Current Literature

In Basic Science

“It Was the Interneuron With the Parvalbumin in the Hippocampus!” “No, It Was the Pyramidal Cell With the Glutamate in the Cortex!” Searching for Clues to the Mechanism of Dravet Syndrome – The Plot Thickens

Strain- and Age-Dependent Hippocampal Neuron Sodium Currents Correlate With Epilepsy Severity in Dravet Syndrome Mice.

Mistry AM, Thompson CH, Miller AR, Vanoye CG, George AL Jr, Kearney JA. *Neurobiol Dis* 201;65:1–11.

Heterozygous loss-of-function SCN1A mutations cause Dravet syndrome, an epileptic encephalopathy of infancy that exhibits variable clinical severity. We utilized a heterozygous *Scn1a* knockout (*Scn1a*^{+/-}) mouse model of Dravet syndrome to investigate the basis for phenotype variability. These animals exhibit strain-dependent seizure severity and survival. *Scn1a*^{+/-} mice on strain 129S6/SvEvTac (129.*Scn1a*^{+/-}) have no overt phenotype and normal survival compared with *Scn1a*^{+/-} mice bred to C57BL/6J (F1.*Scn1a*^{+/-}) that have severe epilepsy and premature lethality. We tested the hypothesis that strain differences in sodium current (I_{Na}) density in hippocampal neurons contribute to these divergent phenotypes. Whole-cell voltage-clamp recording was performed on acutely-dissociated hippocampal neurons from postnatal days 21–24 (P21–24) 129.*Scn1a*^{+/-} or F1.*Scn1a*^{+/-} mice and wild-type littermates. I_{Na} density was lower in GABAergic interneurons from F1.*Scn1a*^{+/-} mice compared to wild-type littermates, while on the 129 strain there was no difference in GABAergic interneuron I_{Na} density between 129.*Scn1a*^{+/-} mice and wild-type littermate controls. By contrast, I_{Na} density was elevated in pyramidal neurons from both 129.*Scn1a*^{+/-} and F1.*Scn1a*^{+/-} mice, and was correlated with more frequent spontaneous action potential firing in these neurons, as well as more sustained firing in F1.*Scn1a*^{+/-} neurons. We also observed age-dependent differences in pyramidal neuron I_{Na} density between wild-type and *Scn1a*^{+/-} animals. We conclude that preserved I_{Na} density in GABAergic interneurons contributes to the milder phenotype of 129.*Scn1a*^{+/-} mice. Furthermore, elevated I_{Na} density in excitatory pyramidal neurons at P21–24 correlates with age-dependent onset of lethality in F1.*Scn1a*^{+/-} mice. Our findings illustrate differences in hippocampal neurons that may underlie strain- and age-dependent phenotype severity in a Dravet syndrome mouse model, and emphasize a contribution of pyramidal neuron excitability.

Commentary

Dravet syndrome (DS, also known as Severe Myoclonic Epilepsy of Infancy) is a catastrophic pediatric epileptic encephalopathy with cognitive, behavioral, and motor impairments, as well as a high risk of sudden unexpected death in epilepsy (SUDEP). The first mutations linked to DS were identified in *SCN1A*, the gene encoding the α subunit of the voltage-gated sodium channel Na_v1.1 (1). To date, the majority of DS patients have de novo *SCN1A* mutations that result in haploinsufficiency (2). This raises some interesting questions: How does reduced expression of a major brain sodium channel gene result in hyperexcitability and network synchrony? Further, why do individuals with the same

heterozygous *SCN1A* mutation, even within the same family, sometimes have divergent seizure phenotypes?

A major breakthrough in understanding the mechanism of DS occurred with the development of the first mouse model of the disease by the Catterall group (3). Global deletion of *Scn1a* yielded a strong seizure phenotype in heterozygous animals and was the first in vivo confirmation of *Scn1a* haploinsufficiency resulting in seizures. Patch clamp recordings in acutely dissociated hippocampal neurons isolated from postnatal day (P) 14–16 *Scn1a*^{+/-} mice demonstrated that morphologically identified bipolar neurons, but not pyramidal neurons, had dramatically reduced sodium current density. This resulted in reduced firing frequency of interneurons in response to injected current. Nav1.1 was postulated to be the major functional sodium channel in interneurons, particularly in fast-spiking, parvalbumin-positive cells (3, 4). In contrast, glutamatergic pyramidal neurons were proposed to rely on other brain sodium

Epilepsy Currents, Vol. 14, No. 6 (November/December) 2014 pp. 350–352
© American Epilepsy Society

OPEN ACCESS Freely available online

channels, possibly Nav1.2 or Nav1.6, for firing. Based on this model, it was hypothesized that pathophysiology in DS is due to dysfunctional inhibitory circuits: the “interneuron hypothesis.”

Since the initial description of *Scn1a*^{+/-} mice by the Catterall group, two competing theories have emerged to explain the mechanism of DS. The interneuron hypothesis is largely supported by work done in mouse models (4–10), while more recent data that challenge it, showing increased sodium current density and hyperexcitability in both inhibitory and excitatory neurons, have been generated using human DS patient-derived induced pluripotent stem cell (iPSC) neurons (11, 12). Is it possible that this discordance results from differences in the model systems themselves? In their paper in *Neurobiology of Disease*, Mistry and colleagues begin to bridge the gap between competing DS mechanistic theories. Prior work from Dr. Kearney's laboratory (13) showed that phenotypic severity of the *Scn1a*^{+/-} mutation in mice is highly dependent on genetic background. *Scn1a*^{+/-} mice maintained on the 129 strain exhibited no seizures and lived normal life spans. In contrast, crossing *Scn1a*^{+/-} mice on the 129 background to the *C57Bl/6J* strain, referred to as *F1.Sc1a*^{+/-}, resulted in animals with a severe phenotype, including overt seizures and SUDEP. Thus, genetic background is key to the severity of pathophysiology in DS mouse models. In the Mistry et al. study, the authors compare sodium current properties and neuronal firing in acutely dissociated hippocampal neurons from *Scn1a*^{+/-} mice on the 129 versus the 129 x *C57Bl/6J* F1 backgrounds at two different developmental time points. In all of their experiments, the identity of acutely dissociated bipolar interneurons was confirmed by single cell RT-PCR. Using the criteria of *Gad67* but not *Gfap* positivity, only 48% of morphologically identified “bipolar” neurons were confirmed as such. This is an important consideration for the interpretation of previous work in which a proportion of morphologically identified “bipolar neurons” may have been, in reality, astrocytes.

Patch clamp recording of hippocampal neurons showed that sodium current density was significantly lower in identified P21–24 GABAergic interneurons from the phenotypically more severe *F1.Sc1a*^{+/-} strain compared to wild-type littermates, similar to the Catterall et al. (3) study focusing on neurons isolated from P14–16 *Scn1a*^{+/-} mice bred for 6 generations onto *C57Bl/6* from 129SvJ. In contrast, GABAergic interneuron sodium current densities in 129.*Scn1a*^{+/-} mice (which had no spontaneous seizures) and their corresponding wild-type littermates were indistinguishable. Surprisingly, and similar to more recently published findings in human iPSC forebrain neuron models of DS (11), sodium current density was elevated in pyramidal neurons from both P21–24 129.*Scn1a*^{+/-} and *F1.Sc1a*^{+/-} mice compared to strain-matched wild-type littermates. Importantly, pyramidal neurons from the phenotypically more severe *F1.Sc1a*^{+/-} strain displayed a significantly larger increase in sodium current density compared to pyramidal neurons from the less severe 129.*Scn1a*^{+/-} strain. In addition, *F1.Sc1a*^{+/-}, but not 129.*Scn1a*^{+/-} P21–24 pyramidal neurons exhibited a significant hyperpolarizing shift in the voltage-dependence of activation compared to wild-type. Consistent with this observation and in agreement with previous studies (8), P21–24 *F1.Sc1a*^{+/-} pyramidal neurons exhibited high frequency spontaneous firing and greater excitability

across a range of current injections. P21–24 pyramidal neurons from the less phenotypically severe 129.*Scn1a*^{+/-} strain also exhibited higher spontaneous firing frequencies than wild-type, but their rate of firing was significantly less than that observed in the *F1.Sc1a*^{+/-} animals.

These observed increases in pyramidal neuron sodium current density were age-dependent, occurring later in development than the observed reduction in inhibitory neuron sodium current density. Sodium current densities in P14–15 pyramidal neurons were not significantly different between mutant and wild-type on either background. Interestingly, the exaggerated increase in sodium current density in the P21–24 *F1.Sc1a*^{+/-} excitatory pyramidal neurons correlated with age-dependent onset of lethality. Finally, the authors reported strain-dependent trends in compensatory up- or down-regulation of other brain sodium channel transcripts in response to *Scn1a* haploinsufficiency, results that may shed light on patient-specific difference in phenotype severity.

This study provides important new clues to solving the mechanism of DS. First, it is now clear from multiple models of DS that both inhibitory and excitatory neurons have altered sodium currents, suggesting that both inhibitory and excitatory networks contribute to seizure severity. Inhibitory neuron sodium channel loss-of-function combined with excitatory neuron sodium channel gain-of-function may result in an inhibition-excitation imbalance during the P21–24 developmental period in *F1.Sc1a*^{+/-} mice leading to severe seizures. Second, similar to human DS patients with variable seizure severity and susceptibility to SUDEP, the phenotypic severity of DS mouse models is highly dependent on genetic background.

In spite of these advances, important questions remain. A detailed developmental time course of sodium channel protein expression in excitatory versus inhibitory neurons (and in different interneuronal subtypes) in specific brain areas and comparing mouse strains is a necessary, yet difficult, next step. A detailed examination of sodium channel expression in the developmental period surrounding P18, the time point at which *F1.Sc1a*^{+/-} begin to seize, would be particularly informative. Not only sodium channel pore-forming α subunits, but also β subunits, need to be investigated. It will be important to know whether impaired inhibitory neuron GABAergic signaling through loss of Nav1.1-mediated sodium current density during early development (e.g., P14–16) can subsequently influence sodium current expression in excitatory neurons, presumably through synaptic signaling, at later time points (P21–24). If the expression of other sodium channel subunits is indeed altered in response to *Scn1a* haploinsufficiency, as suggested here and in the human iPSC neuron models, what is underlying the mechanism for this compensatory gene expression? Does the developmental switch of early GABAergic excitatory to inhibitory signaling occur normally in *Scn1a*^{+/-} mouse and iPSC neuron models? If not, this in itself changes the prevailing ideas surrounding disinhibition. Finally, what other genes—that is, encoding other ion channels, cell adhesion molecules, or synaptic proteins—are altered in response to *Scn1a* haploinsufficiency? New animal models, as well as analysis of DS patient-derived iPSC neurons, which allow correlation of human epilepsy phenotypes with cell autonomous electrophysiological, genetic, and channel protein

complex characterization, will shed further light on this rapidly progressing field.

by Lori L. Isom, PhD

References

1. Claes L, Del Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* 2001;68:1327–1332.
2. Marini C, Scheffer IE, Nabbout R, Suls A, De Jonghe P, Zara F, Guerrini R. The genetics of Dravet syndrome. *Epilepsia* 2011;52(suppl 2):24–29.
3. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci* 2006;9:1142–1149.
4. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K. Na(v)1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: A circuit basis for epileptic seizures in mice carrying an *Scn1a* gene mutation. *J Neurosci* 2007;27:5903–5914.
5. Cheah CS, Yu FH, Westenbroek RE, Kalume FK, Oakley JC, Potter GB, Rubenstein JL, Catterall WA. Specific deletion of NaV1.1 sodium channels in inhibitory interneurons causes seizures and premature death in a mouse model of Dravet syndrome. *Proc Natl Acad Sci U S A* 2012;109:14646–14651.
6. Kalume F, Westenbroek RE, Cheah CS, Yu FH, Oakley JC, Scheuer T, Catterall WA. Sudden unexpected death in a mouse model of Dravet syndrome. *J Clin Invest* 2013;123:1798–1808.
7. Yu FH, Mantegazza M, Westenbroek R, Robbins CA, Spain WJ, Burton KA, McKnight GS, Scheuer T, Catterall WA. Deletion of the Na_v1.1 channel: A mouse model for severe myoclonic epilepsy of infancy. Abstract Viewer/Itinerary Planner Washington, DC: Society for Neuroscience. 2004; Program No. 479.5.
8. Liautard C, Scalmani P, Carriero G, de Curtis M, Franceschetti S, Mantegazza M. Hippocampal hyperexcitability and specific epileptiform activity in a mouse model of Dravet syndrome. *Epilepsia* 2013;54:1251–1261.
9. Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, Rubenstein JL, Scheuer T, de la Iglesia HO, Catterall WA. Autistic-like behaviour in *Scn1a*^{+/-} mice and rescue by enhanced GABA-mediated neurotransmission. *Nature* 2012;489:385–390.
10. Han S, Yu FH, Schwartz MD, Linton JD, Bosma MM, Hurley JB, Catterall WA, de la Iglesia HO. Na(V)1.1 channels are critical for intercellular communication in the suprachiasmatic nucleus and for normal circadian rhythms. *Proc Natl Acad Sci U S A* 2012;109:E368–377.
11. Liu Y, Lopez-Santiago LF, Yuan Y, Jones JM, Zhang H, O'Malley HA, Patino GA, O'Brien JE, Rusconi R, Gupta A, Thompson RC, Natowicz MR, Meisler MH, Isom LL, Parent JM. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. *Ann Neurol* 2013;74:128–139.
12. Jiao J, Yang Y, Shi Y, Chen J, Gao R, Fan Y, Yao H, Liao W, Sun XF, Gao S. Modeling Dravet syndrome using induced pluripotent stem cells (iPSCs) and directly converted neurons. *Hum Mol Genet* 2013; 22: 4241–4252.
13. Miller AR, Hawkins NA, McCollom CE, Kearney JA. Mapping genetic modifiers of survival in a mouse model of Dravet syndrome. *Genes Brain Behav* 2014;13:163–172.