Is Targeting of Compensatory Ion Channel Gene Expression a Viable Therapeutic Strategy for Dravet Syndrome?

Augmented Reticular Thalamic Bursting and Seizures in *Scn1a*-Dravet Syndrome

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Loss of function in the *Scn1a* gene leads to a severe epileptic encephalopathy called Dravet syndrome (DS). Reduced excitability in cortical inhibitory neurons is thought to be the major cause of DS seizures. Here, in contrast, we show enhanced excitability in thalamic inhibitory neurons that promotes the nonconvulsive seizures that are a prominent yet poorly understood feature of DS. In a mouse model of DS with a loss of function in *Scn1a*, reticular thalamic cells exhibited abnormally long bursts of firing caused by the downregulation of calcium-activated potassium SK channels. Our study supports a mechanism in which loss of SK activity causes the reticular thalamic neurons to become hyperexcitable and promote nonconvulsive seizures in DS. We propose that reduced excitability of inhibitory neurons is not global in DS and that non-GABAergic mechanisms such as SK channels may be important targets for treatment.

Commentary

Dravet syndrome (DS) is a catastrophic developmental and epileptic encephalopathy with cognitive, behavioral, and motor impairments, as well as a high risk of sudden unexpected death in epilepsy (SUDEP). The majority of patients with DS have *de novo* mutations in *Scn1a*, encoding the α subunit of the voltage-gated sodium channel Na$_V$1.1, resulting in haploinsufficiency.[1] *Scn1a* haploinsufficiency in mice results in severe seizures and SUDEP.[2] Neonatal (postnatal day [P] 14-16) *Scn1a*$^{+/-}$ mice have reduced sodium current density and reduced action potential firing in hippocampal GABAergic inhibitory neurons with no detectable effects on excitatory pyramidal neurons.[2] Similarly, reduced sodium currents, reduced action potential firing, and hypoexcitability have been reported in *Scn1a*$^{+/-}$ mouse cerebellar Purkinje neurons[3] and in neurons isolated from the reticular nucleus of the thalamus (nRT).[4]

Importantly, however, there is more to the story. Experiments in both mouse and human DS models have shown that compensatory overexpression of other sodium channel genes or sodium channel activity contributes to the mechanism of disease. Moreover, changes in sodium current density are dependent on the stage of brain development, at least in mice. Sodium current density, which was selectively reduced in *Scn1a*$^{+/-}$ mouse hippocampal GABAergic neurons at P14-16,[2] becomes elevated in pyramidal neurons but not in GABAergic neurons, at P21-24, resulting in spontaneous firing and hyperexcitability.[5] A study of human DS patient-derived induced pluripotent stem cell (iPSC) forebrain neurons showed increased sodium current density in both pyramidal and bipolar neurons.[6] Finally, intercrossing *Scn1a*$^{+/-}$ mice with *Scn8a*$^{+/-}$ mice resulted in reduced seizures and increased mouse survival compared to *Scn1a*$^{+/-}$ mice.[7] While there are a number of potential interpretations of these data, it is possible that *Scn8a* expression and/or that Nav1.6 activity become upregulated in pyramidal neurons and/or interneurons in response to *Scn1a* haploinsufficiency in DS mice, resulting in hyperexcitability and hypersynchrony.[8] Lowering *Scn8a* expression thus reduces seizures in this DS model. Taken together, these results suggest that there may be compensatory overexpression of other sodium channel genes or sodium channel activity in response to *Scn1a* haploinsufficiency that is dependent on brain developmental stage and further, that compensatory upregulation of *Scn8a*, encoding Nav1.6, may be critical to the mechanism of DS.

Understanding the complexity of differential gene expression, changes in channel subcellular localization or altered channel activity in DS may be critical to the generation of effective therapeutics. Previous work by Favero et al examined fast-spiking parvalbulin-positive (PV+) interneuron excitability over the life span of *Scn1a*$^{+/-}$ DS mice.[9] Early in brain development, at P10, there were no observable differences in excitability in these neurons between genotypes. At P18-20,

$Scn1a^{+/-}$ fast-spiking PV+ interneurons were observed to be hypoexcitable, in agreement with the Catterall group.[10] However, consistent with the idea of changes in the activity of another sodium channel in the mechanism of DS, no changes in fast-spiking PV+ interneuron excitability between genotypes could be detected from P35-56, suggesting that interneuron excitability normalizes in DS mouse brain with development. This result confirmed other work showing that interneuron hypoexcitability was not detectable in adult $Scn1a^{+/-}$ DS mice during spontaneous activities using *in vivo* recording techniques.[11]

The new Ritter-Makinson et al study suggests that altered expression of genes encoding other types of ion channels may also contribute to the mechanism of DS. Specifically, these investigators demonstrate that the gene, *Kcnn2S*, encoding SK2 calcium-activated small potassium channels, is selectively downregulated in nRT neurons in $Scn1a^{R1407+/-}$ knock-in DS mice[12] bred on the 80% to 88%:12% to 20% C57BL6J:C3HeB/FeJ background and assayed from P18-80. *Kcnn2S* messenger RNA (mRNA) expression was assayed in 3- to 5-month-old animals. The authors propose that this decrease in gene expression resulted in reduced SK currents, an increase in the posthyperpolarization rebound bursting known to be characteristic of nRT neurons, hyperexcitability of the intrathalamic microcircuit connecting the nRT with somatosensory ventrobasal thalamus, and ultimately the promotion of nonconvulsive seizures as measured by electroencephalogram. However, because the mRNA experiments were performed in "survivor" animals, rather than in neonates, it is not possible to determine whether changes in *Kcnn2s* expression contributed to the mechanism of disease.

Interestingly, this result is in contrast to previous work by Kalume et al, which demonstrated that nRT neurons acutely isolated from P13-14 $Scn1a^{+/-}$ mice on the 50:50 C57Bl/6:129SvJ background have reduced sodium current density, reduced posthyperpolarization rebound burst firing, and hypoexcitability.[4] Is it possible that, as sodium channel genes become upregulated with brain development in the forebrain in response to *Scn1a* haploinsufficiency, *Kcnn2s* becomes downregulated in the nRT? To resolve these opposing results, it would be interesting to compare the developmental time course of *Kcnn2s* expression in nRT neurons, as well as in the forebrain, in the 2 DS mouse models, for example, P13 through 5 months of age, and assess changes in nRT excitability as well as the incidence of nonconvulsive seizures with time. It would also be important to measure sodium current density in acutely isolated nRT neurons in both mouse models versus age-matched controls over the P13 through 5-month age range. In addition to developmental changes, could there be an effect of genetic background on the regulation of intrathalamic microcircuit excitability in DS mice? Finally, is *Kcnn2s* expression also downregulated in DS patient iPSC-derived neurons?

A confound in the Ritter-Makinson et al study's experimental design is that only a modest percentage (1/4 to 1/3) of $Scn1a^{R1407+/-}$ mice exhibit the full DS phenotype, which includes spontaneous seizures and SUDEP. Thus, the majority of mice studied in the 3- to 5-month range, for example, as reported for the mRNA expression assays, may have belonged to the non-DS phenotypic cohort. This may also be the case for the P73 and older mice used for chronic multioptrode recordings in the study. Because of this potential data acquisition bias, it would have been informative to control for mouse age in each experiment and to assess specific developmental changes in gene expression, channel function, and response to pharmacology. In addition, providing evidence of spontaneous seizures, and thus demonstrating that the mice being analyzed had the full DS phenotype, would have strengthened the work.

DS is an intractable developmental and epileptic encephalopathy. Ritter-Makinson and colleagues suggest that an effective therapeutic strategy moving forward may be to target ion channels like SK2 that undergo compensatory expression in DS brain. This approach may be more effective than activating Nav1.1 or modulating GABAergic signaling, as discussed in the article and previously suggested by other groups. The SK channel agonist, 1-ethyl-2-benzimidazolinone, used in this study to normalize the rebound bursting properties of DS nRT neurons and to treat nonconvulsive seizures in the mice, is not a Food and Drug Association (FDA)-approved drug. However, related SK agonist benzoxazolone derivatives, such as the centrally acting muscle relaxant, chlorzoxazone, also discussed in the article, are FDA approved and thus available for repurposing. Other groups have taken a similar strategy. For example, Wulff and coworkers discovered that 2-amino-6-trifluoromethylthio-benzothiazole (SKA-19), a thioanalog of riluzole, is a potent and novel anticonvulsant in preclinical models. SKA-19 is a use-dependent sodium channel blocker as well as an activator of SK channels.[13] Taken together, these new preclinical results may inform future drug discovery or repurposing efforts for DS.

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