

NEW EVIDENCE SUPPORTING A ROLE FOR T-TYPE Ca^{2+} CHANNELS IN ABSENCE EPILEPSY AND IN THE ACTION OF ETHOSUXIMIDE

Lack of the Burst Firing of Thalamocortical Relay Neurons and Resistance to Absence Seizures in Mice Lacking $\alpha 1G$ T-Type Ca^{2+} Channels

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T-type Ca^{2+} currents have been proposed to be involved in the genesis of spike-and-wave discharges, a sign of absence seizures, but direct evidence *in vivo* to support this hypothesis has been lacking. To address this question, we generated a null mutation of the $\alpha 1G$ subunit of T-type Ca^{2+} channels. The thalamocortical relay neurons of the $\alpha 1G$ -deficient mice lacked the burst mode firing of action potentials, whereas they showed the normal pattern of tonic mode firing. The $\alpha 1G$ -deficient thalamus was specifically resistant to the generation of spike-and-wave discharges in response to $GABA_B$ receptor activation. Thus, the modulation of the intrinsic firing pattern mediated by $\alpha 1G$ T-type Ca^{2+} channels plays a critical role in the genesis of absence seizures in the thalamocortical pathway.

Block of Cloned Human T-Type Calcium Channels by Succinimide Antiepileptic Drugs

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Inhibition of T-type Ca^{2+} channels has been proposed to play a role in the therapeutic action of succinimide antiepileptic drugs. Despite the widespread acceptance of this hypothesis, recent studies using rat and cat neurons have failed to confirm inhibition of T-type currents at therapeutically relevant concentrations. The present study re-examines this issue using the three cloned human channels that constitute the T-type family: $\alpha 1G$, $\alpha 1H$,

and $\alpha 1I$. The cloned cDNAs were stably transfected and expressed into mammalian cells, leading to the appearance of typical T-type currents. The results demonstrate that both ethosuximide and the active metabolite of methsuximide, α -methyl- α -phenylsuccinimide (MPS), block human T-type channels in a state-dependent manner, with higher affinity for inactivated channels. In contrast, succinimide analogs that are not anticonvulsive were relatively poor blockers. The apparent affinity of MPS for inactivated states of the three channels was estimated using two independent measures: K_I for $\alpha 1G$ and $\alpha 1I$ was 0.3 to 0.5 mM and for $\alpha 1H$ was 0.6 to 1.2 mM. T-type channels display current at the end of long pulses (persistent current), and this current was especially sensitive to block (ethosuximide $IC_{50} = 0.6$ mM). These drugs also reduced both the size of the T-type window current region and the currents elicited by a mock low threshold spike. We conclude that succinimide antiepileptic drugs are capable of blocking human T-type channels at therapeutically relevant concentrations.

COMMENTARY

Generalized absence (“petit mal”) seizures are characterized by short lapses of consciousness that start and stop abruptly. The seizures have minimal motor and autonomic manifestations, there is no postictal depression, and the EEG exhibits a stereotyped bilaterally synchronous 3 Hz spike-and-wave rhythm that is coincident with the behavioral absences (1). In the 1960s, Pierre Gloor at the Montreal Neurological Institute formulated the “corticoreticular hypothesis” of generalized spike-and-wave seizures, which proposed that the seizures are generated by an essential interplay between thalamus and cortex (2). This set the stage for the current view that the generalized spike-and-wave discharge results from hypersynchronization in the loop formed by the massive projections from thalamus to cortex and back to thalamus. Two decades later, Henrik Jahnsen and Rodolfo Llinás noticed that strong hyperpolarization of thalamocortical relay neurons in slices of guinea pig brain allows a slow spike-like response to be gener-

ated that typically evokes a burst of fast Na^+ -dependent action potentials (3). This burst firing is believed to be critical for spindle oscillations in slow-wave sleep and also for the rhythmic discharges in generalized absence seizures. The slow spikes are generated by a unique type of voltage-dependent Ca^{2+} channel that is distinct from other Ca^{2+} channels in that it requires hyperpolarization to become primed for activation and is therefore said to have a “low threshold” (4,5). The channels were referred to as T-type because of their “transient” nature (they inactivate within 10s of ms) and also because of their “tiny” single channel conductance. The molecular characterization of T-type Ca^{2+} channels was finally accomplished in 1998 when Edward Perez-Reyes and his group at Loyola University (now the University of Virginia) identified a family of genes (CavT: $\alpha 1\text{G}$, $\alpha 1\text{H}$, and $\alpha 1\text{I}$) that encode ion channels with characteristics that are similar to T-type Ca^{2+} channels in neurons. Thalamic neurons seem to express mainly the $\alpha 1\text{G}$ form. Now, Kim et al. have generated mice in which the $\alpha 1\text{G}$ gene has been disrupted by gene targeting so that the $\alpha 1\text{G}$ protein is not expressed. Thalamic relay neurons from these knockout mice lack T-type Ca^{2+} currents, but the high-threshold Ca^{2+} currents necessary for neurotransmitter release and other critical functions are unaffected. The knockout mice are outwardly normal appearing. However, they fail to exhibit spike-and-wave discharges in response to some chemical agents known to induce absence-like seizures, thus elegantly confirming the essential role of T-type Ca^{2+} channels. Nevertheless, because $\alpha 1\text{G}$ channels are expressed in brain regions other than the thalamus—most notably the neocortex—this new research still does not address the decades-old issue of whether absence seizures require involvement of both thalamus and cortex or whether cortex alone is sufficient.

If T-type Ca^{2+} channels are crucial to absences, then inhibiting these channels should protect against the seizures. This is exactly what Douglas Coulter, John Huguenard, and David Prince showed occurs with the antiabsence agents ethosuximide, dimethadione, and α -methyl- α -phenylsuccinimide (see Current Controversies, in this issue). In recent years, the idea that succinimides act through their effects on T-type Ca^{2+} channel has had numerous challenges from investigators who failed to confirm that ethosuximide affects T-type currents at therapeutically relevant concentrations. However, the hypoth-

esis has now received renewed support from the work of Gómora et al., who show that ethosuximide and α -methyl- α -phenylsuccinimide do block recombinant human $\alpha 1\text{G}$ T-type Ca^{2+} channels. As discussed by Huguenard and also by Crunelli and Leresche (see Huguenard, in this issue), the explanation for the discrepancies in the literature is not immediately apparent. However, part of the problem could be that succinimides bind in an apparent voltage-dependent fashion so that a substantial block is only present at relatively depolarized potentials. This, in part, occurs because the drugs bind preferentially to the inactivated state of the channel. As is the case for phenytoin block of voltage-activated Na^+ channels, such preferential binding may allow the succinimides to inhibit selectively the pathological firing without affecting normal neuronal activity. In addition, a persistent portion of the T current (“window” current)—which could play a role in biasing thalamic neurons towards burst firing mode (6)—may be more sensitive to block than the phasic current examined in previous studies. In the end, ethosuximide, like most other antiepileptic drugs, probably acts through multiple mechanisms. Although effects on T-type Ca^{2+} channels are likely to be crucial, other actions discussed by Crunelli and Leresche may also play a role.

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