

TRAFFIC JAM AT THE SODIUM CHANNEL

Modulatory Proteins Can Rescue a Trafficking Defective Epileptogenic Nav1.1 Na⁺ Channel Mutant. Rusconi R, Scalmani P, Cassulini RR, Giunti G, Gambardella A, Franceschetti S, Annesi G, Wanke E, Mantegazza M. *J Neurosci* 2007;27(41):11037–11046. Familial epilepsies are often caused by mutations of voltage-gated Na⁺ channels, but correlation genotype–phenotype is not yet clear. In particular, the cause of phenotypic variability observed in some epileptic families is unclear. We studied Nav1.1 (SCN1A) Na⁺ channel subunit M1841T mutation, identified in a family characterized by a particularly large phenotypic spectrum. The mutant is a loss of function because when expressed alone, the current was no greater than background. Function was restored by incubation at temperature <30°C, showing that the mutant is trafficking defective, thus far the first case among neuronal Na⁺ channels. Importantly, also molecular interactions with modulatory proteins or drugs were able to rescue the mutant. Protein–protein interactions may modulate the effect of the mutation in vivo and thus phenotype; variability in their strength may be one of the causes of phenotypic variability in familial epilepsy. Interacting drugs may be used to rescue the mutant in vivo.

COMMENTARY

Neuronal sodium channels subserve action potential generation and are critical for membrane excitability. Sodium channels are large transmembrane proteins comprised of α and β subunits. The α subunit consists of four homologous domains with intracellular amino- and carboxy-terminus tails (1) and forms the voltage-sensing region of the channel as well as the pore through which sodium ions diffuse. Each α subunit is associated with one or more β subunits that modulate the voltage dependence, kinetics, and expression of the sodium channel (2). More than 150 mutations of genes coding for α or β subunits have been reported in patients with epilepsy; these mutations are found throughout the protein structure. Nevertheless, despite the growing recognition of the role of sodium channel mutations in epilepsy, much remains to be discovered regarding the correlation of genotype with phenotype.

The gene *SCN1A* codes for the neuronal sodium channel α protein Nav1.1. Mutations in *SCN1A* have been documented in a spectrum of clinical epilepsy syndromes, ranging from relatively benign generalized epilepsy with febrile seizures plus (GEFS+) to intractable epilepsy with mental retardation and ataxia seen in severe myoclonic epilepsy of infancy (SMEI, or Dravet syndrome). GEFS+ is mainly familial, occurring in large pedigrees, within which a wide range of epilepsy severity exists; most GEFS+ mutations are missense. SMEI usually arises from a de novo truncation mutation, though some inherited cases have been reported. The overlap between these syndromes is yielding important information for the elucidation of genotype/phenotype mechanisms.

The paper by Rusconi and colleagues examines the cellular functional consequences of a specific *SCN1A* mutation,

M1841T, in which methionine is replaced by threonine at the amino acid position 1841; the mutation is located on the C-terminus tail region of the Nav1.1 α subunit. This missense mutation was previously found in an Italian family exhibiting a variety of epilepsy phenotypes, ranging from simple febrile seizures to SMEI (3). Here, Rusconi et al. show that the M1841T mutation results in loss of function of the sodium channel. When the mutated protein is transfected into human embryonic kidney cells, the cells harboring mutant channels pass almost no sodium current. There was no difference in biophysical characteristics of sodium channels in wild type and mutants, such as activation, inactivation, recovery, and persistent Na⁺ current (I_{NaP}) (4).

The investigators showed that several manipulations could rescue the M1841T loss of function. That is, the sodium current could be restored to more than 50% of the amplitude seen in wild type control cells by decreasing temperature or adding $\beta 1$ (or other β subunits), calmodulin, or phenytoin. How do these disparate compounds rescue the mutant? Interestingly, each one binds to the intracellular tail of the C-terminus region of the α subunit, near the M1841T mutated region. It has been shown previously that the C-terminus region subserves interactions between sodium channel α and β subunits, suggesting that the M1841T mutation disrupts channel function by altering that interaction.

In particular, the ability of decreased temperature (in this case, a permissive temperature of 27°C) to rescue channel function suggests that the mutation causes a defect in protein trafficking from the endoplasmic reticulum to the plasma membrane. Improperly folded proteins cannot pass from the endoplasmic reticulum to the plasma membrane. Lowering temperature allows a greater proportion of misfolded protein to bypass endoplasmic reticulum quality control mechanisms and reach the target location (5). Thus, a mutation in protein trafficking would prevent translocation of the abnormally folded sodium channel α subunits from reaching its final destination in the

plasma membrane, where it can exert its excitability function. Such a loss of excitability might seem paradoxical to the development of epilepsy, but the exact pathophysiological consequences depend on a number of factors, including the specific cells and specific brain regions that harbor the mutation. For example, it recently has been shown that *SCN1A* mutations cause epilepsy in $\text{Na}_v1.1$ knockout mice by decreasing the excitability of inhibitory GABA interneurons (6).

The authors speculate that in vivo variability of protein–protein interactions may underlie phenotypic variation in some epileptic families and that loss of function of $\text{Na}_v1.1$ function, as a result of a M1841T, facilitates development of SMEI—similar to loss of function truncation mutations. Whether novel therapeutics could be developed by targeting protein trafficking is uncertain. An intrinsic problem would need to be overcome: the therapy would need to be able to rescue the mutant proteins but not alter the rescued channels already extant in the plasma membrane. Since phenytoin, a therapeutic agent already in widespread use as an antiepileptic, partially rescues mutant channel function, these findings widen the possible mechanisms by which this drug reduces excitability. However, caution is raised because phenytoin acts by blocking activated sodium channels and other sodium channel blockers (carbamazepine and lamotrigine) seem to be contraindicated in SMEI (7).

This paper provides an intriguing potential mechanism for a specific *SCN1A* sodium channel mutation—a defect in protein trafficking. Aberrant protein trafficking has already been demonstrated for several GABA-receptor subunits including $\gamma 2$ (8) and $\alpha 1$ (9). For example, mutations of the GABA_A-receptor $\gamma 2$ subunit, which mediates receptor trafficking, show temperature dependence, with temperature increases causing rapid trafficking impairment and receptor dysfunction, possibly contributing to genetic susceptibility to febrile seizures (8).

Therefore, these studies raise the intriguing possibility that genetic mutations causing defective protein trafficking comprise a common motif for genetic epilepsies, especially channelopathies. Protein folding and trafficking defects are well described in other disorders of excitability regulation, including long QT syndromes (involving mutations of the *SCN5A* sodium channel gene causing inherited arrhythmias (10)) and cystic fibrosis, in which a mutation of the transmembrane conductance regulator (CFTR) results in abnormal

chloride secretion (11). Protein trafficking defects now join a multitude of other pathogenetic mechanisms underlying hyperexcitability caused by *SCN1A* mutations suggest that manifold opportunities for novel therapeutic interventions exist. The challenge is now to unravel the traffic jam at the sodium channel.

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