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T-CHANNELS: SHORT-TERM UP-REGULATION CAUSES LONG-TERM CONSEQUENCES IN EPILEPSY

Transcriptional Upregulation of Ca_v3.2 Mediates Epileptogenesis in the Pilocarpine Model of Epilepsy. Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC, Campbell KP, Schoch S, Yaari Y, Beck H. *J Neurosci* 2008 Dec 3;28(49):13341–13353. In both humans and animals, an insult to the brain can lead, after a variable latent period, to the appearance of spontaneous epileptic seizures that persist for life. The underlying processes, collectively referred to as epileptogenesis, include multiple structural and functional neuronal alterations. We have identified the T-type Ca²⁺ channel Ca_v3.2 as a central player in epileptogenesis. We show that a transient and selective upregulation of Ca_v3.2 subunits on the mRNA and protein levels after status epilepticus causes an increase in cellular T-type Ca²⁺ currents and a transitional increase in intrinsic burst firing. These functional changes are absent in mice lacking Ca_v3.2 subunits. Intriguingly, the development of neuropathological hallmarks of chronic epilepsy, such as subfield-specific neuron loss in the hippocampal formation and mossy fiber sprouting, was virtually completely absent in Ca_v3.2^{-/-} mice. In addition, the appearance of spontaneous seizures was dramatically reduced in these mice. Together, these data establish transcriptional induction of Ca_v3.2 as a critical step in epileptogenesis and neuronal vulnerability.

COMMENTARY

It is clear that ion channel dysfunction, or channelopathy, causes epilepsy in a number of relatively uncommon human genetic epilepsy syndromes. Channelopathy is suspected to be causative in the acquired epilepsies, which are considered

to be far more common, but this assertion is far from proven. A number of studies in animal models of acquired epilepsy have found associated alterations in ion channels (1). However, is association causation? Minimal criteria to answer this question might include evidence that: 1) ion channel dysfunction occurs following a neural insult but preceding the onset of spontaneous seizures, 2) the alteration produces neuronal hyperexcitability, and 3) reversal of the alteration inhibits epileptogenesis or the development of the epileptic state. The recent paper by Becker

et al. takes on these issues by exploring the dysregulation of T-type Ca^{2+} ($\text{Ca}_v3.2$) channel expression after status epilepticus. This impressive study provides compelling evidence for a proepileptic effect of $\text{Ca}_v3.2$ channels and challenges common assumptions of how acquired ion channelopathy may produce epilepsy.

T-type Ca^{2+} channels have long been implicated in epilepsy. Their biophysical characteristics of low activation voltage, rapid kinetics, and voltage-dependent inactivation yield transient depolarizing currents that predispose neurons to burst firing. Their enriched distribution in the dendrites of pyramidal neurons places them in the company of other intrinsic conductances, such as A-type K^+ channels and hyperpolarization-activated cation channels, which also are dynamically regulated in epilepsy (2,3). Previous work has shown up-regulation of T-channels in CA1 pyramidal neurons occurs in chronic epilepsy (4), T-channels mediate thalamocortical bursting phenomena underlying generalized seizures (5), and inhibition of these channels by ethosuximide likely mediates the drug's antiepileptic effect in absence epilepsy (6).

The present study by Becker and colleagues analyzes in great detail the regulation of T-channels in CA1 pyramidal neurons following an episode of pilocarpine-induced status epilepticus (the sheer scope of the methodology of this study, filling two and a half published pages of Methods, alone is impressive). The authors begin by demonstrating that a single subtype of T-channel, $\text{Ca}_v3.2$, underwent transient transcriptional up-regulation, beginning within 2 days after induced status epilepticus and terminating by day 10. By day 5 post-status epilepticus, protein expression of $\text{Ca}_v3.2$ increased five-fold; this effect too returned to normal levels a month later. Bursting behavior of CA1 neurons followed $\text{Ca}_v3.2$ levels, with a transient increase in the proportion of cells able to produce action potential bursts that returned to normal levels 20 days after status epilepticus. Importantly, mice with genetic deletions of the $\text{Ca}_v3.2$ subunit did not show the up-regulation of bursting behavior, proving its dependence on this subtype of T-channels. Interestingly, $\text{Ca}_v3.2^{-/-}$ mice still evidence T-type currents, suggesting that other Ca_v subunits may contribute to T-currents as well.

These data demonstrated a transient up-regulation of Ca^{2+} channel expression following status epilepticus. What role does $\text{Ca}_v3.2$ channel up-regulation play in epileptogenesis? The answer is surprising. $\text{Ca}_v3.2$ knockout mice develop epilepsy at a similar rate as wild-type mice, but seizure severity is markedly reduced. Other sequelae of status epilepticus, such as neuronal loss and mossy fiber sprouting, were also markedly attenuated by $\text{Ca}_v3.2$ deletion. The authors interpreted the findings as showing that the transient up-regulation of $\text{Ca}_v3.2$ channels after status epilepticus sets into motion unknown events that result, at later time points, in neurodegenerative changes and

an increase in severity of the epilepsy phenotype. The objection could be raised that the knockout animals may have experienced a reduced intensity of provoked status epilepticus, which would in turn, have led to a milder epileptic condition. However, the authors quantified the status epilepticus duration and EEG spectrum intensity to show this was not the case.

In several ways, these findings challenge current conceptions of ion channelopathy in acquired epilepsy. The notion that ion-channel expression may transiently change, yet produce long-lasting epilepsy results, is contrary to the assumption that ion channelopathy occurs early and is persistent. Also, the specificity of these findings to a single ion channel subtype, while other subtypes mediating similar currents remain unchanged, is puzzling. So, does $\text{Ca}_v3.2$ mediate epileptogenesis? In the strict sense of the term, the answer is unclear. $\text{Ca}_v3.2$ knockouts ultimately develop epilepsy, just as wild types do, and with a similar time course. Thus, it does not appear that $\text{Ca}_v3.2$ deletion inhibits or delays the development of epilepsy. Because $\text{Ca}_v3.2$ channels are constitutively deleted in the knockouts, we cannot exclude the confounding possibility that these channels are exerting an *antiepileptic*—not antiepileptogenic—effect on seizure frequency and other neurodegenerative markers of epilepsy. Of note, the T-channel blocker ethosuximide does not produce significant antiepileptic actions in human acquired epilepsy syndromes, which may argue against this theory.

The authors acknowledge that development of a conditional knockout for $\text{Ca}_v3.2$ channels could address these issues by allowing transient suppression of $\text{Ca}_v3.2$ expression to occur only during the key post-status epilepticus period, while maintaining normal levels during epilepsy induction and chronic epilepsy. Such a difficult experiment would add important additional proof that $\text{Ca}_v3.2$ channels mediate epileptogenic neuronal plasticity, without producing antiepileptic actions as well.

Despite these limitations, the present study is one of the most complete descriptions of an acquired channelopathy in an animal model of epilepsy, tracing the development of $\text{Ca}_v3.2$ up-regulation from gene to protein to neuron to behavior. The thoroughness of the work by Becker et al. sets a new standard for studies of channelopathy and acquired epilepsy. Given the variety of ion channel alterations that already have been described, it is unrealistic to think that acquired epileptogenesis will depend on a single channel subtype, as it does in some human genetic syndromes. However, this study demonstrates that alteration of one ion channel species can have dramatic effects on the expression of chronic epilepsy. Further work to understand what early events link neural insults with subsequent derangement of channel expression and function may uncover common mechanisms of epileptogenesis that could be

used to therapeutically target true preventative measures against epilepsy.

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