Characterization of Ethosuximide Reduction of Low-Threshold Calcium Current in Thalamic Neurons

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The mechanism by which ethosuximide reduces thalamic low-threshold calcium current (LTCC) was analyzed using voltage-clamp techniques in acutely isolated ventrobasal complex neurons from rats and guinea pigs. The ethosuximide-induced reduction of LTCC was voltage dependent: it was most pronounced at more-hyperpolarized potentials and did not affect the time course of activation or inactivation of the current. Ethosuximide reduced LTCC without altering the voltage dependence of steady-state inactivation or the time course of recovery from inactivation. Dimethadione reduced LTCC by a similar mechanism, while valproic acid had no effect on LTCC. We conclude that ethosuximide reduction of LTCC in thalamic neurons is consistent with a reduction in the number of available LTCC channels or in the single LTCC channel conductance, perhaps indicating a direct channel-blocking action of this drug. Given the importance of LTCC in thalamic oscillatory behavior, a reduction in this current by ethosuximide would be a mechanism of action compatible with the known anticonvulsant effects of this drug in typical absence seizures.

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Ethosuximide is an anticonvulsant frequently used in the treatment of typical generalized absence (petit mal) epilepsy [1, 2]. In fact, its anticonvulsant action is specific for this type of epilepsy; it is ineffective in the treatment of other types of generalized seizures and against all types of partial seizures [1, 2]. However, few cellular actions of ethosuximide have been described that are consistent with its specific anticonvulsant effects (reviewed in [3]). Characterization of the cellular mechanism of action of ethosuximide could have direct bearing on our understanding of the pathogenesis of petit mal epilepsy, and could also aid in the development of other anticonvulsant drugs.

Evidence from depth recordings in petit mal patients [4], and extracellular recordings in animal models of petit mal [5–7], indicates that the 3-Hz spike-wave rhythms in the electroencephalogram, characteristic of absence seizures, are generated by rhythmic reverberating interactions between mutually interconnected thalamic and cortical areas. In different experimental models of petit mal the thalamus may either lead or follow the cortex in the genesis of these spike-wave rhythms. In rat genetic models of petit mal the discharge in the thalamus may lead that in the cortex [7], whereas in feline generalized penicillin epilepsy the reverse is true [5, 6]. However, what is clear in all of these models, and in recordings from petit mal patients [4], is that the thalamus plays an important role in the generation of 3-Hz spike-wave rhythms characteristic of petit mal epilepsy.

Most studies of the cellular actions of ethosuximide have employed cultured cortical mammalian [8] and spinal neurons [8]; in some cases neurons from invertebrate species have been used [9]. No cellular mechanisms consistent with the anticonvulsant action of ethosuximide have been demonstrated in these types of cells when the drug is applied in concentration ranges appropriate for its clinical action [2, 3]. We chose to examine ethosuximide actions on thalamic neurons because of the important role of the thalamus in generation of petit mal epilepsy. Neurons in virtually all areas of the thalamus are characterized physiologically by the presence of a large-amplitude, low-threshold calcium spike [10–14]. This conductance underlies bursts of action potentials generated in thalamic neurons and plays an important role in thalamic oscillatory behavior (e.g., in the generation of thalamic sleep spindles, as reviewed in [14]).

We have previously shown that the petit mal anticonvulsant ethosuximide reduces the low-threshold calcium current (LTCC) underlying the low-threshold calcium spike in rodent thalamic neurons in vitro [15]. In the present study, we further examined this action of ethosuximide and also tested the effects of another

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specific petit mal anticonvulsant, dimethadione, on calcium currents in thalamic neurons. Our goal was to investigate the mechanism by which these anticonvulsants reduce the LTCC.

Methods

Dissociation

All experiments were performed on thalamic neurons acutely isolated from the ventrobasal complex of rats and guinea pigs (ages 1 day–adult) using the methods described by Kay and Wong [16]. Animals were anaesthetized with pentobarbital and decapitated. A portion of the brain containing the thalamus was removed, trimmed, and glued to the stage of a vibratome (Lancer) for slicing. Thalamic slices (600 μm thick) were then cut and placed in an oxygenated PIPES (piperazine-N, N'-bis[2 ethanesulphonic acid])-buffered solution containing trypsin (0.8 mg/ml, Sigma, St Louis, MO) for 45 minutes to 1 hour. The PIPES solution contained the following (in mM): NaCl, 120; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; D-glucose, 25; PIPES 20 (pH 7.0). Slices were then removed from the enzyme solution, rinsed, cut into chunks, and triturated using fire-polished Pasteur pipettes. Cells were plated onto culture dishes and stored in an oxygenated incubator for 0.5 to 12 hours prior to use.

Recording

Patch recordings of calcium currents were made using the whole-cell voltage-clamp method described by Hamill and colleagues [17]. The intracellular solution used to isolate calcium currents was a variation of one previously described [18] and consisted of the following (in mM): Trizma phosphate (dibasic), 110; Trizma base, 28; ethylene glycol bis-(β aminoethylether)-N, N, N', N'-tetraacetic acid, 11; $MgCl_2$, 2; $CaCl_2$, 0.5; Na–adenosine triphosphate, 4; pH 7.35. The external solution was composed of the following (in mM): NaCl, 155; KCl, 3; $MgCl_2$, 1; $CaCl_2$, 3; HEPES-Na^+, 10; tetrodotoxin, 0.0005; pH 7.4. All recordings were conducted at room temperature (20–22°C). A 8-mV liquid junction potential was measurable between the electrode and bath solutions, so all voltage command levels were compensated accordingly. Patch electrodes were pulled on a List L/M-3P-A puller (Darmstadt, FRG) using a two-stage pull, and had resistances of 6 to 8 MΩ. Currents were monitored either with a List L/M-EPC-7 or with an Axopatch 1A (Axon Instruments, Burlingame, CA) and filtered at 5 KHz with an 8-pole Bessel filter prior to digitization. All data were stored and analyzed using a DEC PDP-11/73 computer with a Cheshire data interface (Indec, Sunnyvale, CA). A backup copy of most data was recorded on videotape using a pulse-code modulated interface (Neurocorder, Neurodata Instruments, New York, NY). Leak and capacitance currents are subtracted from active currents in all figures.

Series Resistance Errors and Escape

Compensation circuitry was used whenever possible to minimize potential series resistance errors. This circuitry could compensate 90% of the series resistance. Typical series resistance (R_s) errors with a 10 MΩ R_s (compensated to 1 mΩ) and a 500 pA current would be 0.5 mV. When drugs were applied using bath perfusion with high flow rates (for example, in obtaining ethosuximide dose-response data), fluctuations in solution level often induced oscillations due to the R_s compensation circuitry. For this reason, R_s compensation was not used in these instances, and the potential R_s error therefore increased to 5 mV. Voltages during command potentials eliciting calcium currents were judged to be well controlled based on smooth voltage-dependent activation and activation and inactivation kinetics, which were independent of current magnitude (for example, see current traces in steady-state inactivation data).

Drug Concentrations and Method of Application

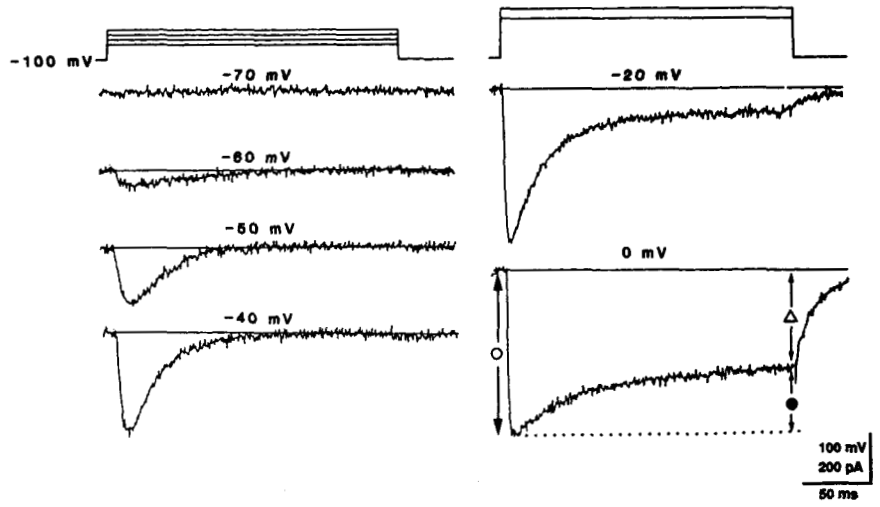
All anticonvulsants were applied in concentration ranges that were clinically relevant (i.e., in concentrations that are achieved as free serum levels in epileptic patients medicated with a particular anticonvulsant). For ethosuximide and dimethadione this concentration range was 280 to 700 μM (40–100 μg/ml) [1, 2] and 5 to 9 mM (700–1,200 μg/ml) [19, 20], respectively. Drugs were applied either by changing the extracellular bathing medium or by perfusion onto the cell produced by applying pressure to the back of drug-containing broken micropipettes (tip diameter 2–4 μm).

Results

Results presented in this paper are summarized from recordings of drug effects in 143 cells: 80 from rat, and 63 from guinea pig, ventrobasal complex neurons. Results from the two species were indistinguishable and so were pooled for subsequent data analysis.

Thalamic Calcium Currents

It was possible to identify at least two, and possibly three, distinct calcium currents in whole-cell voltage-clamp recordings from thalamic neurons on the basis of activation and inactivation kinetics, voltage thresholds, and pharmacological sensitivity [21]. Figure 1 illustrates the properties of the two largest, most easily distinguishable calcium currents, which were the focus of our studies of anticonvulsant pharmacological action. At holding potentials of −100 mV, depolarizing voltage commands to −60 mV or more positive potentials elicited a transient calcium current that inactivated rapidly during the time course of the 200-msec voltage command (see Fig 1A, left traces). This current was similar to the "T" [22], type I [23], or l.v.a. [24] calcium current described by others, and will henceforth be called the low-threshold calcium current. The LTCC became larger with increasing voltage commands and peaked in isolation during voltage commands to −40 mV (see Fig 1A, left traces and Fig 1B, IV plot). During voltage commands to −30 mV or more positive potentials, a sustained noninactivating or slowly inactivating calcium current began to appear that peaked at 0 mV (see Fig 1B, right traces and IV plot). This current was similar to the "L" [22], type II [23], or h.v.a. [24] calcium current described by



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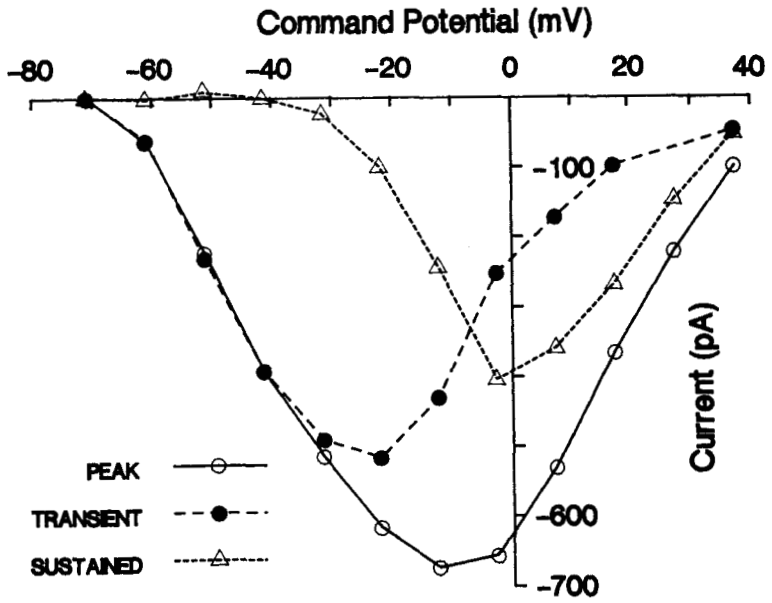

B

Fig 1. Calcium currents in thalamic neurons. (A) Calcium currents elicited by depolarizing voltage commands from a holding potential of −100 mV. The amplitude of the voltage command step eliciting the inward current is shown above each current trace. Note that at the most hyperpolarizing voltage commands (−60 to −40 mV) a fully inactivating, transient calcium current is evoked (left traces). *A sustained calcium current begins to appear during voltage commands to −30 mV or more depolarized levels* (right traces). *(B) Plot of calcium current from the cell illustrated in A. The peak, sustained, and transient components of calcium current (calculated as illustrated in* right bottom trace *of A) are plotted versus the voltage command eliciting the current. Note that the transient component of calcium current is evoked at a lower threshold and is equal in maximal amplitude or greater than the sustained component in the same cell. In this and all subsequent figures the command voltage eliciting the current is above the current trace, and a downward deflection is an inward current.*

others, and will be referred to as the high-threshold calcium current (HTCC). Steps to potentials more depolarizing than 0 mV resulted in a gradual decrease in the size of the HTCC, as the voltage command approached the reversal potential for the calcium current (see Fig 1, IV plot). At holding potentials of −30 mV the LTCC was inactivated, and the HTCC could be evoked in isolation by depolarizing steps (not shown). The effects of anticonvulsants on these two components of whole-cell calcium current could thus be examined by eliciting responses from a holding potential of −100 mV to step commands of −40 mV (to illustrate effects on the LTCC in isolation), and to 0 mV (where the HTCC is maximal). Both of these currents were blocked by perfusion of 500 μM Cd^{2+} (not shown).

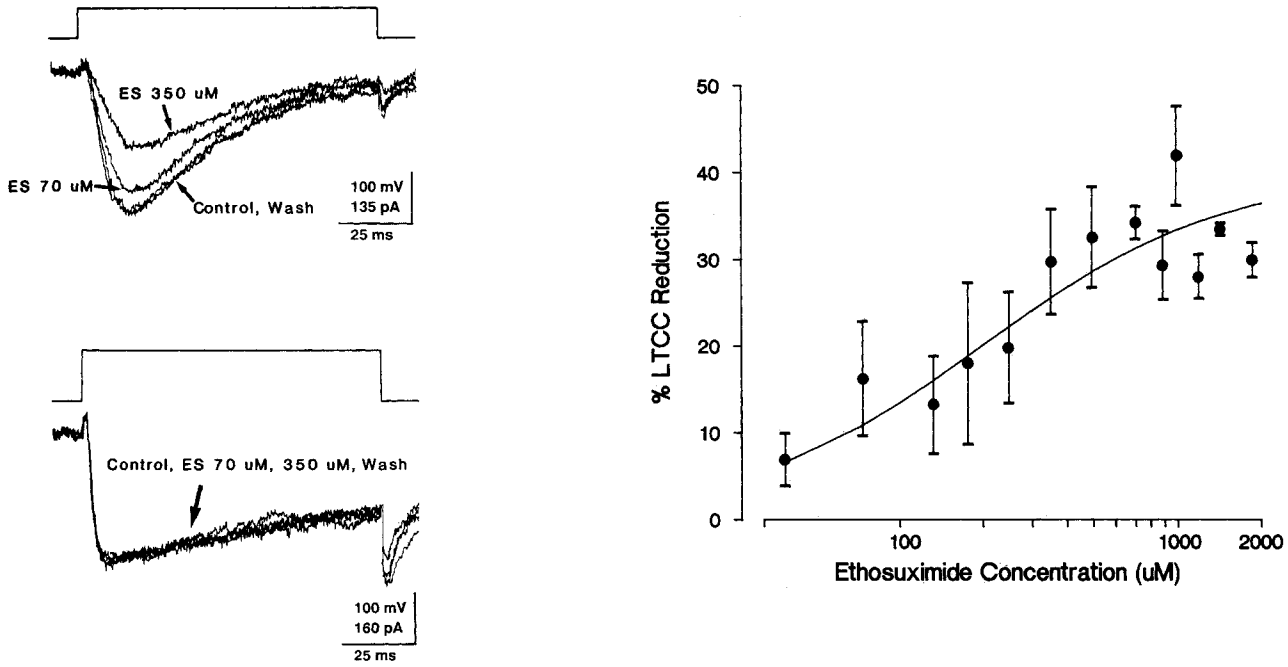

Fig 2. Ethosuximide (ES)-induced reduction of low-threshold transient calcium current (LTCC). (A) Upper sweeps: *Superimposed traces of LTCC evoked by a depolarizing voltage command to −40 mV from a holding potential of −100 mV in control solution, ES 70 μM (reducing LTCC 16%), ES 350 μM (reducing LTCC 46%), and washout of ES (complete recovery).* Lower sweeps: *Superimposed traces of high-threshold calcium current (HTCC) evoked in the same cell by a depolarizing voltage command to 0 mV from a holding potential of −100 mV, following application of ES 70 and 350 μM and washout. No effect of ethosuximide application on HTCC was visible in this neuron. (B) Plot of ES bath concentration (logarithmic scale) verus percent reduction of LTCC (at −40 mV command potential) for 23 ES-responsive thalamic neurons (error bars show mean ± SE, mean of 2–7 cells per point). The curve, which was fitted to the data by eye, is constructed from the standard dose-response equation: effect = maximal effect * ES concentration/(ES concentration + EC_{50}), using an EC_{50} estimate of 200 μM and a maximal effect estimate of 40%. This equation assumes a one-to-one stoichiometry between ES concentration and effect on calcium current.*

Ethosuximide Reductions of Calcium Currents

Applications of ethosuximide in concentrations of 50 to 1,000 μM reversibly reduced the LTCC and in some cases the HTCC (Fig 2A) in 77 of 101 neurons, as we have previously described [15]. A portion of the population (23%) was unresponsive to ethosuximide. No differences in calcium current properties between ethosuximide-unresponsive and ethosuximide-responsive neurons were detected. Ethosuximide reduction of LTCC was maximal at concentrations of 500 μM. For 10 ethosuximide-responsive cells, application of 500 μM ethosuximide resulted in a reduction of LTCC (assessed by voltage commands to −40 mV from a holding potential of −100 mV) of 31.9 ± 4% (mean ± SE). For the same cells, ethosuximide reduced the HTCC (assessed by voltage commands to 0 mV from a holding potential of −100 mV) by 2.8 ± 7.5% (cf., upper and lower traces of Fig 2A). Ethosuximide reduction of LTCC was dose dependent and could be fit by a curve generated by an equation that assumed one-to-one stoichiometry, a maximal reduction of LTCC of 40%, and an EC_{50} of 200 μM (curve in Fig 2B).

Ethosuximide reduced calcium currents over the full range of activation potentials (Fig 3), although the percentage reduction was largest at more hyperpolarized step commands and decreased with more depolarized step commands. This effect is illustrated in Figure 4A, where percentage reduction in calcium currents by ethosuximide is plotted versus step command potential for the ethosuximide application illustrated in Figure 3. This plot clearly shows that the ethosuximide effect on the LTCC is voltage dependent. This voltage dependence may explain some of the specificity of ethosuximide action on the LTCC as compared with the HTCC: the LTCC is evoked by more hyperpolarized step commands than the HTCC, and therefore would be more affected by ethosuximide. In Figure 4B, the amplitude of the ethosuximide-blocked current is plotted versus the voltage eliciting the current for the cell shown in Figures 3 and 4A. Ethosuximide reduction of calcium currents occurred only in potential ranges overlapping the activation range of the LTCC (cf., Figs 1, 3C).

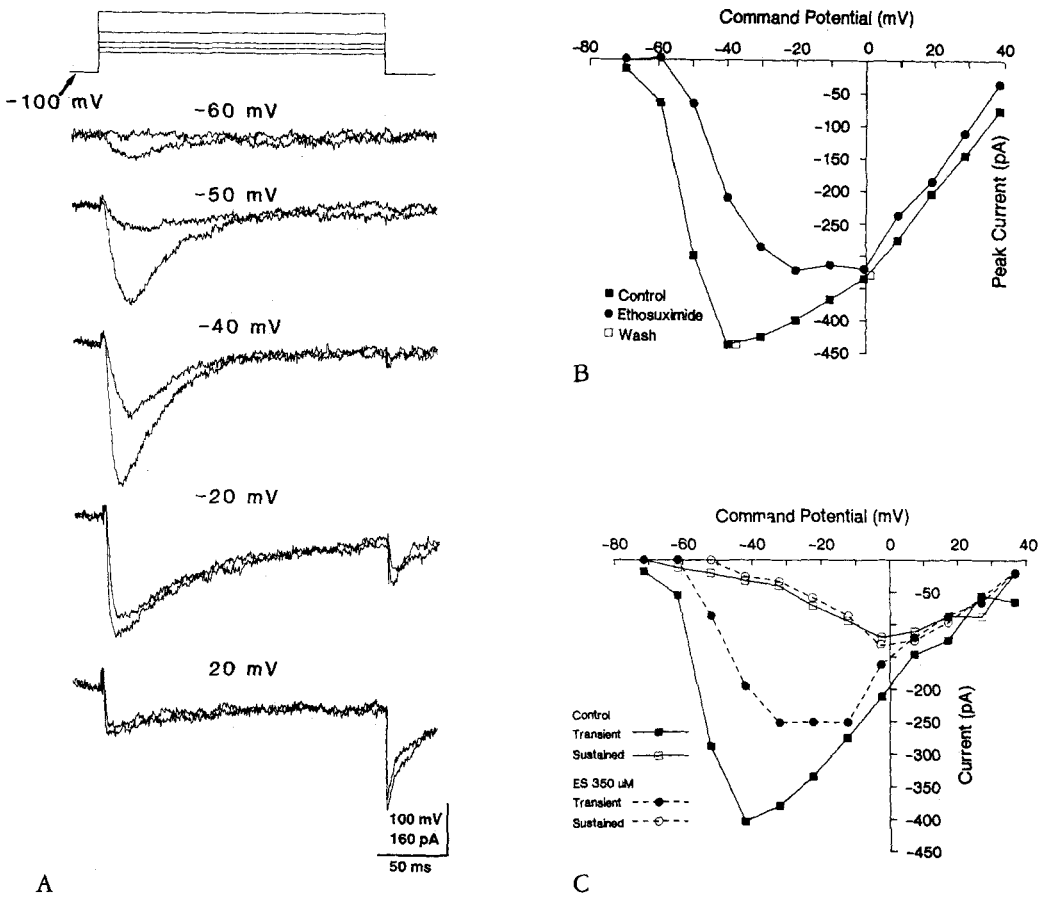

Fig 3. (A) Effect of ethosuximide (ES, 350 μM) on calcium current evoked by varying depolarizing commands from a holding potential of −100 mV. Depolarizing command level eliciting the current accompanies each trace. Two superimposed traces in each line show the control current and the current after ES application. Note that ES reduction of calcium current is maximal at more hyperpolarized command potentials. (B) Plot of voltage command amplitude versus peak calcium current under control, ES-exposed (350 μM), and wash conditions for the same cell as in A. (C) Plot of voltage command amplitude versus transient and sustained calcium current for the same cell as in A, separated as in Figure 1. Note that ES reduces calcium current in a voltage range overlapping the activation range for low-threshold calcium current (LTCC) and that this effect is voltage dependent, fully reversible, and specific for the LTCC (there is no apparent effect on the sustained component of calcium current).

Mechanism of Ethosuximide Block of Calcium Currents

The ethosuximide-induced reductions in LTCC might be produced by a change in the activation or inactivation kinetics or voltage dependence of the current, a decrease of the number of available LTCC channels, or a decrease in the single channel conductance. We performed experiments to test some of these possibilities. Records of control and ethosuximide-reduced LTCC traces were normalized so that the peak amplitudes of both traces were the same. The time course of activation and inactivation of the LTCC could then be directly compared under control and ethosuximide-exposed conditions. If ethosuximide reduced LTCC by slowing activation or speeding inactivation kinetics, a change in the time course of the current would be expected. The time course of the LTCC was identical in normalized current traces from control and drug-treated neurons (Fig 5), suggesting that ethosuximide reduces the LTCC without affecting the kinetics of activation or inactivation.

A second mechanism by which ethosuximide could reduce the LTCC might be to shift the voltage dependence of steady-state inactivation of this conductance to more hyperpolarized levels. This would reduce the number of available LTCC channels that could be activated by a given depolarizing command and consequently reduce the amplitude of the total LTCC. Figure 6 shows a comparison of steady-state inactivation curves under control and ethosuximide (350 μM)-exposed conditions (in a neuron where the drug reduced the LTCC by 46%). When the available fraction of LTCC was plotted against the holding potential, no shift in the voltage dependence of steady-state inactivation was found (see Fig 6B).

Another possibility is that ethosuximide reduces LTCC by prolonging the time course of recovery from

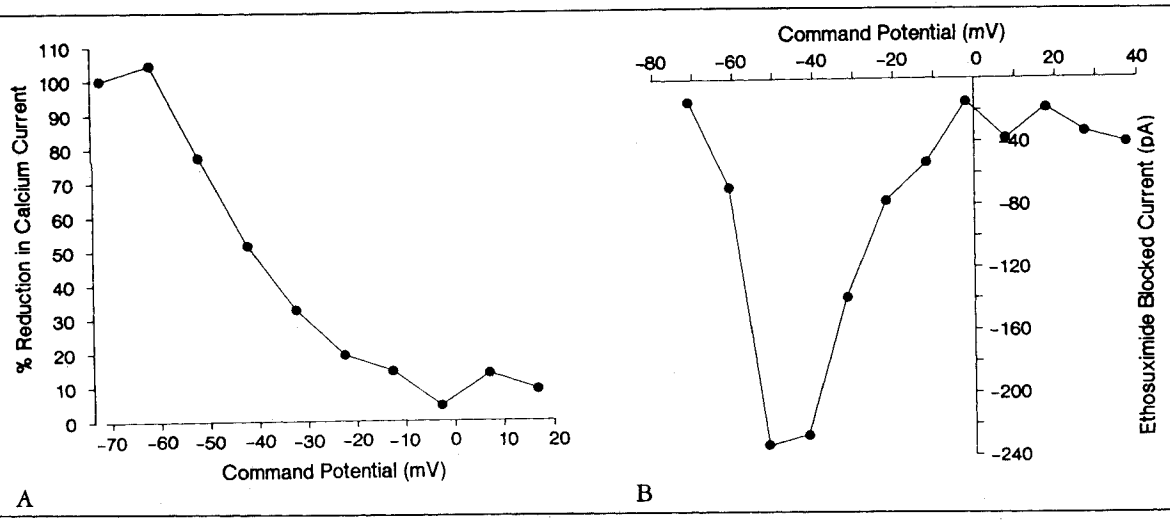

Fig 4. Voltage dependence of ethosuximide low-threshold calcium current (LTCC) reduction. (A) Plot of percent reduction of peak calcium current versus command potential eliciting the current. Note that the percentage reduction is largest at more hyperpolarized command potentials (−60 to −40 mV). (B) Plot of amplitude of ethosuximide-blocked calcium current versus command potential eliciting the current. The ethosuximide-blocked current amplitude is derived by subtracting the amplitude of calcium current under ethosuximide-exposed conditions from that under control conditions. Note that the ethosuximide-reduced current peaks at −50 to −40 mV and has a voltage activation range identical to that of the LTCC (cf., Fig 1B). Both A and B are derived from the IV plot of Figure 3B. Holding potential = −100 mV.

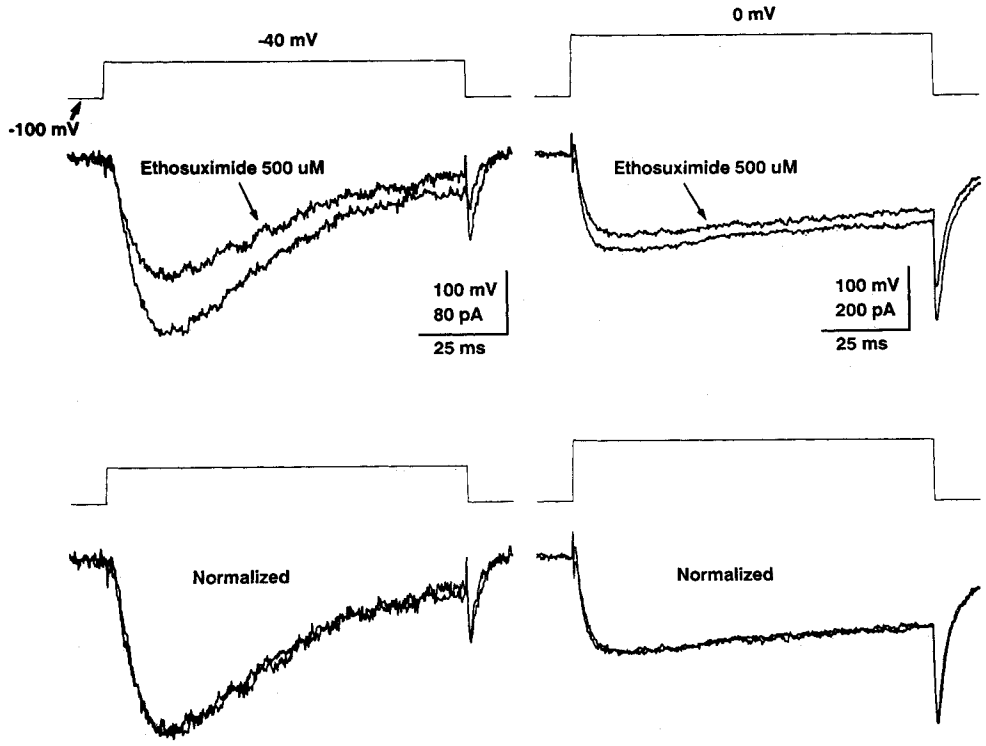

Fig 5. Ethosuximide calcium current reduction is accomplished without altering the time course of activation or inactivation of the current. Top sweeps: *Control and ethosuximide-reduced low-threshold calcium current (LTCC)* (left) *and high-threshold calcium current (HTCC)* (right) *used to obtain normalized traces.* Bottom sweeps: *Superimposed traces of control and ethosuximide-reduced calcium currents normalized to peak amplitude. Note that the ethosuximide-reduced LTCC* (left traces, *32% reduction) and HTCC* (right traces, *15% reduction) have time courses that are identical to control currents (i.e., normalized current traces overlap almost completely). Ethosuximide concentration was 500 μM. Voltage commands and holding potential are the same in bottom and top sweeps of each column.*

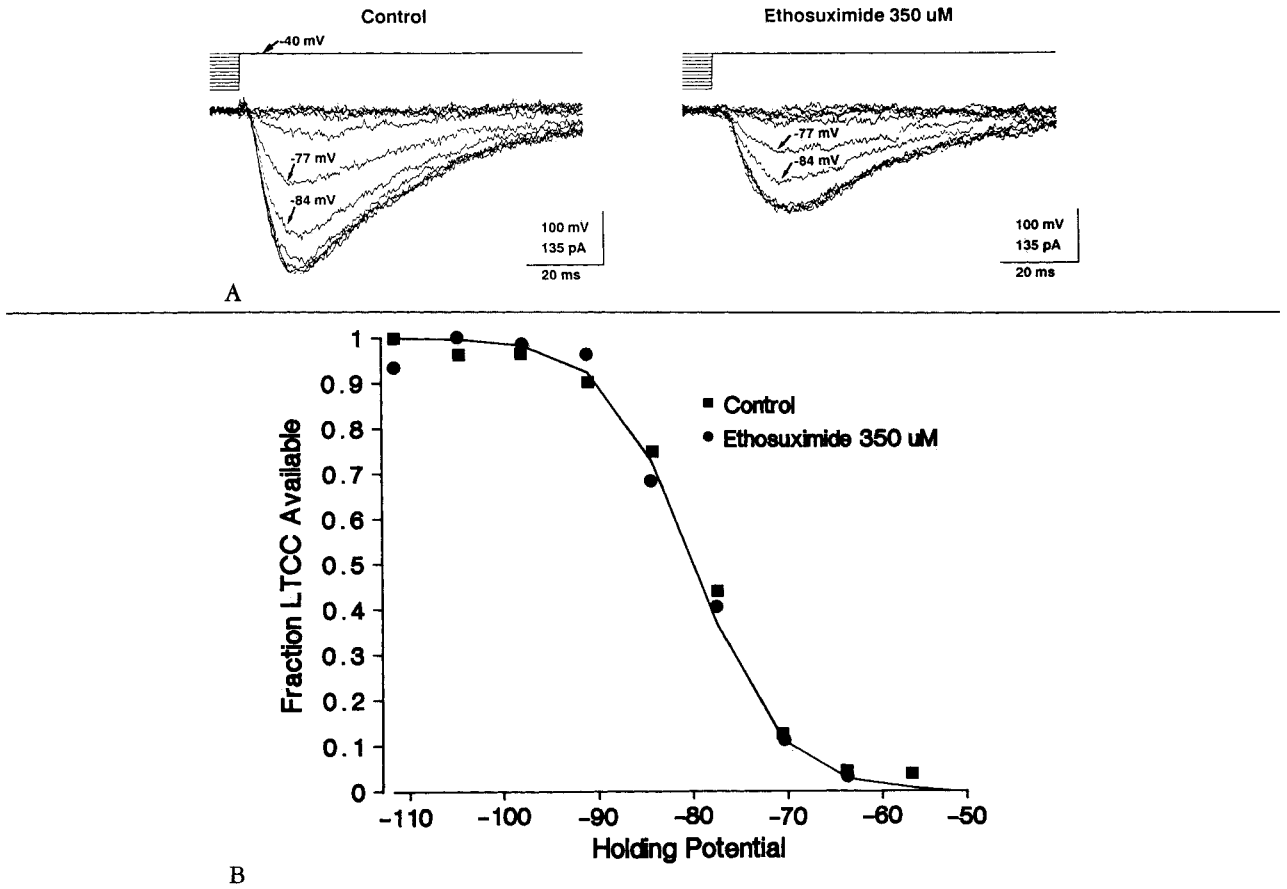

Fig 6. Steady-state inactivation of control and ethosuximide-reduced low-threshold calcium current (LTCC). Top: *Superimposed sweeps of control* (left) *and ethosuximide-reduced* (right) *LTCC evoked by depolarizing commands to −40 mV from varying holding potentials* (upper traces). *Note the dependence of LTCC amplitude (but not time course) on holding potential.* Bottom: *Plot of holding potential versus fraction of LTCC available (relative to LTCC evoked from −112 mV) derived from the superimposed traces above. Note that ethosuximide has no effect on the voltage dependence of steady-state inactivation. Records are from a cell where 350 μM ethosuximide reduced LTCC by 46% in a completely reversible manner.*

inactivation to such an extent that subsequent voltage steps find the neuron still recovering from the previous depolarization and a portion of the LTCC channels unavailable for activation. This explanation for the ethosuximide effects on LTCC was also inadequate, since the time course of recovery from inactivation under control and ethosuximide-exposed conditions was similar (Fig 7) in a cell where ethosuximide (500 μM) reduced the LTCC by 30%.

Dimethadione Reductions of Calcium Currents

Trimethadione was a frequently prescribed specific petit mal anticonvulsant prior to the development of ethosuximide and valproic acid. In the body, it is *N*-demethylated to dimethadione, the active metabolite, which is only poorly metabolized and accumulates in high concentrations (therapeutic free serum levels are 5–9 mM) [19, 20]. When applied to acutely isolated thalamic neurons in concentrations of 4 or 8 mM, dimethadione reversibly reduced both the LTCC and HTCC (LTCC reduction 52.4 ± 3.6%, HTCC reduction 34 ± 5.8%, concentration 8 mM, n = 10; LTCC reduction 40.0 ± 5.5%, HTCC reduction 31.2 ± 4.5%, concentration 4 mM, n = 5) (Fig 8). Like ethosuximide, dimethadione caused a reduction of LTCC over the full range of activation voltages (see Fig 8A,B). Dimethadione-reduced calcium currents showed no changes in their time course of activation or inactivation when compared with control currents (see Fig 8A). The reductions of LTCC showed similar voltage dependence to those produced by ethosuximide (see Fig 8C). Dimethadione also had no effect on the voltage dependence of steady-state inactivation (Fig 8D) or on the time course of recovery from inactivation (not shown). These findings suggest that dimethadione may reduce calcium currents in thalamic neurons through a mechanism similar to ethosuximide's. Structural parallels between these two molecules may be related to their similar actions on calcium currents (Fig 9).

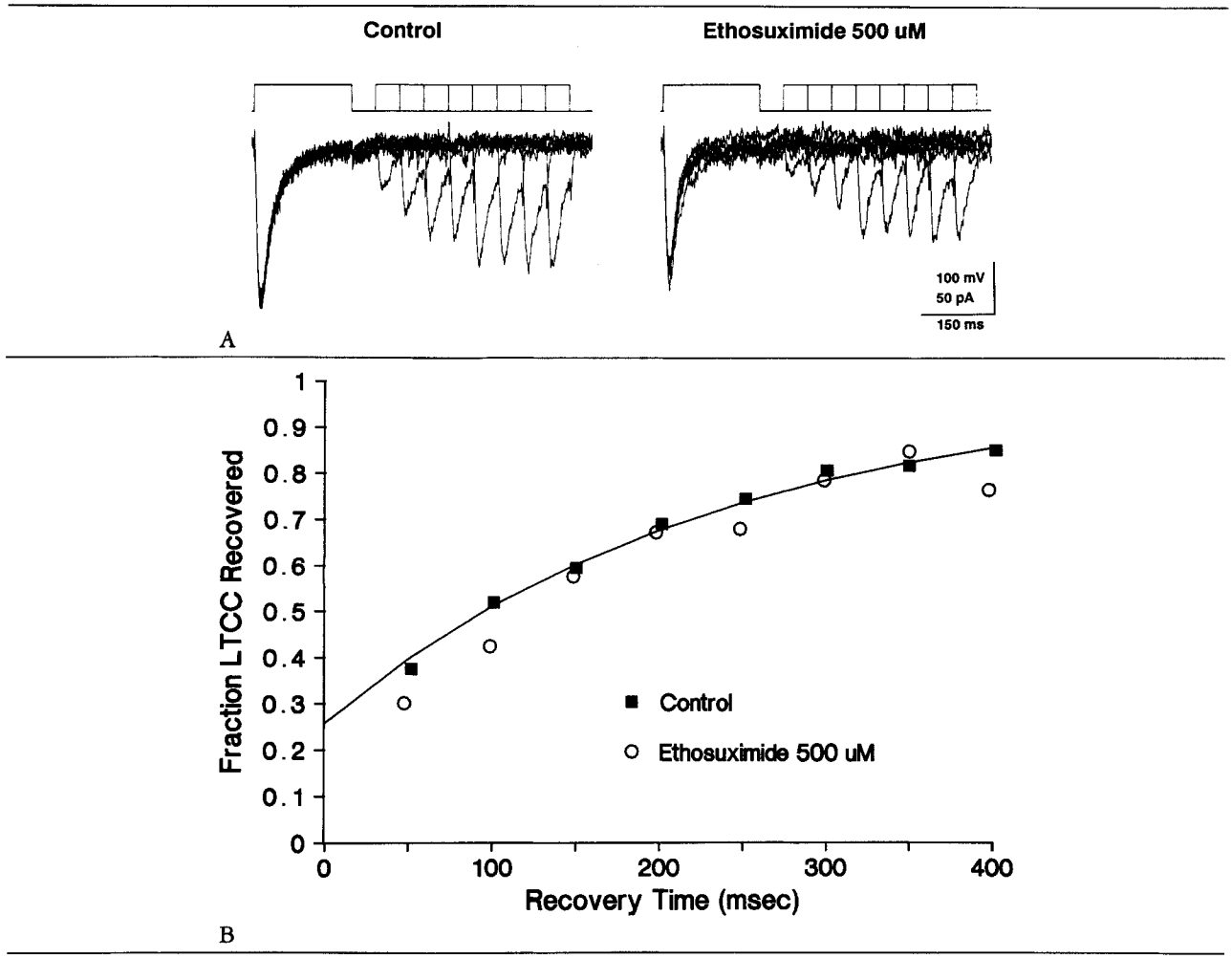

Fig 7. Recovery from inactivation of control and ethosuximide-reduced low-threshold calcium current (LTCC). Top: *superimposed traces illustrating the time course of recovery from inactivation of control* (left) *and ethosuximide-reduced* (right) *LTCC. The LTCC was first inactivated by a 200 msec depolarizing command to −40 mV from a holding potential of −100 mV. At varying times after the offset of this command, the degree of recovery of the LTCC was assessed by a 50-msec depolarizing command to 40 mV.* Bottom: *Plot of recovery time versus fraction of LTCC recovered for control and ethosuximide-reduced LTCC derived from the top traces. Note that ethosuximide has little effect on the time course of recovery from inactivtion. Records are from a cell where ethosuximide (500 μM) reduced LTCC by 30% in a completely reversible manner.*

Other Anticonvulsants

Valproic acid, another anticonvulsant effective against petit mal, is structurally and functionally quite different from ethosuximide and dimethadione (see Fig 9). It has a very broad spectrum of action, including effectiveness in control of other types of generalized seizures [25]. When applied to thalamic neurons, valproic acid had no effect on calcium currents at concentrations up to 1 mM (n = 12). Phenytoin, a drug effective in partial or generalized motor seizures, but ineffective in the control of petit mal, shares some structural features with ethosuximide (Fig 9). These structural similarities, and reports that phenytoin reduces LTCC in cultured hippocampal neurons [26] and neuroblastoma cells [27], prompted us to examine the effects of this agent on calcium currents in thalamic cells. At concentrations of 4 to 8 μM (therapeutic free serum levels), this drug had only small effects on calcium currents (less than 10% reductions of the LTCC, n = 8). At 100 μM, a concentration that far exceeds clinically relevant levels, phenytoin reduced the LTCC by 44 ± 2.8% and the HTCC by 3.3 ± 2.3% (n = 6) (Fig 10).

Discussion

We have previously reported that ethosuximide reduces the LTCC in enzymatically dissociated thalamic neurons [15] in a dose-dependent manner at concentrations very similar to the free serum levels achieved in petit mal patients whose seizures were controlled [2]. Our present results provide additional information about the nature of this reduction of LTCC by ethosuximide. Ethosuximide effects occurred without

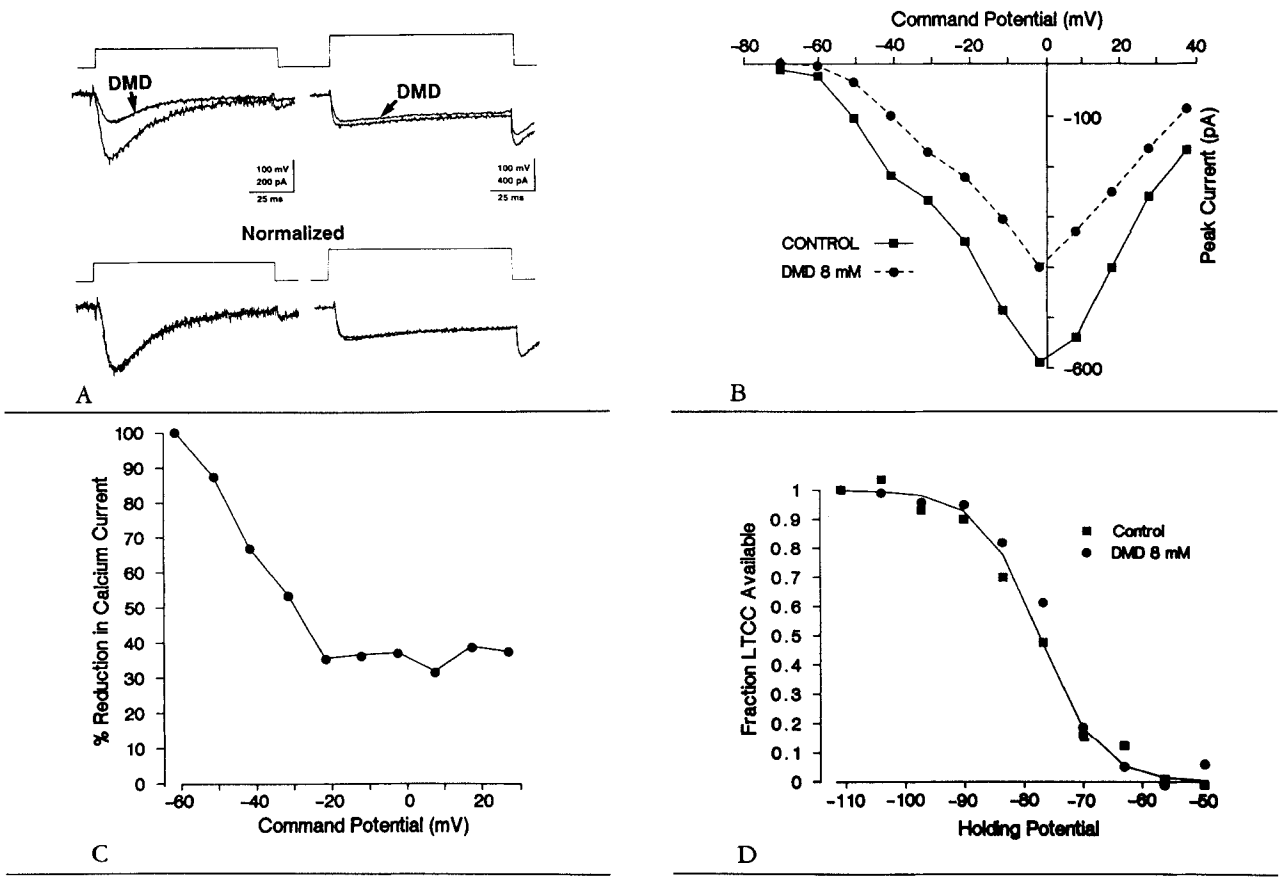

Fig 8. Dimethadione (DMD) reduction of calcium currents. (A) DMD reduces low (LTCC) and high-threshold calcium currents (HTCC) without altering the time course of activation or inactivation of either current. Top traces: *Control and DMD-reduced LTCC* (left, *55% reduction) and HTCC* (right, *19% reduction).* Bottom traces: *Superimposed normalized traces of control and DMD-reduced LTCC* (left) *and HTCC* (right). *Note that the drug-reduced and control traces have identical time courses. (B) IV plot of calcium currents under control and DMD-exposed conditions. Voltage command potential (from a holding potential of −100 mV) is plotted versus the peak amplitude of the elicited calcium current. Note that DMD reduces calcium current over the full range of activation potentials. (C) Plot of percent reduction of calcium current by DMD versus the command potential eliciting the current for the IV plot in B. Note that the DMD reduction of calcium current is voltage dependent. (D) Steady-state inactivation of control and DMD-reduced LTCC, derived as in Figure 6 for ethosuximide. Plot of holding potential versus fraction of LTCC available (relative to LTCC evoked from −112 mV). Note that DMD has no effect on the voltage dependence of steady-state inactivation. Records are from a cell where 9 mM DMD reduced LTCC by 55% in a completely reversible manner.*

changes in the time course of activation or inactivation of the LTCC and were evident over the entire voltage activation range. Further, reductions in LTCC occurred without alterations in the voltage dependence of activation or steady-state inactivation, or the time dependence of recovery from inactivation. Taken together, these data suggest that ethosuximide does not reduce the LTCC by exerting direct effects on channel kinetics or gating mechanisms. Rather, the results are compatible with a decrease in the number of available LTCC channels or in the elementary single LTCC channel conductance. Direct studies of LTCC channels will be required to confirm this conclusion. Dimethadione appeared to reduce LTCC by a similar voltage-dependent mechanism, but was less specific in that it significantly reduced the HTCC as well as the LTCC.

Twenty-three percent of the thalamic neurons studied did not respond to any concentration of ethosuximide with a reduction in calcium current, even though they possessed an LTCC that was indistinguishable from that in other, responsive neurons. This finding appears inconsistent with the LTCC channel–blocking hypothesis: ethosuximide channel–blocking actions would be expected to occur in all cells with low-threshold calcium channels. It is possible that the enzymatic treatment in some way altered the nonre-

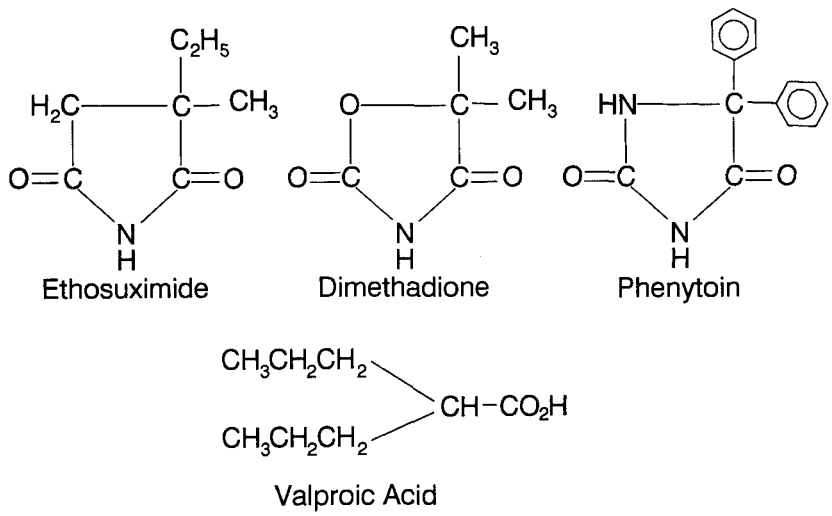

Fig 9. Structures of the anticonvulsants whose actions on calcium currents were examined in this study. Note the similar ring structures and substitutions of ethosuximide, dimethadione, and phenytoin, and the dissimilar structure of valproic acid.

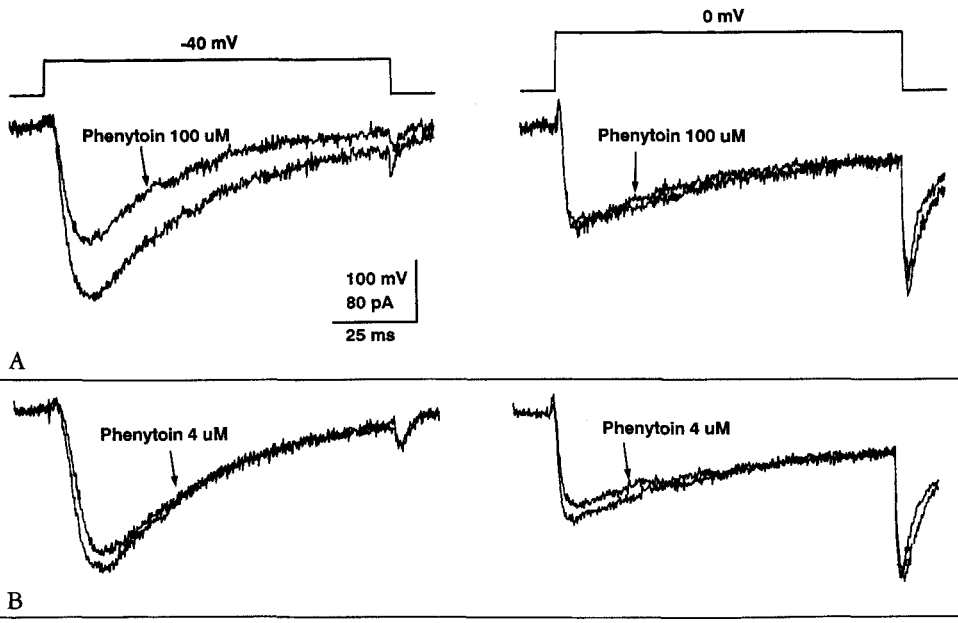

Fig 10. Phenytoin reductions of calcium currents. (A) At high concentration (100 μM), phenytoin specifically reduced low-threshold calcium current (LTCC) by 33% (left) *without having an effect on the high-threshold calcium current* (right). *(B) In the same cell, following washout of 100 μM phenytoin, low, clinically relevant concentrations of phenytoin (4 μM) had only small effects on LTCC (< 10% reduction,* left). *Control and drug sweeps are superimposed.*

sponsive cells so that they could not bind ethosuximide. By analogy with the actions of dihydropyridines on sustained calcium current in other cells [28], the receptor for ethosuximide could be the low-threshold calcium channel itself, somehow modified in a subset of the cells so as to be inaccessible to ethosuximide. Alternatively, there may be subsets of relay neurons that differ in their pharmacological sensitivity to these agents.

Consideration of the normal role of LTCC in the behavior of thalamic neurons may provide insights about the mechanisms by which ethosuximide-induced depression of this current could lead to control of spike-wave discharges. In contrast to its size in other mammalian neurons [18, 22, 24], the LTCC is particularly prominent in thalamic cells, where its overall peak amplitude is usually equal to or greater than that of the HTCC (e.g., Figs 1, 4C, and [21]). This proportionally large LTCC influences the behavior of thalamic neurons, as seen in current-clamp recordings, where large, low-threshold calcium-dependent spikes (LTCSs) are a prominent feature of cell behavior [10–13]. Given the kinetics of its voltage- and time-dependence of activation, inactivation, and deinactivation (see Figs 1–7) [11, 17, 22, 24], the LTCC is the only calcium conductance that could be responsible for the LTCSs. The LTCS is particularly important in regulating the normal oscillatory behavior of thalamic neurons [10, 12–14]. Normal afferent barrage, as occurs in the waking state, will maintain the resting membrane potential at a depolarized level sufficient to inactivate the LTCS, so that simple relay behavior without bursting predominates. Under conditions where the membrane potential is hyperpolarized, either by reduction of afferent drive (as in quiet sleep) or by strong hyperpolarizing inputs (e.g., [13]), the LTCS will be deinactivated and tend to promote bursting. Sequential activation of the LTCC and repolarizing potassium conductances can produce intrinsic oscillatory behavior [10–12]. These properties, together with the tight reciprocal connectivity between the thalamus and cortex, predispose the network to thalamocortical oscillations (reviewed in [14]) such as occur during sleep spindles.

Although direct evidence is not available in animal models of petit mal, or in humans, the above considerations make it likely that the LTCS and underlying LTCC play in important role in generating the abnormal rhythmicity that characterizes spike-wave discharges. This conclusion is further supported by the close relationship between spindles and spike waves present both in the feline penicillin model of generalized spike-wave discharge [6] and in human petit mal during spindle stage sleep (see Kellaway [29] for review) when spike-wave discharges are most prominent. Synchronous corticothalamic volleys such as occur during experimental spike-wave discharges [5, 7] can activate the LTCS in a postsynaptic population (e.g., [30]), with resulting spike burst generation in thalamic neurons that would propagate over thalamocortical axons and reactivate the cortical population. Even small reductions in the LTCS with ethosuximide would tend to dampen or abolish this type of oscillation and hence reduce spike-wave discharges and absence seizures. These effects would be significantly enhanced by the voltage dependence of the ethosuximide-induced LTCC reduction (see Fig 4), which would cause threshold calcium conductances, evoked by small depolarizations from hyperpolarized potentials, to be particularly affected. Thus, the action of ethosuximide on LTCC of thalamic neurons seems consistent with its anticonvulsant action in petit mal seizures, as does its ability to reduce thalamocortical transmission selectively at 3 to 4 Hz in vivo in cats [31, 32].

Dimethadione reduced the LTCC to a greater extent than did ethosuximide (maximal LTCC reductions, assessed during a voltage command to -40 mV, were 52.4% versus 31.9% for dimethadione and ethosuximide, respectively), although both drugs appeared to act through similar mechanisms (cf., Figs 3–6, 8). This greater efficacy of dimethadione appears to be at odds with the relative clinical effectiveness of these two agents as anticonvulsants. Because of their voltage-dependent actions, however, these two agents were equally effective in reducing calcium currents at threshold potentials (cf., Figs 4A, 8C). Phenytoin, which is also structurally similar to ethosuximide (see Fig 9), reduces LTCC only at toxic concentrations (see Fig 10), and so its actions on LTCC are most likely unrelated to its anticonvulsant action.

Not all petit mal anticonvulsants share the action of ethosuximide on calcium currents in thalamic neurons. Valproic acid had no effect on LTCC in concentrations up to 1 mM. Other effects of valproate [8, 33, 34] may be responsible for its diverse anticonvulsant actions. It is also conceivable that valproate metabolites not tested in our acute experiments (e.g., 2-en-valproate, 3-keto-valproate, or various hydroxy-valproates; see Löscher and colleagues [35]) have effects on the LTCC.

The finding that specific petit mal anticonvulsants reduce LTCC in thalamic neurons does not necessarily imply that abnormalities of LTCC regulation or function play a role in the pathogenesis of petit mal epilepsy, although this will certainly be an interesting hypothesis to explore. This result does suggest, however, that the LTCC is important in the generation and maintenance of spike-wave discharges underlying petit mal seizures and that modulation of the properties of the LTCC could influence petit mal attacks. The well-known effects of the level of arousal on both petit mal seizures [29] and thalamic LTCSs [14] may be an example of such modulatory influences. We are not implying that reductions in the LTCC are the only mech-

anism of action of ethosuximide, since other possible effects in the thalamus or at other sites have not been examined in this study (but see [3] for review). The important finding is an effect of ethosuximide that is consistent with its anticonvulsant action. The LTCC reduction occurs in clinically relevant drug concentration ranges and is shared by another specific petit mal anticonvulsant (dimethadione). The effects of these compounds on the LTCC are not mimicked by succinimide [15], a close structural relative that is inactive as an anticonvulsant [3]. Thus, LTCC reduction appears to be closely related to specific petit mal anticonvulsant effectiveness.

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