiwara et al., 2007), thereby impairing their in vivo inhibitory function that depends on generation of high-frequency bursts of action potentials. An immunocytochemical survey also revealed a specific upregulation of $Na_V1.3$ channels in a subset of hippocampal interneurons, but this upregulation was insufficient to compensate for the loss of the sodium current of $Na_V1.1$ channels (Yu et al., 2006). These results suggest that reduced sodium currents in GABAergic inhibitory interneurons in $Na_V1.1$ (+/−) heterozygotes may cause the hyperexcitability that leads to epilepsy in patients with SMEI.

Ataxia may also be due to GABAergic cell-specific loss of sodium current. Cerebellar Purkinje cells are GABAergic inhibitory neurons that serve as the output pathway for information on movement, coordination, and balance from the cerebellar cortex. Degeneration of Purkinje neurons and abnormal expression of voltage-gated ion channels in them are associated with ataxia (Fletcher et al., 1996; Raman & Bean, 1997; Grusser-Cornehls & Baurle, 2001; Sausbier et al., 2004). Immunohistochemical studies showed that $Na_V1.1$ and $Na_V1.6$ channels are the primary sodium channel isoforms expressed in cerebellar Purkinje neurons (Kalume et al., 2007). To determine whether loss of sodium current occurs in Purkinje cells in mSMEI, the amplitudes of whole-cell peak, persistent and resurgent sodium currents were studied. Sodium currents were reduced by 58–69% without detectable change in the kinetics or voltage dependence of channel activation or inactivation. Current-clamp recordings revealed that the firing rates of Purkinje neurons from mutant mice were substantially reduced, with no effect on threshold for action potential generation, and that the ability to sustain firing against hyperpolarizing stimuli is greatly impaired (Kalume et al., 2007). The results show that $Na_V1.1$ channels play a crucial role in the excitability of cerebellar Purkinje neurons, with major contributions to peak, persistent, and resurgent forms of sodium current and to sustained action potential firing. Loss of these channels in Purkinje neurons of mutant mice and SMEI patients may be sufficient to cause their ataxia. Loss of sodium currents in different classes of GABAergic neurons may underlie the multiple comorbidities in SMEI, including light hypersensitivity, altered circadian rhythms and sleep disturbance, and cognitive impairment.

Can Pharmacologic Treatments Rebalance Excitation and Inhibition in mSMEI?

Because SMEI is apparently caused by loss of sodium current and failure of firing of GABAergic interneurons (Yu et al., 2006; Kalume et al., 2007), it may be compensated by rebalancing excitation and inhibition, either by reducing the sodium current and thus action potential firing of excitatory neurons or increasing GABA neuron excitability and GABAergic transmission. Decreasing excitation was studied by mating mouse lines having

different well-defined genetic deficiencies. $Na_V1.6$ channels encoded by the *Scn8a* gene are highly expressed in excitatory neurons, and their functional properties are well-suited to driving repetitive firing (Raman & Bean, 1997, 1999; Chen et al., 2008). Double heterozygous mice with haploinsufficiency for both *Scn1a* and *Scn8a* did indeed have reduced susceptibility to drug-induced seizures and improved lifespan compared to $Na_V1.1$ heterozygotes (Martin et al., 2007). Pharmacologically enhanced inhibition was studied using febrile seizures in a mouse model of SMEI as a test system (Oakley et al., 2009). Drugs such as tiagabine increase the concentration of GABA in the synaptic cleft by inhibiting its reuptake into nerve terminals and glia, and benzodiazepines such as clonazepam increase the response of the postsynaptic $GABA_A$ receptors to GABA. Combinations of tiagabine and clonazepam are effective in preventing thermally induced myoclonic and generalized tonic–clonic seizures and prolong the lifespan of mSMEI (Oakley et al., American Epilepsy Society Abstract 2010). These encouraging preliminary results suggest that similar combination drug therapies may be useful for children with SMEI.

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