JCI The Journal of Clinical Investigation

Epilepsy channelopathies go neddy: stabilizing $Na_V1.1$ channels by neddylation

Stephen C. Cannon

J Clin Invest. 2021;131(8):e148370. https://doi.org/10.1172/JCI148370.

Commentary

Loss-of-function mutations of *SCN1A* encoding the pore-forming α subunit of the $Na_V1.1$ neuronal sodium channel cause a severe developmental epileptic encephalopathy, Dravet syndrome (DS). In this issue of the *JCI*, Chen, Luo, Gao, et al. describe a phenocopy for DS in mice deficient for posttranslational conjugation with neural precursor cell expressed, developmentally downregulated 8 (NEDD8) (neddylation), selectively engineered in inhibitory interneurons. Pursuing the possibility that this phenotype is also caused by loss of $Na_V1.1$, Chen, Luo, Gao, and colleagues show that interneuron excitability and GABA release are impaired, $Na_V1.1$ degradation rate is increased with a commensurate decrease of $Na_V1.1$ protein, and $Na_V1.1$ is a substrate for neddylation. These findings establish neddylation as a mechanism for stabilizing $Na_V1.1$ subunits and suggest another pathomechanism for epileptic sodium channelopathy.

Epilepsy channelopathies go neddy: stabilizing $Na_V1.1$ channels by neddylation

Stephen C. Cannon

Department of Physiology, David Geffen School of Medicine, UCLA, Los Angeles, California, USA.

Loss-of-function mutations of *SCN1A* encoding the pore-forming α subunit of the $Na_V1.1$ neuronal sodium channel cause a severe developmental epileptic encephalopathy, Dravet syndrome (DS). In this issue of the *JCI*, Chen, Luo, Gao, et al. describe a phenocopy for DS in mice deficient for posttranslational conjugation with neural precursor cell expressed, developmentally downregulated 8 (NEDD8) (neddylation), selectively engineered in inhibitory interneurons. Pursuing the possibility that this phenotype is also caused by loss of $Na_V1.1$, Chen, Luo, Gao, and colleagues show that interneuron excitability and GABA release are impaired, $Na_V1.1$ degradation rate is increased with a commensurate decrease of $Na_V1.1$ protein, and $Na_V1.1$ is a substrate for neddylation. These findings establish neddylation as a mechanism for stabilizing $Na_V1.1$ subunits and suggest another pathomechanism for epileptic sodium channelopathy.

The $Na_V1.1$ sodium channel in epilepsy

Epilepsy is a disorder of brain function of which the cardinal feature is an enduring propensity for generating seizures. These anomalous paroxysms of hypersynchronous network activity may arise from a variety of brain insults, including developmental defects, acquired lesions (trauma, stroke, infection), and a myriad of associated gene defects. Mutations that disrupt ion channel genes, so-called channelopathies, are often identified in familial epilepsy syndromes or as de novo lesions in developmental epileptic encephalopathy.

Not surprisingly, epilepsy mutations often occur in genes coding for the pore-forming α subunits of voltage-gated sodium channels that generate action potentials in excitable cells (1–3). Mutant α subunits may have altered function or may fail to form a functional channel, and these discoveries have served as the foundation for understanding epileptogenesis at a molecular level. Accessory subunits of the channel complex and channel-interacting proteins have also emerged as culprits in epileptic channelopathies (4). In this issue of the *JCI*, Chen, Luo, Gao, et al. (5) report on uncovering a new potential mechanism for sodium-channel epilepsy, wherein disrupted neddylation of the channel α subunit increases the protein degradation rate and results in lower Na^+ current density with reduced GABAergic inhibition by interneurons.

The human genome contains nine distinct α subunit genes (*SCN1A*, *SCN2A*, etc.) coding for the main pore-forming subunits $Na_V1.1$, $Na_V1.2$, etc. and four accessory β subunit genes (*SCN1B*, etc.). Epilepsy syndromes have been associated with mutations of $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.6$, and the β_1 subunit. The number of identified epilepsy mutations for $Na_V1.1$ greatly exceeds that for all the other sodium-channel subunits, and mutations of $Na_V1.1$ cause a wide variety of clinical phenotypes (Figure 1) ranging from mild to severe epilepsy with seizures refractory to drug treatment, developmental delay, and cognitive impairment (i.e., epileptic encephalopathy). A genotype/phenotype pattern is emerging wherein missense mutations of $Na_V1.1$ with modest alterations of channel function are found in mild syndromes (generalized epilepsy with febrile seizures [GEFS] and generalized epilepsy with febrile seizures plus [$GEFS^+$]). In contrast, haploinsufficiency from a single null allele (e.g., frameshift, nonsense with premature truncation) causes a severe developmental epileptic encephalopathy with onset at six months of age (Dravet syndrome [DS]) (3). Over 700 pathogenic or likely pathogenic variants of *SCN1A* are listed for DS on ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as of March 2021.

The association of a severe epilepsy phenotype with a sodium-channel loss of function was initially puzzling because epilepsy is a disorder of anomalously enhanced excitability of neuronal activity. A major clue was provided by the $Na_V1.1$ knockout mouse (6). Global haploinsufficiency from heterozygous deletion of exon 26 (*Scn1a*$^{+/-}$) leads to early onset seizures (spontaneous and temperature induced), mild ataxia, and sudden unexplained death in epilepsy (SUDEP), which is similar to what occurs in individuals with DS. $Na_V1.1$-deficient mice had a reduction of Na^+ current density and decreased firing rates of parvalbumin expressing inhibitory GABAergic neurons (PVINs), but not excitatory neurons, in the cerebral cortex, hippocampus, cerebellar Purkinje cells, and reticular nucleus of the thalamus. A similar epilepsy phenotype occurred with selective haploinsufficiency (PV-*Scn1a*$^{+/-}$) restricted to PV-positive interneurons, while the epilepsy phenotype was milder with *Scn1a*$^{+/-}$ selective for somatostatin-positive inhibitory interneurons (7, 8). Conversely, haploinsufficiency of *Scn1a* in excitatory neurons ameliorates the DS phenotype (9). Exceptions to this geno-

▶ **Related Article: https://doi.org/10.1172/JCI136956**

Conflict of interest: The author has declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2021;131(8):e148370. https://doi.org/10.1172/JCI148370.

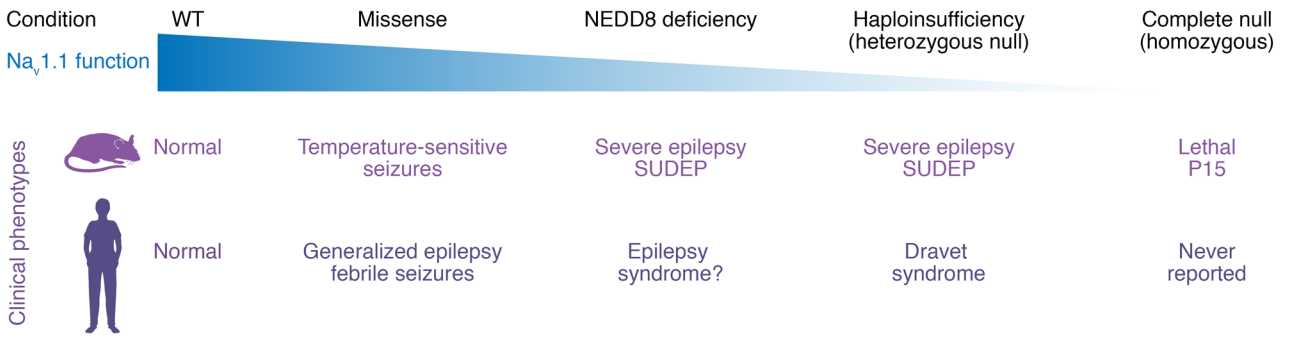

Figure 1. Spectrum of epilepsy syndromes from $Na_V1.1$ loss of function. $Na_V1.1$ loss of function caused by *SCN1A* mutations or NEDD8 deficiency induces a wide variety of clinical phenotypes with varying severity.

type/phenotype paradigm indicate more work needs to be done. For example, about half of the *SCN1A* variants reported in DS cases are missense mutations, some of which caused gain-of-function changes in expression studies (10); the reduced excitability of PVINs in *Scn1a*$^{+/-}$ mice at P14 later resolved at P18–P21, whereas seizures persisted (11). Others reported hyperexcitability and increased Na^+ currents in P21–P24 hippocampal pyramidal neurons, suggesting overexpression of other sodium-channel genes (12). Nevertheless, the effects of murine $Na_V1.1$ loss of function coupled to severe early onset seizures and SUDEP are robust and were the basis for implicating $Na_V1.1$ in the neddylation studies by Chen, Luo, Gao, et al. reported in this issue (5).

Epilepsy phenotype from disrupted neddylation in mouse interneurons

Posttranslational modification by the ubiquitin-like protein neural precursor cell expressed, developmentally downregulated 8 (NEDD8) regulates many cellular functions (transcription, proliferation, differentiation, apoptosis), is essential for dendritic spine maturation and stability (13), and has been implicated in disorders of the central nervous system (14). To interrogate the role of NEDD8 in parvalbumin-positive inhibitory interneurons (PVINs), Chen, Luo, Gao, et al. (5) generated a conditional knockout of an obligatory subunit for the NEDD8-specific E1 (NAE1). Unexpectedly, the mice had severe epilepsy, ataxia, and a median survival of only 60 days. The abundance of PVINs was unchanged in *PV-Nae1*$^{-/-}$ mice, but inhibitory GABAergic neurotransmission was reduced and intrinsic excitability was reduced for PVINs, but not pyramidal neurons. Further investigation for the cause of reduced excitability in PVINs revealed a Na^+ current density of 62% of WT. When measured by immunoblot, $Na_V1.1$ protein amounts were decreased, whereas the abundance of the other major α subunit ($Na_V1.6$) remained unchanged. By comparison, Na^+ current density for PVINs in the DS *Scn1a*$^{+/-}$ mice was 47% of WT and 38% of WT for homozygous *Scna1*$^{-/-}$ (6). Epileptic seizures began around P30 for *PV-Nae1*$^{-/-}$ mice, whereas for DS *Scna1*$^{+/-}$ mice, seizure onset was earlier (P21), consistent with a more severe loss of Na^+ current for *Scna1*$^{+/-}$.

The observed reduction in Na^+ current density is sufficient to explain the reduced excitability of PVINs, but why does impaired neddylation lead to lower amounts of $Na_V1.1$? There is no previously established role for regulating the abundance of voltage-gated ion channel proteins by neddylation, although for the epithelial sodium channel (α-ENaC), NEDD8 conjugated to cullin-1 increases ubiquitination and proteolytic degradation of the channel (15). Chen, Luo, Gao, and colleagues excluded a NEDD8 effect on *$Na_V1.1$* transcript levels in *PV-Nae1*$^{-/-}$ mice (5). Instead, expression studies in a human cell line (tsA-201 cells) showed an increased rate of proteasome-dependent $Na_V1.1$ degradation when neddylation was inhibited using MLN4924. The interpretation is that neddylation stabilizes $Na_V1.1$ by preventing ubiquitination, just the inverse of how NEDD8/cullin-1 regulates α-ENaC.

What is the evidence $Na_V1.1$ is a substrate for neddylation? NEDD8 immunoreactivity was detected after immunoprecipitation of tagged $Na_V1.1$ expressed in a human cell line (HEK cells) or for native $Na_V1.1$ from brain. Moreover, the NEDD8 signal was reduced in the presence of the NAE inhibitor MLN-4924 and for brain homogenates from *PV-Nae1*$^{-/-}$ mice, consistent with neddylation of $Na_V1.1$. To search for potential neddylation sites at lysines in $Na_V1.1$, the authors screened for variants of Lys residues in ClinVar and an epilepsy database. Thirteen epilepsy variants were identified, two of which were studied further because they are located in cytoplasmic loops. The $Na_V1.1$ K1936E variant, 74 residues upstream from the C-terminal Lys, was expressed in HEK cells and found to have increased rates of $Na_V1.1$ degradation, reduced NEDD8 immunostaining, and lower Na^+ current density, all of which are consistent with impaired neddylation and destabilized $Na_V1.1$ protein. $Na_V1.1$ K1936 is not conserved in $Na_V1.6$ (established using the multiple sequence alignment program CLUSTAL 1.2.4), which may account for the lack of a detectable change for $Na_V1.6$ expression in *Nae1*$^{-/-}$ mice. Unfortunately, the clinical annotation for $Na_V1.1$ K1936E was insufficient to ascertain the confidence level showing that this is indeed a pathogenic mutation for epilepsy.

Many substrates for neddylation are surely present in PVINs, and the downstream effects of this posttranslational modification may be diverse and extensive. The authors acknowledge that effects beyond the reduction of $Na_V1.1$ may contribute to the ataxia and severe epilepsy in the *PV-Nae1*$^{-/-}$ mouse. A proteome screen identified 5167 proteins with 169 downregulated and 279 upregulated for *PV-Nae1*$^{-/-}$ mice compared with WT. The former group is implicated in neural development, synaptic plasticity, and glutamatergic neu-

rotransmission, whereas the upregulated set is involved with ubiquitin-regulated catabolism and the metabolic pathway. Interestingly, of the 53 ion channel genes identified in this analysis, only the reduction of $Na_v1.1$ could readily account for the decreased excitability observed in PVINs.

Open questions and clinical implications

The study by Chen, Luo, Gao, et al. in this issue of the *JCI* identifies a mechanism for fine-tuning neuronal excitability by neddylation-dependent stabilization of ion channel proteins and also reveals that selective disruption of this regulatory pathway in PVINs leads to ataxia, severe epilepsy, and SUDEP in mice (5). This phenotype highly resembles that of DS models in which the coding potential of *Scn1a* is destroyed and lends credence to the notion that loss-of-function defects for $Na_v1.1$ in PVINs produces susceptibility to severe epilepsy. It remains to be established that epilepsy in humans is attributable to impaired neddylation of $Na_v1.1$. One question is whether haploinsufficiency of Nae1 or some other critical component for NEDD8 conjugation will be sufficient to cause epilepsy. A global null of *Nae1* is embryonic lethal (16). If neddylation is also deficient in excitatory pyramidal neurons, will the balance between excitation and inhibition be restored to ameliorate the risk of seizures as occurs in *Scn1a*$^{+/-}$ mice (9)? Similarly, it is unknown whether a single $Na_v1.1$ K1936E allele is sufficient to cause an epilepsy phenotype in mice or humans. The probability of homozygous $Na_v1.1$ K1936E would be exceedingly small, except for cases of consanguinity. More studies in murine models may shed light on whether the heterozygous cases are sufficient to cause epilepsy.

Acknowledgments

SCC is supported by NIH grant R01 AR 063182 and the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at the UCLA Research Award Program.

Address correspondence to: Stephen Cannon, Department of Physiology, David Geffen School of Medicine, 10833 Le Conte Ave., Los Angeles, California 90095, USA. Phone: 310.825.5882; Email: sccannon@mednet.ucla.edu.

1. Mantegazza M, et al. Sodium channelopathies of skeletal muscle and brain [published online March 26, 2021]. *Physiol Rev*. https://doi.org/10.1152/physrev.00025.2020.
2. Escayg A, et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet*. 2000;24(4):343–345.
3. Claes L, et al. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet*. 2001;68(6):1327–1332.
4. Wallace RH, et al. Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. *Nat Genet*. 1998;19(4):366–370.
5. Chen W, et al. Neddylation stabilizes $Na_v1.1$ to maintain interneuron excitability and prevent seizures in murine epilepsy models. *J Clin Invest*. 2021;131(8):e136956.
6. Yu FH, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006;9(9):1142–1149.
7. Cheah CS, et al. 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(36):14646–14651.
8. Rubinstein M, et al. Dissecting the phenotypes of Dravet syndrome by gene deletion. *Brain*. 2015;138(Pt 8):2219–2233.
9. Ogiwara I, et al. Nav1.1 haploinsufficiency in excitatory neurons ameliorates seizure-associated sudden death in a mouse model of Dravet syndrome. *Hum Mol Genet*. 2013;22(23):4784–4804.
10. Rhodes TH, et al. Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. *Proc Natl Acad Sci U S A*. 2004;101(30):11147–11152.
11. Favero M, et al. A transient developmental window of fast-spiking interneuron dysfunction in a mouse model of Dravet syndrome. *J Neurosci*. 2018;38(36):7912–7927.
12. Mistry AM, et al. Strain- and age-dependent hippocampal neuron sodium currents correlate with epilepsy severity in Dravet syndrome mice. *Neurobiol Dis*. 2014;65:1–11.
13. Vogl AM, et al. Neddylation inhibition impairs spine development, destabilizes synapses and deteriorates cognition. *Nat Neurosci*. 2015;18(2):239–251.
14. Mori F, et al. Accumulation of NEDD8 in neuronal and glial inclusions of neurodegenerative disorders. *Neuropathol Appl Neurobiol*. 2005;31(1):53–61.
15. Downs CA, et al. H2O2 regulates lung epithelial sodium channel (ENaC) via ubiquitin-like protein Nedd8. *J Biol Chem*. 2013;288(12):8136–8145.
16. Zou J, et al. Neddylation mediates ventricular chamber maturation through repression of Hippo signaling. *Proc Natl Acad Sci U S A*. 2018;115(17):E4101–E4110.