



R U OK?

The Novel Therapeutic Potential of R Channels in Epilepsy

Ca_v2.3 Channels Are Critical for Oscillatory Burst Discharges in the Reticular Thalamus and Absence Epilepsy.

Zaman T, Lee K, Park C, Paydar A, Choi JH, Cheong E, Lee CJ, Shin HS. *Neuron* 2011;70(1):95–108.

Neurons of the reticular thalamus (RT) display oscillatory burst discharges that are believed to be critical for thalamocortical network oscillations related to absence epilepsy. Ca²⁺-dependent mechanisms underlie such oscillatory discharges. However, involvement of high-voltage activated (HVA) Ca²⁺ channels in this process has been discounted. We examined this issue closely using mice deficient for the HVA Ca_v2.3 channels. In brain slices of Ca_v2.3^{-/-}, a hyperpolarizing current injection initiated a low-threshold burst of spikes in RT neurons; however, subsequent oscillatory burst discharges were severely suppressed, with a significantly reduced slow afterhyperpolarization (AHP). Consequently, the lack of Ca_v2.3 resulted in a marked decrease in the sensitivity of the animal to γ -butyrolactone-induced absence epilepsy. Local blockade of Ca_v2.3 channels in the RT mimicked the results of Ca_v2.3^{-/-} mice. These results provide strong evidence that Ca_v2.3 channels are critical for oscillatory burst discharges in RT neurons and for the expression of absence epilepsy.

Commentary

Absence epilepsy—the most common form of childhood epileptic disorders—is characterized by a brief and frequent loss of consciousness associated with generalized spike-and-wave discharges (1). These discharges represent synchronized oscillations in the corticothalamic system in which the thalamus is proposed to be an essential rhythm generator (2), although the role of the thalamus versus the cortex in absence seizure initiation remains controversial (3–5). The corticothalamic system is composed of reciprocally connected excitatory thalamocortical and corticothalamic pathways along with a key intervening structure, the reticular thalamus (RT). Both sets of long-range excitatory projections—the cortical and thalamocortical glutamatergic axons—emit axon collateral branches en route to their final targets to activate the GABAergic neurons within the RT. This leads to burst firing of RT cells and powerful inhibitory output to thalamocortical relay neurons, which paradoxically leads to reentrant circuit activation through postinhibitory burst firing. Oscillations are thought to arise and be sustained in the thalamocortical network through cyclical excitation, which is phased by the inhibitory output of RT. Thus a key element in generation of neural oscillations in thalamocortical circuits is the oscillatory burst activity in RT neurons, which is critical for the expression of spike-and-wave discharges (6). Therefore, the mechanisms underlying the bursting activity in this nucleus are of paramount importance. In particular, T-type calcium currents are thought to underlie

the low-threshold calcium burst firing mode. Indeed, T currents and the bursts are reduced by ethosuximide (7, 8), which is a first choice anti-absence therapy (9).

In 1993, Soong et al. reported the cloning of a T-type voltage-gated calcium channel: “rBE-II channel” (10). This channel ultimately turned out to be the R-type channel (Ca_v2.3) (11), and distinct from the “classic” T-type channel mediated by Ca_v3 channel family (12). In a recent study, Zaman et al. provided the first compelling evidence that the R current does act as a T-type calcium current in that it strongly contributes to the calcium-dependent burst firing mode in the RT neurons and to absence epilepsy.

R channels are densely expressed in the cortex and RT but not in thalamocortical neurons. Although R channels are structurally related to high-voltage-activated calcium channels, some of their electrophysiological properties are closer to those of T channels (10), yet their activation threshold is ~25 mV more depolarized (11). Given the differences in their biophysical properties, T channels are more suitable for supporting slow pacemaker activity such as postinhibitory rebound, whereas R channels do provide more rapid, transient surges of calcium (11). While the role of T current in bursting of RT cells has been extensively studied, the involvement of R channels in this activity remained obscure.

Zaman et al. examined this issue using Ca_v2.3^{-/-} mice. The investigators demonstrate for the first time that R channels play a major role in both supporting the burst firing mode in RT neurons and in experimental absence epilepsy. Using the state-of-art electrophysiological patch clamp techniques from brain slice preparations, Zaman et al. show that in wild-type RT cells postinhibitory rebound activation of T channels leads to depolarization and recruitment of R channels with a resultant



enhanced burst response. In addition, R channel activation appears to be particularly effective in promoting calcium-dependent slow afterhyperpolarization (AHP) in RT cells, which enhances repetitive burst firing and is critical for intrinsic rhythmic discharge in the RT neurons that would reinforce synaptic network activity. In $Ca_v2.3^{-/-}$ mice, the strength of the rebound burst as well as the postburst AHP were reduced, and the ability of the neuron to discharge oscillatory bursts was abolished. All these alterations in bursting properties of RT cells were faithfully mimicked in the wild-type mouse by SNX-482—a specific blocker of R channels. Thus, the authors convincingly showed that the reduced bursting properties in the $Ca_v2.3^{-/-}$ mouse resulted directly from lack of R channels rather than from compensatory reduction in the T current and confirmed that T currents were not reduced.

Given that bursting firing properties in RT neurons are critical in the expression of absence seizures (6), and that R channels enhance the burst strength and the propensity of the cell to fire rhythmic bursts (see above), the authors asked whether blocking R channels in RT could be antiepileptic. In order to answer this question, EEG recordings were performed in wild-type and $Ca_v2.3^{-/-}$ mice to compare the susceptibility of these mice to gamma-butyrolactone (GBL)-induced spike-and-wave discharges—a well-established pharmacologic model of absence epilepsy. Although systemic administration of GBL induced typical spike-and-wave discharges in all genotypes, their duration was significantly reduced in $Ca_v2.3^{-/-}$ mice compared with wild-type $Ca_v2.3^{+/+}$ mice and was intermediate in $Ca_v2.3^{+/-}$ mice, suggesting a gene-dosage effect. Also, the lack of $Ca_v2.3$ was associated with a tendency toward a delay in the onset of GBL-induced seizures.

Based on these results alone, it was not possible to exclude the possibility that the reduced severity of seizures in $Ca_v2.3^{-/-}$ mice resulted at least in part from lack of R channels in the cerebral cortex—a major factor in the expression of absence seizures. To determine whether the reduced seizure susceptibility of $Ca_v2.3^{-/-}$ mice resulted from lack of R channels in RT, the authors performed a technically challenging experiment consisting of local injections of the R-type selective channel blocker SNX-482 bilaterally into the RT of wild-type mice while examining susceptibility to GBL-induced seizures. Strikingly, SNX-482 injections in the RT of wild-type mice phenocopied the results from the $Ca_v2.3^{-/-}$ mice (i.e., reduced the duration of GBL-induced spike-and-wave discharges and induced a tendency to a delayed onset). These results are interesting in the context of the recent controversy regarding the role of the cortex versus the thalamus in the expression of absence seizures, as they reinforce the concept that the thalamus plays an active rhythmogenic role. Several studies in genetic rat models of absence epilepsy have reported that the cortex has a leading role in seizure initiation (13, 14). While abnormalities in either the cortex or thalamus could initiate absence seizures (5), the present study demonstrates that specifically targeting the RT excitability could be a therapeutic approach for treating the seizures.

Absence epilepsy can have multifactorial genetic origins (1). Whether or not mutations in genes encoding for R channels are associated with the human epilepsy is unknown. Given the seizure-suppressing effects of R channel blockade by Zaman et al., one could speculate that upregulation of this

channel could be pro-epileptic and could be associated with human absence epilepsy.

Finally, this study leads to the idea that targeting R channels could be a novel therapeutic approach, potentially useful in cases where absence epilepsy is refractory to conventional antiepileptic medications. Indeed, combining R-channel blockers with T-channel blockers would have a synergistic effect on thalamic burst firing and might increase the efficacy over current antiepileptic monotherapies.

by Jeanne T. Paz and John R. Huguenard

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