

Block of T-Type Ca^{2+} Channels Is an Important Action of Succinimide Antiabsence Drugs

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The role of calcium channel blockade in the antiepileptic action of ethosuximide is controversial, especially regarding the potency and efficacy of block. However, recent evidence obtained from transgenic animals and heterologous expression systems supports a major role of T-type calcium channels in both the generation of absence seizures and the action of ethosuximide in human absence epilepsy.

A series of articles by Douglas Coulter and colleagues in the late 1980s demonstrated a new cellular mechanism for ethosuximide (ES), an antiepileptic medication with specific utility in the treatment of generalized absences (1–3). ES and related compounds were shown to block a specific type of voltage-gated calcium channel, the T channel, in thalamic relay neurons. These findings led to a series of follow-up studies (4–6) that elucidated cellular and synaptic mechanisms within thalamic cortical circuits responsible for driving thalamocortical epilepsies. In recent years, some aspects of the original ES studies have come into question. Specifically, it has been reported that ES has no direct effect on calcium channels in cat and rat thalamic neurons (7). Two reports published in 2001, along with abstracts presented at the recent Society for Neuroscience Meeting in San Diego, have provided a number of interesting new findings relevant to this issue. At issue is whether the lead succinimide, ES, does indeed block neuronal T channels and whether this leads to its antiepileptic action. This is of continued interest because pharmacotherapy for absence seizures re-

mains imperfect. It is critical to know whether targeted blockade of T channels, specifically those in thalamic neurons, would be an effective strategy for improved drug treatment of absence epilepsies.

The original study, which used voltage-clamp recordings of acutely isolated neurons of guinea pig and rat (1), showed that when all other ion channels were blocked, ES produced a variable and incomplete blockade of the T-type but not other voltage-gated calcium channels. T channels are expressed at high levels in thalamic relay cells (8), which results in their robust ability to fire phasic bursts of action potentials. Calcium-dependent burst firing driven by T-type calcium channels is important in promoting thalamic oscillatory activity thought to be necessary for the genesis of spike wave discharge (SWD) of absence seizures (4,5,9). The original findings (1–3) show that ES produced a maximal T-channel blockade of approximately 20% to 30%, that this blockade occurred in a clinically relevant concentration range (between 0.125 and 1.0 mmol/L, 20 to 140 $\mu\text{g}/\text{mL}$), that the effect was duplicated by other antiabsence medications, including the active metabolites of methosuximide (methoxyphenylsuccinimide) and tridione (dimethadione), and not by other structurally related compounds such as the succinimide parent ring structure, which has no antiepileptic action, and tetramethylsuccinimide, which is a convulsant compound.

Recent work has provided further support for potential roles for both upregulation and downregulation of T currents in regulating absence seizures. For example, studies of thalamic and thalamocortical circuitry in vitro (4,10) showed that the ES-induced T-channel blockade strongly suppresses thalamic excitability in ways specifically relevant to the activation of recurrent slow (2 to 6 Hz) thalamocortical responses. In addition, the experimental compound U92032 is a full antagonist of T-type calcium channels in thalamic relay cells and essentially abolishes in vitro epileptiform activity (11). This contrasts with the effects of ES, characterized by partial antagonism of T channels (2), and reduction, not blockade, of epileptiform activity in vitro (4). The cloning of three T-channel genes ($\alpha 1\text{G}$, $\alpha 1\text{H}$, and $\alpha 1\text{I}$) led to the demonstration of a prominent expression of the $\alpha 1\text{G}$ in dorsal thalamus (12), which plays a central role in absence seizure genesis (13). An increased expression of T channels in rodent mutants and inbred strains with absence epilepsy phenotypes provides support for a role of T channels, per se, in the genesis of absence seizures. For example, functional expression of T channels in

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thalamic reticular neurons is increased in GAERS, an inbred rat strain with spontaneous SWD and behavioral absences (14). This finding is supported by an increased expression of $\alpha 1G$ and $\alpha 1H$ transcripts (15) in adult GAERS rats. Recent studies in a variety of absence mouse models also find increased T channel expression in thalamic neurons (discussed later here).

However, since the time of publication of the first reports (1–3) describing the antagonistic effect of ES on calcium currents, recent studies have indicated that the specific antagonism of T channels can be a quite variable or even absent in different cell types. For example, in acutely isolated hippocampus CA3 (16) and neocortical neurons (17), no effect on ES was observed with concentrations up to millimolar levels. Similarly, Leresche et al. (7) found no evidence for ES action on calcium currents in rat and cat thalamic neurons. Their results suggested rather that the actions of ES were mainly on sodium and potassium conductances. However, other studies have confirmed blocking effects of ES on the T-type calcium channels, including studies in mouse $\alpha 1G$ channels expressed in HEK293 cells (18), cultured dorsal ganglion cells (19), GH3 cells (20), thalamic reticular neuron (14), and freshly dissociated dorsal root ganglia neurons (21). These studies have demonstrated widely varying effects in different preparations, with efficacies ranging between 0% and 100% and binding affinities varying between submillimolar to high millimolar ranges. The variability in responsiveness is yet to be explained. Interestingly, a comparison of results from studies of rat $\alpha 1G$ and $\alpha 1H$ channels stably expressed in HEK293 cells with those obtained with rat DRG neurons has led to the suggestion that other factors; for example, channel accessory subunits may contribute to subtle variations in pharmacologic sensitivity of T channels (22).

Two studies published last year have had a significant impact on this field. In one, human T-type calcium channels were cloned, and their sensitivities to antiepileptic drugs were tested (23). It was found that ES had antagonistic effects on all three cloned human T-type calcium channels: $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$. Interestingly, the concentration response curves suggest a biphasic function with two apparent binding sites: (a) a high-affinity (approximately 0.1 mmol/L) site that accounts for approximately 20% of total channel blockade and (b) a low-affinity site (approximately 10 mmol/L) that accounts for the remainder. Interestingly, the low-affinity binding of ES could be shifted to a much higher binding affinity through manipulations in the experimental conditions. Specifically, depolarizing conditioning steps dramatically enhanced the effect of succinimides on the T-type calcium current. Such depolarizations increased the sensitivity to methyl-phenyl-succinimide and ES by a factor of more than 10. The resultant affinity is in the submillimolar range, which is consistent with a

potential clinically relevant action on T channels. This interesting finding suggests that for neurons in normal behavioral states (i.e., with resting membrane potentials varying between -55 and -70 mV), the sensitivity of T channels to succinimide block will be greatly enhanced. Thus, ES would have powerful effects during physiologic or experimental conditions in which the membrane potential evolves slowly over time, such as during gradual voltage ramp (7) or during slow, GABA-dependent network activity (4), as is expected to occur during absence seizures.

The second recently published study of interest is from the Chin group at Pohang University in Korea (24). A mouse deficient in the $\alpha 1G$ gene was generated, and the effects on thalamic burst firing and absence seizures were examined. Transient calcium currents were completely abolished in thalamic relay neurons, as was calcium-dependent burst firing. These results were expected, as $\alpha 1G$ is the major T-type channel expressed in dorsal thalamic relay neurons (12). Other aspects of basic neuronal excitability in thalamus, such as repetitive firing ability, were apparently unaffected in the knockout. Perhaps most interestingly, the $\alpha 1G$ knockouts were insensitive to GABA_B receptor agonist-induced SWD. Baclofen and gamma-hydroxybutyrate are two such agonists with well-known seizure-inducing properties. In control ($\alpha 1G^{+/+}$) mice, a high degree of 3 to 5 Hz SWD, approaching absence status, was observed, compared with very weak and sporadic SWD in $\alpha 1G^{-/-}$ mice. Other forms of experimental seizures were, in contrast, unaffected. For example, systemic treatment with the GABA_A antagonist bicuculline induced wide-ranging epileptic activities in both control and knockout mice, including vibrissal twitching, immobility, and jumping. These were associated with EEG abnormalities that included isolated EEG spikes and runs of epileptiform activity with time-varying frequency components. The potassium channel antagonist 4-aminopyridine was able to produce comparable tonic-clonic seizures in both control and $\alpha 1G^{-/-}$ animals. The overall results of this study suggest a critical role for $\alpha 1G$ T-type calcium channels specifically in the genesis of SWD responsible for absence seizures. In an interesting follow-up, the same group has now reported in abstract form (25) that an $\alpha 1A$ calcium channel knockout mouse, which has previously been characterized as having major motor defects, including dystonia and weakness, also expresses an absence epilepsy phenotype— $\alpha 1A^{-/-}$ mice exhibit spontaneous, ES-sensitive, behavioral absences with 3.5 to 5.0 Hz SWD. Furthermore, there is an increased expression of T channels in thalamic relay neurons. This is surprising because $\alpha 1A$ encodes a high-voltage-activated calcium channel that has no direct relevance to T-channel function. To test whether the increased expression of T channels was necessary for the spontaneous absence seizures in the $\alpha 1A^{-/-}$ mice, they crossed these with the $\alpha 1G^{-/-}$ mice to create double knockouts. As ex-

pected, if $\alpha 1G$ was necessary for expression of SWD and absences, the double knockouts were rescued from the epileptic phenotype. The latter finding must be interpreted with caution because the two mice strains were created on different genetic backgrounds, and therefore, confounds caused by strain differences must be excluded. However, at this point, the results from the $\alpha 1G^{-/-}$ mice further support the idea that T channels are an essential component of the cellular machinery that drives absence seizures. Two other abstracts at the same meeting (26,27) provided additional evidence for altered T-channel function in absence seizures. In one abstract, two $\alpha 1A$ -related channel mutants, lethargic and tottering mice, were reported to exhibit increased expression of T channels in thalamocortical relay cells (27), which is consistent with the results of the $\alpha 1A^{-/-}$ study (25). In the second, intrathalamic infusion of ES was shown to suppress SWD in GAERS rats (26). These findings, together with those of Song et al. (25), provide new support for the idea that either upregulation or downregulation of T channels in thalamic relay cells will have functional consequences for absence seizures.

In summary, T channels are now known to be expressed at very high levels in thalamic cells, both in thalamic relay cells ($\alpha 1G$) and in thalamic reticular cells ($\alpha 1H$ and $\alpha 1I$). These are two cell groups known to participate strongly in absence seizure genesis (13). These channels have been shown in both acutely isolated-neuronal preparations and in slices to be sensitive to blockade by ES and related compounds, with a resultant reduction in burst-firing activity (4,10). Other actions of ES may contribute to its antiepileptic action, but the effects on T channels remain the most likely antiabsence action of this drug. The recent findings of sensitivity of human T channels to ES and especially the enhanced sensitivity observed under physiological conditions are particularly compelling in this regard. Decreases in $\alpha 1G$, $\alpha 1H$, and $\alpha 1I$ function by antiepileptic drugs will reduce excitability in thalamic circuits and reduce the ability to recruit network oscillatory actions in the thalamic and thalamocortical circuits. In contrast, increases in the excitability of these cells through upregulation of T channels (14,15,25,27) has the opposite effect to increase the likelihood of abnormal thalamocortical discharge. Thus, thalamic T channels remain an important target for pharmacotherapy of absence seizures.

References

- Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol* 1989;25:582–593.
- Coulter DA, Huguenard JR, Prince DA. Specific petit mal anticonvulsants reduce calcium currents in thalamic neurons. *Neurosci Lett* 1989;98:74–78.
- Coulter DA, Huguenard JR, Prince DA. Differential effects of petit mal anticonvulsants and convulsants on thalamic neurones: calcium current reduction. *Br J Pharmacol* 1990;100:800–806.
- Huguenard JR, Prince DA. Intrathalamic rhythmicity studied in vitro: nominal T-current modulation causes robust antioscillatory effects. *J Neurosci* 1994;14:5485–5502.
- von Krosigk M, Bal T, McCormick DA. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science* 1993;261:361–364.
- Warren RA, Agmon A, Jones EG. Oscillatory synaptic interactions between ventroposterior and reticular neurons in mouse thalamus in vitro. *J Neurophysiol* 1994;72:1993–2003.
- Leresche N, Parri HR, Erdemli G, Guyon A, Turner JB, Williams SR, Asprodingi E, Crunelli V. On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. *J Neurosci* 1998;18:4842–4853.
- Coulter DA, Huguenard JR, Prince DA. Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *J Physiol (Lond)* 1989;414:587–604.
- Steriade M, McCormick DA, Sejnowski TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 1993;262:679–685.
- Kao CQ, Coulter DA. Physiology and pharmacology of corticothalamic stimulation-evoked responses in rat somatosensory thalamic neurons in vitro. *J Neurophysiol* 1997;77:2661–2676.
- Smith SD, Huguenard JR. The specific T-type Ca^{2+} channel blocker U92032 reduces synchronous phasic discharge in thalamus. *Epilepsia* 1996;37:S5.
- Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA. Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. *J Neurosci* 1999;19:1895–1911.
- Huguenard JR. Neuronal circuitry of thalamocortical epilepsy and mechanisms of anti-absence drug action. In: Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ, eds. *Basic mechanisms of the epilepsies*. New York: Lippincott-Raven, 1999.
- Tsakiridou E, Bertollini L, de Curtis M, Avanzini G, Pape HC. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. *J Neurosci* 1995;15:3110–3117.
- Talley EM, Solorzano G, Depaulis A, Perez-Reyes E, Bayliss DA. Low-voltage-activated calcium channel subunit expression in a genetic model of absence epilepsy in the rat. *Brain Res Mol Brain Res* 2000;75:159–165.
- Avery RB, Johnston D. Multiple channel types contribute to the low-voltage-activated calcium current in hippocampal CA3 pyramidal neurons. *J Neurosci* 1996;16:5567–5582.
- Sayer RJ, Brown AM, Schwandt PC, Crill WE. Calcium currents in acutely isolated human neocortical neurons. *J Neurophysiol* 1993;69:1596–1606.
- Lacinova L, Klugbauer N, Hofmann F. Regulation of the calcium channel $\alpha 1G$ subunit by divalent cations and organic blockers. *Neuropharmacology* 2000;39:1254–1266.
- Gross RA, Covey DF, Ferrendelli JA. Voltage-dependent calcium channels as targets for convulsant and anticonvulsant alkyl-substituted thiobutylolactones. *J Pharmacol Exp Ther* 1997;280:686–694.
- Herrington J, Lingle CJ. Kinetic and pharmacological properties of low voltage-activated Ca^{2+} current in rat clonal (GH3) pituitary cells. *J Neurophysiol* 1992;68:213–232.
- Todorovic SM, Lingle CJ. Pharmacological properties of T-type Ca^{2+} current in adult rat sensory neurons: effects of anticonvulsant and anesthetic agents. *J Neurophysiol* 1998;79:240–252.
- Todorovic SM, Perez-Reyes E, Lingle CJ. Anticonvulsants but not

- general anesthetics have differential blocking effects on different T-type current variants. *Mol Pharmacol* 2000;58:98–108.
23. Gomora JC, Daud AN, Weiergraber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. *Mol Pharmacol* 2001;60:1121–1132.
 24. Kim D, Song I, Keum S, Lee T, Jeong MJ, Kim SS, McEnery MW, Shin HS. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca^{2+} channels. *Neuron* 2001;31:35–45.
 25. Song I, Kim D, Jun K, Shin HS. Role of T-type calcium channels in the genesis of absence seizure in the mutant mice for $\alpha 1a$, the pore-forming subunit of the P/Q-type calcium channel. *Soc Neurosci Abstr* 2001;27:572.14.
 26. Richards DA, Bowery NG, Manning J-PA, Spruce AE, Leresche N, Crunelli V. Weak anti-absence activity of ethosuximide infused directly into different thalamic nuclei of absence epilepsy rats. *Soc Neurosci Abstr* 2001;27:969.8.
 27. Zhang Y, Noebels JL. Altered calcium currents in thalamocortical relay cells of mouse absence models with mutations of $\alpha 1A$ and $\beta 4$ calcium channel subunits. *Soc Neurosci Abstr* 2001;27:151.16..