Current Literature
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Criminal Minds: Cav3.2 Channels Are the Culprits, but NMDAR Are the Co-Conspirators

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Ca$_V$3.2 Calcium Channels Control NMDA Receptor-Mediated Transmission: A New Mechanism for Absence Epilepsy.

Wang G, Bochorishvili G, Chen Y, Salvati KA, Zhang P, Dubel SJ, Perez-Reyes E, Snutch TP, Stornetta RL, Deisseroth K, Erisir A, Todorovic SM, Luo JH, Kapur J, Beenhakker MP, Zhu JJ. *Genes Dev* 2015;29:1535–1551.

Ca$_V$3.2 T-type calcium channels, encoded by *CACNA1H*, are expressed throughout the brain, yet their general function remains unclear. We discovered that Ca$_V$3.2 channels control NMDA-sensitive glutamatergic receptor (NMDA-R)-mediated transmission and subsequent NMDA-R-dependent plasticity of AMPA-R-mediated transmission at rat central synapses. Interestingly, functional Ca$_V$3.2 channels primarily incorporate into synapses, replace existing Ca$_V$3.2 channels, and can induce local calcium influx to control NMDA transmission strength in an activity-dependent manner. Moreover, human childhood absence epilepsy (CAE)-linked hCa$_V$3.2 (C456S) mutant channels have a higher channel open probability, induce more calcium influx, and enhance glutamatergic transmission. Remarkably, cortical expression of hCa$_V$3.2 (C456S) channels in rats induces 2- to 4-Hz spike and wave discharges and absence-like epilepsy characteristic of CAE patients, which can be suppressed by AMPA-R and NMDA-R antagonists but not T-type calcium channel antagonists. These results reveal an unexpected role of Ca$_V$3.2 channels in regulating NMDA-R-mediated transmission and a novel epileptogenic mechanism for human CAE.

Commentary

Childhood absence epilepsy (CAE) accounts for 2 to 10 percent of all epileptic disorders in children. It involves short lapses of consciousness that are highly disruptive for learning and other activities of daily life. In the 1950s, Zimmerman and Burgemeister set out to find novel medications to treat CAE that would be as effective as trimethadione, but with fewer side effects (1). They discovered that among the candidates, ethosuximide had the highest effectiveness against CAE and lowest undesirable side effects. This drug remains the first-choice medication for CAE. One of the generally agreed mechanisms for the effect of ethosuximide is blockade of T-type voltage gated calcium channels, i.e. those in the Cav3 family (2).

The three members of the Cav3 family — Cav3.1, 3.2, and 3.3 — are differentially expressed in various brain regions (3). Studies using knockout models of Cav3.1 and Cav3.3 have documented that these channels are indispensable for burst firing. This is consistent with their ability to open with small depolarization of membrane potential and subsequently promote generation of low-threshold calcium spikes (4, 5). Burst firing has been shown to be a highly reliable method of driving activity in downstream neurons (6), suggesting T-type calcium channels, along with other means of generat-

ing bursts, play critical roles in normal information processing. In pathological conditions, mutations of these channels have been hypothesized to lead to greater calcium influx near resting membrane potential, and enhanced burst firing that would increase the propensity to develop epileptic seizures. Indeed, genetic alterations of CACNA1H (the gene encoding Cav3.2) have been identified in many cases of CAE and also in patients with other forms of idiopathic generalized epilepsy, suggesting that CACNA1H is a susceptibility gene for epileptic disorders (7, 8). It is unknown, however, whether Cav3.2 actually affects the firing property of neurons and how mutations of Cav3.2 protein could lead to different types of epilepsy.

In this comprehensive study published in *Genes and Development*, Wang et al. addressed these questions by using an elegant combination of complementary approaches, ranging from genetic manipulation of neurons in vivo or in culture, T-channel specific pharmacology, electrophysiology, calcium imaging, and pathway-specific optogenetic stimulation, as well as ultrastructural studies utilizing immunogold-electron microscopy. In contrast to previous findings that Cav3 channels modulate neuronal excitability through boosting intrinsic excitability by stimulating burst firing (4), the authors demonstrated that overexpression of Cav3.2 — with either gain-of-function mutation linked to CAE-linked hCav3.2 (C456S) or the wild-type form, surprisingly had no effect on GABA-ergic responses, paired-pulse facilitation, membrane potential, bursting activity or spiking frequency. These results differ from those in a recent study in cultured neurons showing

that Cav3.2 mutations directly promote enhanced neuronal excitability (9), and the author propose that these contradictory findings can be reconciled by the fact that T-type calcium channel expression may not be as tightly controlled in culture as in vivo.

The major novel finding of this study is that overexpression of Cav3.2 C456S increased the open probability of functional CaV3.2 channels, but surprisingly not the amount of calcium influx per opening. Furthermore, genetic overexpression did not seem to alter the number of channels expressed, suggesting that channel density is very tightly regulated, such that the overexpressed channels simply replaced, one-for-one, existing channels. This alteration in the channel open probability in turn enhanced net calcium influx at synapses, which further resulted in increased synaptic incorporation of NMDAR. The increase in NMDAR expression was dependent on synaptic activity, as it could be blocked by sodium channel (with the sodium channel toxin TTX) or NMDAR (with elevated $[MgCl_2]_o$) blockade. Thus, activation of synaptic Cav3.2 channels ultimately led to the potentiation of NMDAR and, subsequently, AMPAR activity. Overexpression of the dominant negative form of this channel resulted in the opposite effect (a reduction in NMDAR function), while overexpression of the wild-type form produced no detectable changes. The effects on synaptic NMDARs were observed in many cell types implicated in seizures, including hippocampal CA1 pyramidal neurons, neocortical L5 pyramidal neurons, and thalamic reticular neurons. No differences among cell types were detected, suggesting that Cav3.2-dependent regulation of synaptic NMDARs is a common neuronal mechanism. Taken together, the authors convincingly showed that endogenous Cav3.2 activity contributes to regulation of glutamatergic synaptic transmission by regulating NMDAR numbers at synapses. Thus, Cav3.2, despite being classified as T-type "burst related" calcium channel, actually plays a role similar to that of L-type calcium channel regarding its relationship with regulation of NMDAR (10). These findings by Wang et al. add to our fairly limited understanding of how NMDAR number at synapses is regulated. Additionally, it suggests that Cav3.2 may be a new promising target to treat brain disorders related to NMDAR dysfunction. Finally, the availability of several human disease related Cav3.2 mutations may lead to mechanistic insights regarding molecular pathway regulating synaptic NMDAR expression.

Although mutations in Cav3.2 have been associated with epilepsy, there is no direct evidence documenting whether these genetic changes could actually induce epileptogenesis. To address this question, chronic EEG recordings were performed. Experiments were performed in control rats and those overexpressing versions of hCav3.2 including C456S or wild-type forms. The majority of animals (~60%) overexpressing the mutant form of Cav3.2 displayed brief rhythmic (2–4 Hz) discharges resembling those occurring during generalized spike-and-wave discharges. In addition, progressive EEG changes consistent with other seizure types were occasionally observed. Only ten percent of animals overexpressing wild-type Cav3.2 showed these effects, while seizures were absent in control animals [those expressing blank (GFP-only constructs)]. This study provides the first direct in vivo

evidence to provide a basis of how gain-of-function mutation in Cav3.2 might be responsible for epilepsy. There are many other epilepsy-related mutations of the Cav3.2 channel, and the relevant underlying epileptogenic mechanisms will require further studies. This new animal model, created by overexpression of a specific disease-relevant human mutation, may be useful in studying the general process of epileptogenesis.

The authors propose an interesting and novel epileptogenic mechanism: alterations of Cav3.2 activity might lead to long-lasting changes in NMDAR and AMPAR expression and function, with these being the actual direct factors leading to epilepsy. Accordingly, once chronic alteration in synapses and circuits has taken place, subsequent blockade of Cav3.2 channels may not be able to reverse the process. Indeed, following overexpression of the epilepsy related hCav3.2 mutation (C456S) in rat cortex, acute administration of a selective antagonist for Cav3 (TTA-P2) failed to block the epileptiform 2 to 4 Hz activities. Consistent with the hypothesis of a persistent epileptogenic change in iGluR expression, in the same animals, blockade of the putative downstream iGluR effectors, AMPAR or NMDAR (with pharmacological GYKI 52466 or MK 801 treatments, respectively) significantly reduced the occurrence of epileptiform responses. To test whether chronic treatment might in part reverse the hyperexcitability, the authors used a four repeated dose treatment with TTA-P2 and found that it was antiepileptic – it reduced expression of epileptiform responses. Whether the effects of T-channel blockers (such as ethosuximide) — on absence epilepsy, and perhaps especially the proposed anti-epileptogenic effects (11) — might result in part from chronic rescaling of NMDAR function is an intriguing hypothesis that remains to be tested. Another related question is how T-channel blockade that would counteract the NMDAR dysfunction might do so in a way that would lead to a specific antiepileptic effect without significant cognitive impairments that might be expected from global alterations of these receptors. Finally, one major unresolved issue is how other Cav3.2 mutations might differently affect NMDA function to result in either CAE or other genetic generalized epilepsies. The newly found role of this co-conspirator is likely to be highly relevant to the process of epileptogenesis, as it provides a means to induce long term changes in synaptic weights relevant to maladaptive circuit reorganization.

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