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

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ABSTRACT

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: α1G, α1H, and α1I. 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 α1G and α1I was 0.3 to 0.5 mM and for α1H 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.

Generalized, or petit mal, absence epilepsies are characterized by periods of synchronized neuronal activity, generating a 3-Hz spike-wave pattern on the electroencephalogram. Considerable evidence suggests that spike-wave discharges are produced by synchronized oscillations of cortical, reticular thalamic, and corticothalamic neurons (Gloor and Fariello, 1988; Steriade et al., 1993). Oscillatory firing of corticothalamic neurons requires the activity of low threshold Ca^{2+} spikes (LTS; Steriade and Llinas, 1988; McCormick and Bal, 1997),which are produced by T-type Ca^{2+} channels (Crunelli et al., 1989; Suzuki and Rogawski, 1989).

Ethosuximide is considered the prototypical absence seizure drug that works by inhibition of T-type channels. This hypothesis was based on the finding that ethosuximide could partially block T-type currents in thalamic neurons at therapeutically relevant concentrations (Coulter et al., 1989a). Support for this hypothesis came from studies with related analogs: 1) T-type current block was also observed at therapeutically relevant concentrations of methyl-phenylsuccinimide (MPS), the active metabolite of methsuximide; and 2) no block was observed using either the inactive analog succinimide or the convulsant analog tetra-methylsuccinimide (Coulter et al., 1990a). The sensitivity of T-type channels to MPS has been confirmed using dorsal root ganglion neurons (Todorovic and Lingle, 1998). With one exception (Kostyuk et al., 1992), other studies have failed to confirm ethosuximide block of T-type currents at therapeutically relevant concentrations (Todorovic and Lingle, 1998), leading to the suggestion that block of other ionic channels may be more relevant (Leresche et al., 1998).

Many factors interfere with the pharmacological characterization of native T-type channels, such as interference from other ionic conductances, the lack of highly selective blockers, and adequate voltage control. Most studies have been performed in isolated neurons that have lost their dendritic trees and, hence, most of their T-type channels (Destexhe et al., 1998). We have recently cloned the cDNAs of three T-type Ca^{2+} channels (α1G, $Ca_v3.1$; α1H, $Ca_v3.2$; and α1I, $Ca_v3.3$), and we now report the cloning of human α1I (Perez-Reyes, 1999; Cribbs et al., 2000). Expression of the cloned channels in HEK-293 cells provides an excellent assay system to test the pharmacology of T-type currents, since they contain very little background currents under appropriate assay conditions. We report for the first time the block of human T-type channels by two succinimide antiepileptic

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ABBREVIATIONS: MPS, α-methyl-α-phenylsuccinimide; LTS, low threshold Ca^{2+} spikes; DMEM, Dulbecco's modified Eagle's medium; TEA, tetraethylammonium; TMS, trimethylsuccinimide; DRG, dorsal root ganglion; HEK, human embryonic kidney.

drugs, ethosuximide and MPS, the active metabolite of methsuximide. Our results show that block is state-dependent, with less block observed at highly hyperpolarized holding potentials. This finding may partially explain the controversy of whether ethosuximide block of T-type currents is therapeutically relevant.

Experimental Procedures

Materials. The following stably transfected HEK-293 cell lines were used in this study: hα1G-Q39, containing the human α1G channel, $Ca_v3.1a$ (GenBank accession number AF190860; Cribbs et al., 2000); hα1H-Q31, containing the Hh7 plasmid construct of human α1H, $Ca_v3.2$ (GenBank accession number AF073931; Cribbs et al., 1998); and hα1I-14, containing the LT4 plasmid construct of α1I, $Ca_v3.3$ (described below). All chemicals were from either Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI).

Cloning of a Human α1I-cDNA. Human brain cDNA libraries derived from either fetal brain or cerebellum (CLONTECH, Palo Alto, CA) were screened using the rat α1I as probe. Screening was done by filter hybridization according to the manufacturer's protocol. The cDNA probes were synthesized using [α-^{32}P]dCTP and the Ready-To-Go labeling kit (Amersham Pharmacia Biotech Piscataway, NJ). Positive clones were plaque-purified and then subcloned into pUC18 for sequencing. BLAST searches of the GenBank with the rat α1I identified the genomic DNA encoding the human CACNA1I gene (AL022312), allowing us to design PCR primers to clone the 5′ end. Overlapping clones were selected and ligated in the vector pcDNA3 (Invitrogen, Carlsbad, CA), generating the clone LT4. The nucleotide sequence of human α1I has also been reported by two groups (Mittman et al., 1999; Monteil et al., 2000). The sequence of LT4 is similar to that reported in AF211189, in that it lacks putative exon 9, but is longer at the 3′ end, extending to nucleotide 6214 of AF393329.

Generation of a Stably Transfected Cell Line. Stable cell lines expressing human α1I were constructed by transfecting HEK-293 cells with linearized LT4 plasmid. HEK cells were maintained in DMEM, 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were plated at a density of 1×10^6 per 100-mm plate. One day later, fresh media was added, and the cells were transfected with 10 μg of plasmid DNA by the calcium phosphate method (CalPhos Maximizer Transfection Kit, CLONTECH) according to the manufacturer's protocol. Following transfection, the cells were selected in 1.0 mg/ml G-418 (Invitrogen) for 2 to 3 weeks. Single colonies were isolated, expanded, and then screened electrophysiologically for the expression of T-type Ca^{2+} current. One clone, hα1I-14, was selected for further study.

Electrophysiological Analysis of HEK-293 Transfected Cells. HEK-293 cells were dissociated by digestion with 0.25% trypsin plus 1 mM EDTA for 2 min and then diluted 20-fold with DMEM. The cells were triturated, diluted 2-fold with DMEM, and then plated on cover slips. The cells were incubated at least 4 h and up to 2 days before electrophysiological studies. The standard internal pipette solution contained 135 mM CsCl, 10 mM EGTA, 4 mM Mg-ATP, 0.3 mM Na_3GTP, and 10 mM HEPES, pH adjusted to 7.3 with CsOH. Most experiments were performed using the following solution: 5 mM $CaCl_2$, 155 mM tetraethylammonium (TEA) chloride, and 10 mM HEPES, pH adjusted to 7.4 with TEA-OH. As noted in the figure legends, some experiments were performed using Ba^{2+} as charge carrier in 10 mM $BaCl_2$, 160 mM TEA chloride, 6 mM CsCl, and 10 mM HEPES, pH adjusted to 7.4 with TEA-OH. Addition of 1 mM Mg^{2+} to the external solution had no effect on the block of either α1G or α1I produced by 3 mM MPS.

Whole cell currents were recorded from HEK-293 cells using the ruptured patch method on two electrophysiological set-ups. One set-up consisted of an Axopatch 200B amplifier, Digidata 1200 A/D converter, and pCLAMP 8.0 software (Axon Instruments, Foster City, CA). The second set-up used an Axopatch 200A amplifier, Digidata 1200 A/D converter, and pCLAMP 6.0 software (Axon Instruments). Data were digitized at 4 to 50 kHz and filtered at 1 to 5 kHz. Recording pipettes were made from TW-150-6 capillary tubing (World Precision Instruments, Inc., Sarasota, FL), using a model P-97 Flaming-Brown pipette puller (Sutter Instrument Co., Novato, CA). Once filled with the internal solution the pipette resistance was typically 1.5 to 2.5 MΩ. Series resistance (initially between 2 and 5 MΩ) was compensated 70 to 80% and adjusted between protocols if necessary. Leak currents were minimal; therefore leak subtraction was not used. The average cell capacitance was ~25 pF. All experiments were performed at room temperature.

Dose-Response Analysis. Succinimide analogs were freshly dissolved in external solution at a concentration of either 30 or 100 mM and then diluted in 10-fold increments. The recording chamber was a RC-25 (Warner Instrument Corp., Hampden, CT), which has a volume of 0.15 ml. Each test solution was perfused at 1 to 3 ml/min.

Data Analysis. Peak currents and exponential fits to currents were determined using Clampfit software (Axon Instruments). Dose-response analysis and graphing of the data were with Prism (GraphPad, San Diego, CA). The following equation was used to fit dose-response data

$$Y = 1/(1 + 10^{(\log IC_{50} - X) \cdot \text{Hill slope})})$$

where X is the logarithm of concentration and Y is the response. The voltage dependence of activation was calculated using the solver function of Excel (Microsoft, Redmond, WA) to minimize the residual sum of squares from the simultaneous fit of the data with the Goldman-Hodgkin-Katz equation (Hille, 1992)

$$I = P_{Ca} z^2 (VF^2/RT) \cdot (([Ca^{2+}]_i - [Ca^{2+}]_o) \cdot \exp(-zVF/RT))/ (1 - \exp(-zVF/RT))$$

and a Boltzmann equation

$$G/G_{max} = 1/(1 + \exp((V_{1/2} - V)/k))$$

where P_{Ca} is the Ca^{2+} permeability; z, the valence of the Ca^{2+} ion; V, the test potential; $V_{1/2}$, the mid-point of activation; F, Faraday's constant; R, the gas constant; T, absolute temperature; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration (25 nM); $[Ca^{2+}]_o$, extracellular Ca^{2+} (5 mM); and k is the slope factor. Values are reported as the means ± S.E.M.

Results

Cloning of a Human α1I-cDNA. The human gene encoding $Ca_v3.3$ (α1I), *CACNA1I*, was identified using homology searches of the GenBank HTGS database (Lee et al., 1999a). This sequence information was originally used to clone the rat α1I-cDNA. We then used the rat cDNA to screen human brain cDNA libraries. Overlapping cDNAs were assembled to produce a 6,268-base pair cDNA (LT4-pcDNA3) that encodes a protein of 220,706 Da. The cloning of human α1I was previously reported by Monteil et al. (2000). As noted under *Experimental Procedures*, our clone contains additional coding sequence at the 3′ end. Transient transfection studies indicated that LT4 encoded a functional low voltage-activated Ca^{2+} channel with similar electrophysiological characteristics as observed with the rat (Lee et al., 1999a). The LT4 plasmid was then used to generate stably transfected cell lines, and hα1I-14 was chosen for further study.

Ethosuximide Block of Cloned T-Type Ca^{2+} Channels. The effect of ethosuximide was tested on human T-type

currents using whole cell recording from HEK-293 cell lines that were stably transfected with $Ca_v3.1$ (α1G), $Ca_v3.2$ (α1H), or $Ca_v3.3$ (α1I). Currents were measured using either 5 mM Ca^{2+} or 10 mM Ba^{2+} as charge carrier and Cs-based intracellular solutions that contain ATP. Under these recording conditions the current density is approximately 40 pA/pF, such that the typically sized cell has 1000 pA of current during a test pulse to −30 mV. The size of the currents is quite stable for most recordings, although there is some run-up observed immediately after establishment of the whole cell clamp and some run-down after ~15 min of recording. Therefore, the stability of the currents was monitored in all experiments, and results are only presented for cells whose run-down rate was less than 2%/min. The effects of compounds were tested by dissolving each compound directly in external solution and then perfusing the compound over the cell. Various concentrations were tested on each cell without (Fig. 1, A and B) or with (Fig. 2) washout between concentrations. Little difference was observed among the results obtained with these two protocols, so the results have been pooled. The ethosuximide dose-response curve for block of human α1G currents is shown in Fig. 1C. The data were fit with the dose-response equation, yielding an apparent IC_{50} of 10 ± 2 mM (n = 31 cells). Similar results were obtained using 5 mM Ca^{2+} as the charge carrier (IC_{50} = 12 ± 2 mM, n = 16 cells). The response to concentrations below 1 mM was greater than expected from simple binding of a drug molecule to a single site on the channel. Since the deviation occurred in the therapeutic range of plasma ethosuximide concentrations (40–100 μg/ml or 0.3–0.7 mM; Browne et al., 1975), these measurements were repeated extensively. Clear inhibitory effects of ethosuximide could be detected with concentrations as low as 0.1 mM (Fig. 1D). The response to 100 mM also deviated from the fit. Since such a high concentration alters the osmolarity of the external solution and might cause a nonspecific effect, we adjusted for this by reducing the TEA concentration. In either case the block was 100%. Although the data are consistent with two binding sites, an alternative hypothesis is that ethosuximide block is state-dependent (Hille, 1992).

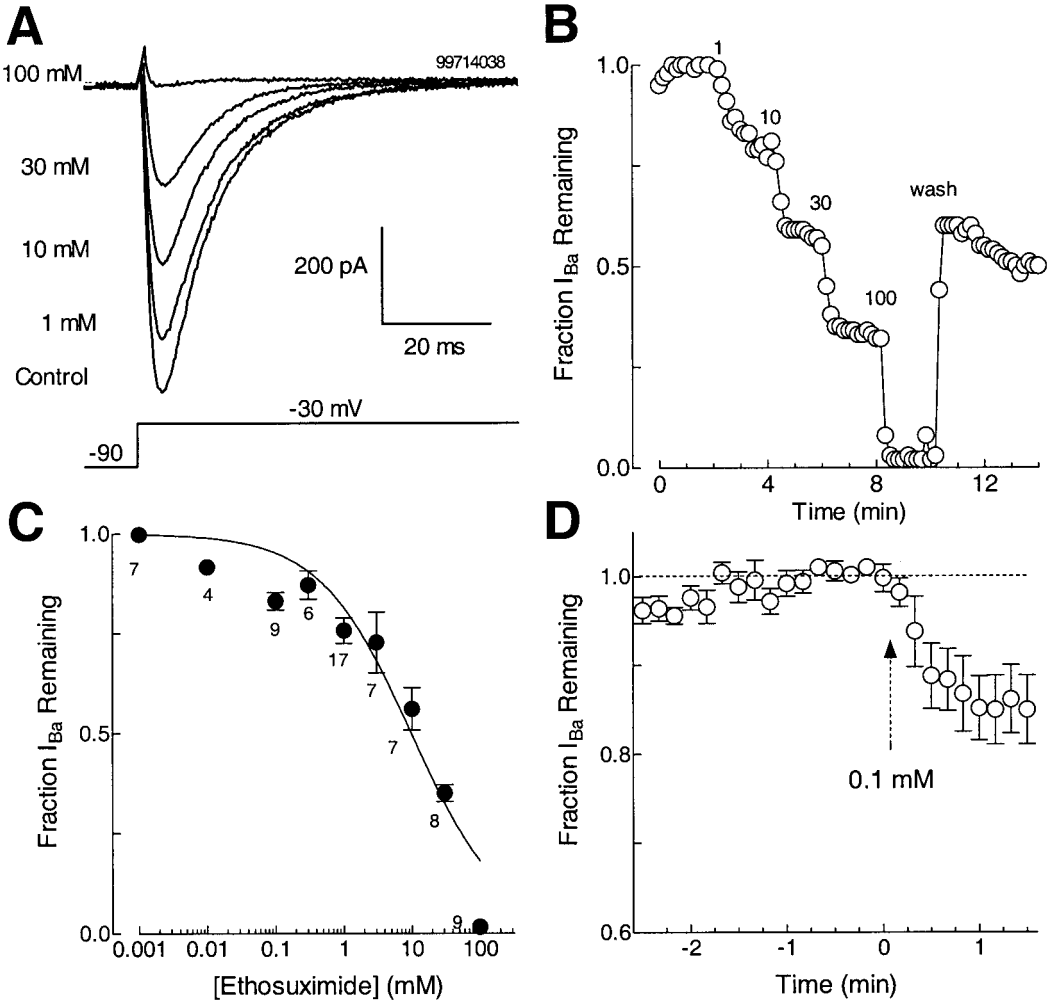

Fig. 1. Inhibition of human α1G T-type Ca^{2+} channels by ethosuximide. A, representative whole cell recordings showing the effect of various concentrations of ethosuximide on T-type currents. Whole cell Ba^{2+} (10 mM) currents recorded from an HEK-293 cell stably transfected with α1G subunit in response to voltage steps to −30 mV from a holding potential of −90 mV applied every 10 s. B, time course of peak currents normalized to control for the same cell shown in A. Ordinate axis, peak current during steady-state exposure to ethosuximide, normalized by the peak current before drug exposure, defined as the fraction of I_{Ba} remaining. C, dose-response relationship for ethosuximide block. Fraction of unblocked peak current is plotted against drug concentration. Number of cells investigated for each concentration is indicated near each data point. Data were fitted (smooth line) using a Hill equation (see *Experimental Procedures*). Note that data points representing drug concentrations lower that 1 mM deviated significantly from the fitted curve. D, inhibitory effect of 0.1 mM ethosuximide on peak currents averaged from five cells.

Methyl-Phenylsuccinimide Block of Cloned T-Type Channels. The antiepileptic drug methsuximide is rapidly demethylated to *N*-desmethylmethsuximide, which is also called MPS. Therapeutic plasma levels of MPS in well controlled patients range between 10 and 40 μg/ml or 50 and 200 μM (Strong et al., 1974; Porter et al., 1979; Wilder and Buchanan, 1981). The sensitivity of human T-type channels to block by MPS was measured as above. Figure 2 shows the time course of a typical experiment using α1I. MPS produced a rapid block of current, which was rapidly and almost completely reversed upon washout. The dose-response relationship of MPS block of the three cloned human T-type channels was measured using the peak Ca^{2+} current elicited during a pulse to −30 mV (Fig. 2C) from a holding potential of −90 mV. The α1G and α1I channels were equally sensitive, displaying apparent IC_{50} values of 1.95 ± 0.19 (n = 7 cells) and 1.82 ± 0.16 mM (n = 14 cells), respectively, whereas α1H channels were slightly less sensitive (IC_{50} = 3.0 ± 0.3 mM, n = 6 cells). The Hill coefficients were significantly greater than one: ~1.3 for α1G and α1I and 1.7 for α1H. As observed with ethosuximide, these results suggested that block was more complex than simple 1:1 binding of drug to channel/receptor; therefore we investigated the mechanisms by which the succinimide antiepileptics blocked the channels.

Block Is Voltage-Dependent. Current-voltage relationships (I-V curves) for α1I were measured using 500-ms step depolarizations to varying potentials from a holding potential of −90 mV. Typical current traces are shown in Fig. 3A, measured before (C) and during (M) exposure to 3 mM MPS. Average I-V curves are shown in Fig. 3B (n = 4 cells). The percent block of the peak current was calculated, and then plotted as a function of the test potential (Fig. 3C). MPS block was greatest during test potentials to −50 mV, and then gradually decreased at more positive potentials. To calculate the $V_{1/2}$ of activation, the permeability of the channels was estimated using constant field equations (Hille, 1992) and fit with a Boltzmann equation. MPS produced an apparent +7 mV shift, which was reversed upon washout ($V_{1/2}$ in mV): control, −41 ± 1; MPS, −34 ± 1; and washout, −41 ± 2. Somewhat surprisingly, the outward currents, which are due to Cs^+ efflux through the T-type channel (Lee et al., 1999a),

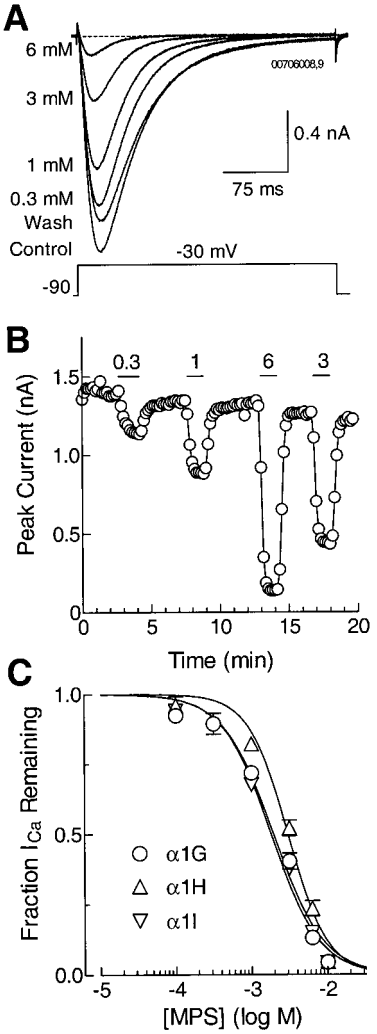

Fig. 2. Inhibition of human T-type Ca^{2+} channels by MPS. A, representative whole cell recordings showing the effect of various concentrations of MPS on T-type currents. Whole cell Ca^{2+} (5 mM) currents recorded from an HEK-293 cell stably transfected with α1I subunit in response to voltage steps to −30 mV from a holding potential of −90 mV applied every 10 s. B, time course of peak current for the same cell shown in A. C, dose-response relationship for MPS block on Ca^{2+} currents expressed by α1G, α1H, and α1I channels. Fraction of unblocked peak current is plotted against drug concentration. IC_{50} values from fitted data (smooth lines) using a Hill equation were: α1G, 1.95 ± 0.2 mM; α1H, 3.03 ± 0.26 mM; and α1I, 1.82 ± 0.16 mM.

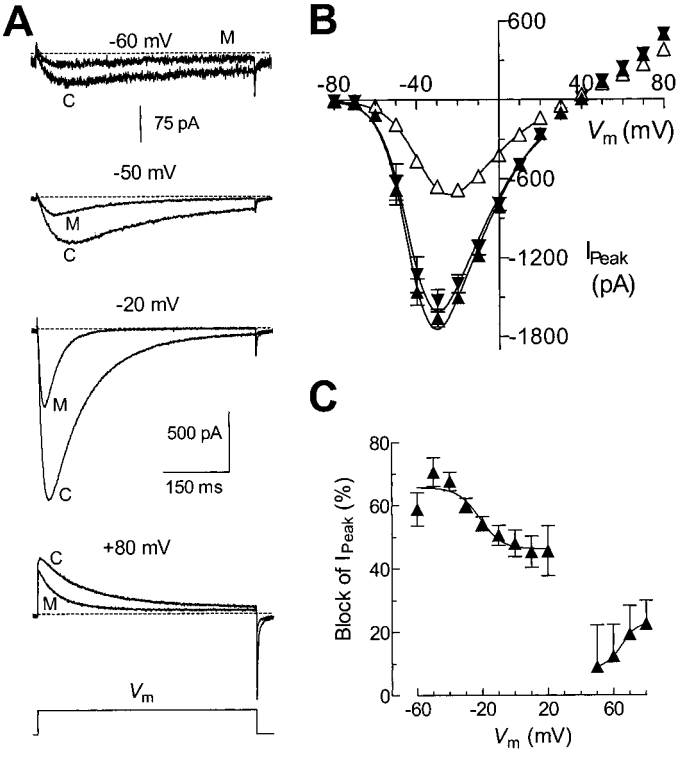

Fig. 3. Voltage-dependent block of MPS. A, sample records obtained at the indicated values of V_m from a holding potential of −100 mV. Current traces were obtained from an HEK-293 cell stably expressing α1I channels in the absence (C) and in the presence of 3 mM MPS (M). Charge carrier was 5 mM Ca^{2+}. B, current-voltage relationships of α1I channels obtained before (▲), in the presence of 3 mM MPS (△), and after washout the drug (▼). Data points are averages of five cells. The smooth lines are spline curves fit to the calculated values of current using the product of the Goldman-Hodgkin-Katz and Boltzmann equations (see *Experimental Procedures*). C, percent block of peak current as a function of test potential; from same cells shown in B. Data are not shown for voltages near the reversal potential.

were hardly blocked at all. These results indicate that MPS affects the voltage dependence of gating, and that it interacts with the permeant ion.

Closer inspection of the current traces revealed that MPS was capable of affecting the decay of the α1I currents. To illustrate this effect, the currents recorded during test pulses to −30 mV were scaled and superimposed (Fig. 4A). Two effects can be observed: 1) MPS accelerated the apparent inactivation rate; and 2) MPS totally abolished the sustained component of current. To quantify the effect on kinetics, the entire current trace was fit with two exponentials, one corresponding to activation and the other to inactivation. The inactivation τ was plotted as a function of test potential in Fig. 4B. MPS had no effect on the relatively slow inactivation rate observed during test pulses to −50 mV, but accelerated the rate greater than 2-fold during pulses in the range of −30 to +20 mV. Currents measured after washout of the drug inactivated in the same manner as control. The MPS effect on inactivation was dose-dependent, with less effect observed using 1 mM (Fig. 4C). MPS was also capable of accelerating the apparent inactivation rate of α1G and α1H in a dose-dependent manner (Fig. 4C).

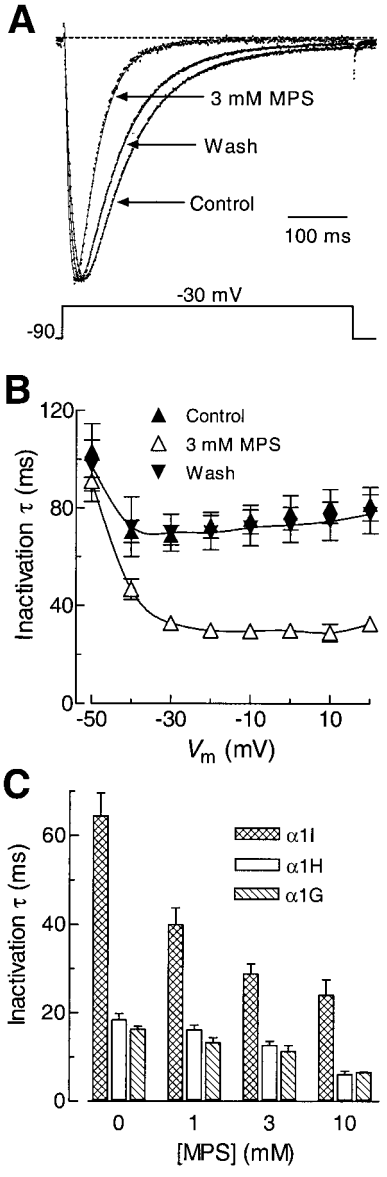

Fig. 4. MPS accelerates decay of human T-type currents. A, superimposed current traces at −30 mV from the same cell illustrated in Fig. 3. MPS (3 mM) and wash traces were normalized to the amplitude of the control trace. Current signals plotted with dots and solid lines represent fits with two exponentials, one for the activation and the other for the inactivation of the current. B, voltage dependence of the inactivation τ in the three conditions. Current traces obtained in response to an *I-V* protocol like in Fig. 3A were fitted as mentioned above, and the respective inactivation time constant was plotted as a function of voltage for the indicated experimental conditions. C, concentration-dependent effect of MPS on inactivation τ. Inactivation time constants were obtained as described in B for α1G, α1H, and α1I currents at −30 mV and were plotted versus the concentration of MPS.

In contrast to its effects on inactivation, MPS did not have a significant effect on the rate of recovery from inactivation of α1G (data not shown). Inactivation was induced by either short (100-ms) prepulses to −30 mV or long (10-s) prepulses to −50 mV. Recovery at −100 mV was measured by changing the interval between the prepulse and the test pulse. Recovery from short prepulses were well fit with a single exponential in both control (87 ± 2 ms) and in the presence of 3 mM MPS (86 ± 2 ms, n = 3 cells). Recovery from long prepulses was significantly better fit with two exponentials in both the absence and presence of 3 mM MPS (τ_1 = 116 ms, τ_2 = 1.2 s, n = 3 cells). In both cases, the percent block at each time point was relatively constant, indicating that drug bound channels remained blocked.

Ethosuximide also accelerated the decay of the current during a depolarizing pulse. Representative current traces are shown in Fig. 5A and were scaled for comparison in Fig. 5B. Although both α1G and α1H exhibit measurable sustained currents using Ca^{2+} as charge carrier (Klöckner et al., 1999; Serrano et al., 1999), these currents are too small to measure drug effects. The α1I currents display more of a sustained component, and this component was extremely sensitive to both succinimide antiepileptics (Figs. 2B, 4A, and 5B). To quantify this effect, we measured the amount of ethosuximide block that occurs after 150 ms of depolarization, and compared that with the block of the peak current. The ethosuximide dose-response relationships on block of α1I channels are shown in Fig. 5C. The apparent IC_{50} for block of the persistent current was 13-fold lower, dropping from 8 ± 2 to 0.6 ± 0.2 mM (n = 8 cells).

Block Is State-Dependent. The modulated receptor hypothesis was based on observations that drugs had varying affinities for closed/rested and inactivated states. Binding to inactivated states is an important property of drugs because it can provide selectivity to their action, as clearly exemplified by dihydropyridines that preferentially block smooth muscle L-type channels with little effect on cardiac channels (Triggle, 1999). Voltage-gated Ca^{2+} channels do not need to open before inactivating, and transitions from closed to inactivated states can be measured using long prepulses at subthreshold potentials, commonly referred to as the steady-state inactivation curve, or h_∞. Drugs that bind and stabilize the inactivated state shift the equilibrium from closed/rested to inactivated states, and hence shift this steady-state inactivation curve to more negative voltages (Bean et al., 1983). The effect of MPS and ethosuximide on the inactivation curves of the three T-type channels was estimated using prepulses of the following duration: 0.3 s for α1G (Fig. 6A); 5 s for α1H prepulses (Fig. 6, C and D); and 30 s for α1I (Fig. 6B) and α1G (Table 1). In each case, MPS was capable of shifting

the curve 5 to 10 mV to more negative potentials. The amount of shift was dose-dependent; 1 mM MPS shifted the α1G curve −5.5 mV (data not shown), whereas 3 mM shifted it −9.5 mV (Table 1). Similar results were obtained using 10 mM ethosuximide, which shifted the α1H inactivation curve 5 mV toward more negative potentials (Fig. 6D). Reversal of this effect by washing out the drug was in many cases incomplete. This is due in part to time-dependent shifts in the inactivation curve, which can be largely prevented by inclusion of ATP in the intracellular pipette solution (Zhang et al., 2000). The lack of full reversal may also be due to residual drug in the bath.

An independent method for measuring binding to inactivated states of the channel is to measure the dose-response relationship at various holding potentials. We chose two potentials to measure the MPS dose response: −110 mV, where all the channels should be in the closed/rested state, and −75 mV, where 50% of the channels should be in inactivated states (see Fig. 6). The potency of MPS for all three T-type channels was enhanced more than 2-fold by holding at −75 mV (Fig. 7 and Table 2). As shown in Fig. 5 for ethosuximide, the potency of MPS was even greater if block was measured on the sustained component (Fig. 7C; IC_{50} = 0.4 ± 0.1 mM, n = 5 cells). Ethosuximide block was also dependent on the holding potential (Fig. 7E), with the apparent IC_{50} decreasing 6-fold from 18.2 ± 1.2 at −100 mV to 3.2 ± 0.4 mM at −75 mV (n = 4 cells).

An important property of T-type Ca^{2+} channels is that they display "window currents", that is, there are voltages where channels do not inactivate totally and are available to open. This property is commonly defined by the presence of an overlap region between the steady-state inactivation curve and the activation curve. The size and position of the window current region is subject to many experimental variables. Short prepulses (<1 s) may not reach steady state, such that increasing the duration of the prepulse shifts the apparent inactivation curve to the left (Herrington and Lingle, 1992). Preliminary experiments with α1I indicated that at least 10 s were required, so we performed experiments with a 30-s prepulse. Activation curves have been estimated by fitting current data with a Boltzmann equation, with the current

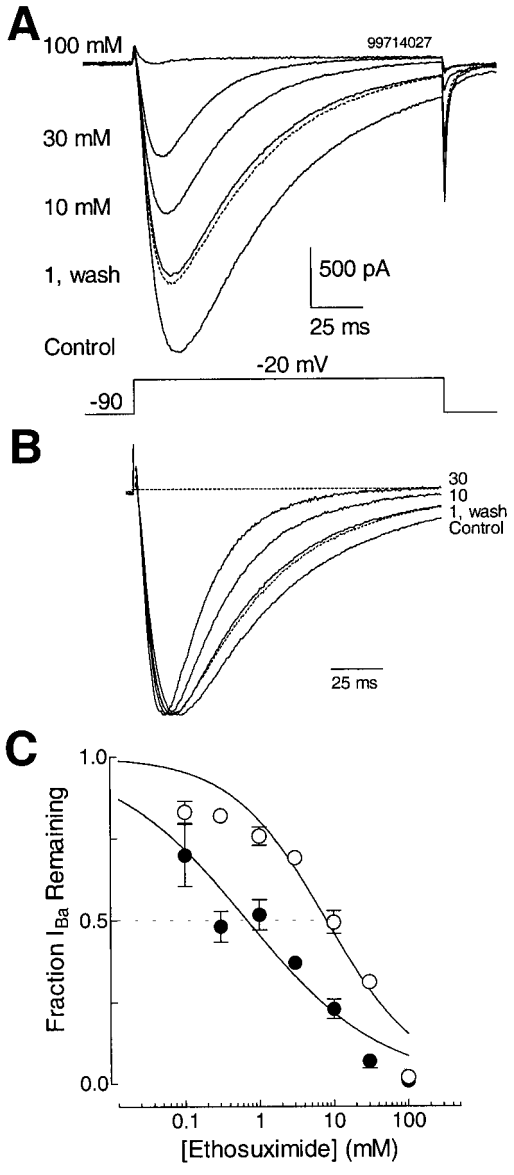

Fig. 5. Ethosuximide accelerates decay of human T-type currents. A, effect of increasing concentrations of ethosuximide on Ba^{2+} currents recorded from a HEK-293 cell stably transfected with α1I subunit in response to voltage steps to −20 mV. Dotted line corresponds to the washout of the drug. B, same traces as in A normalized to the control amplitude. C, dose-response relationship for ethosuximide block of Ba^{2+} currents measured at peak (○) and after 150 ms of depolarization (●). Smooth lines represent the fitted curve using the Hill equation. The calculated IC_{50} values were 8 ± 2 and 0.6 ± 0.2 mM for the peak and the sustained current, respectively.

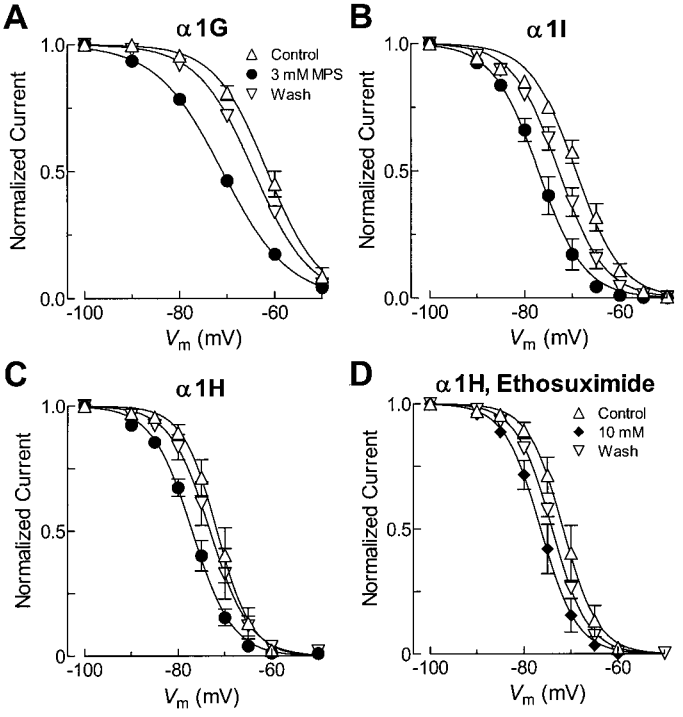

Fig. 6. MPS and ethosuximide shift the voltage dependence of steady-state inactivation of human T-type Ca^{2+} channels. Steps lasting 0.3 s (α1G), 5 s (α1H), and 30 s (α1I) to the indicated voltages were followed by 150-ms test pulses to −30 mV in A and B or −20 mV in C and D. Protocols were run before (△), during, and after (▽) exposure to 3 mM MPS (●) or 10 mM ethosuximide (♦). Charge carrier was 5 mM Ca^{2+} in A and B, and 10 mM Ba^{2+} in C and D. Currents were normalized to the value at −100 mV at each experimental condition. The smooth curves are fits using the Boltzmann equation. The corresponding parameters are shown in Table 1.

TABLE 1

Effect of MPS on inactivation

The effects of MPS on the inactivation properties were determined using a double pulse protocol (see Fig. 6). The prepulse duration is shown in parenthesis. Data from each cell were collected before, in the presence of 3 mM MPS, or after washout. The results from each cell were fit with a Boltzmann equation and then averaged. All $V_{1/2}$ values are in millivolts. Steady-state inactivation curves using 30-s prepulses were also performed on cells that were preincubated (>4 min) in 3 mM MPS before patching the cell and recorded in the continued presence of drug. The double pulse protocol was run 2 min after establishment of the whole cell configuration. As control for these experiments, the protocol was run on other cells a similar time after patching. The $V_{1/2}$ and k values for the curves of Fig. 6D were: control, −71.7, −3.8; 10 mM ethosuximide, −76.5, −3.9; wash, −74.0, −3.8; respectively. Charge carrier was 5 mM Ca^{2+} in α1G and α1I experiments and 10 mM Ba^{2+} in the case of α1H.

T-Type Channel	Control		3 mM MPS		Wash	
	$V_{1/2}$	k	$V_{1/2}$	k	$V_{1/2}$	k
α1G (0.3 s)	−61.5 ± 1.0	−5.5 ± 0.1	−71.0 ± 0.9	−7.0 ± 0.1	−64.4 ± 1.8	−5.9 ± 0.3
α1G (30 s)	−73.0 ± 1.2	−4.0 ± 0.1	−80.0 ± 1.5	−4.9 ± 0.1	−75.7 ± 1.9	−4.0 ± 0.1
α1G (preincubated)	−69.3 ± 1.0	−4.0 ± 0.1	−73.5 ± 1.4	−5.1 ± 0.6		
α1H (5 s)	−71.7 ± 0.2	−3.7 ± 0.1	−77.0 ± 0.2	−4.3 ± 0.2	−73.2 ± 0.2	−4.3 ± 0.1
α1I (30 s)	−69.1 ± 0.7	−5.1 ± 0.2	−75.8 ± 1.5	−4.4 ± 0.1	−72.8 ± 1.0	−4.8 ± 0.1
α1I (preincubated)	−63.7 ± 0.4	−3.8 ± 0.3	−68.5 ± 2.0	−3.9 ± 0.3		

data coming from either *I-V* or tail current measurements. In addition, *I-V* data must take into account changes in driving force, which can be done by either assuming a chord conductance or with constant-field theory. Although these different methods yield different values for $V_{1/2}$ and k, they have surprisingly little effect on the foot of the curve (data not shown) and hence the window current region. We used constant-field theory, which produced a good fit of the *I-V* data (Fig. 3). The effect of 3 mM MPS on the window current region is shown in Fig. 8A. The fraction of α1I channels available for opening in the window current region was reduced by 50% in the presence of 3 mM MPS.

Another important property of T-type channels is that they mediate LTS (Crunelli et al., 1989; Suzuki and Rogawski, 1989). To study the effect of ethosuximide on such activity, we used a voltage protocol that mimics a subthreshold LTS recorded from thalamic neurons (Destexhe et al., 1998). To investigate the effect of a therapeutically relevant concentration, we tested the effect of 0.7 mM ethosuximide on the Ca^{2+} current carried through human

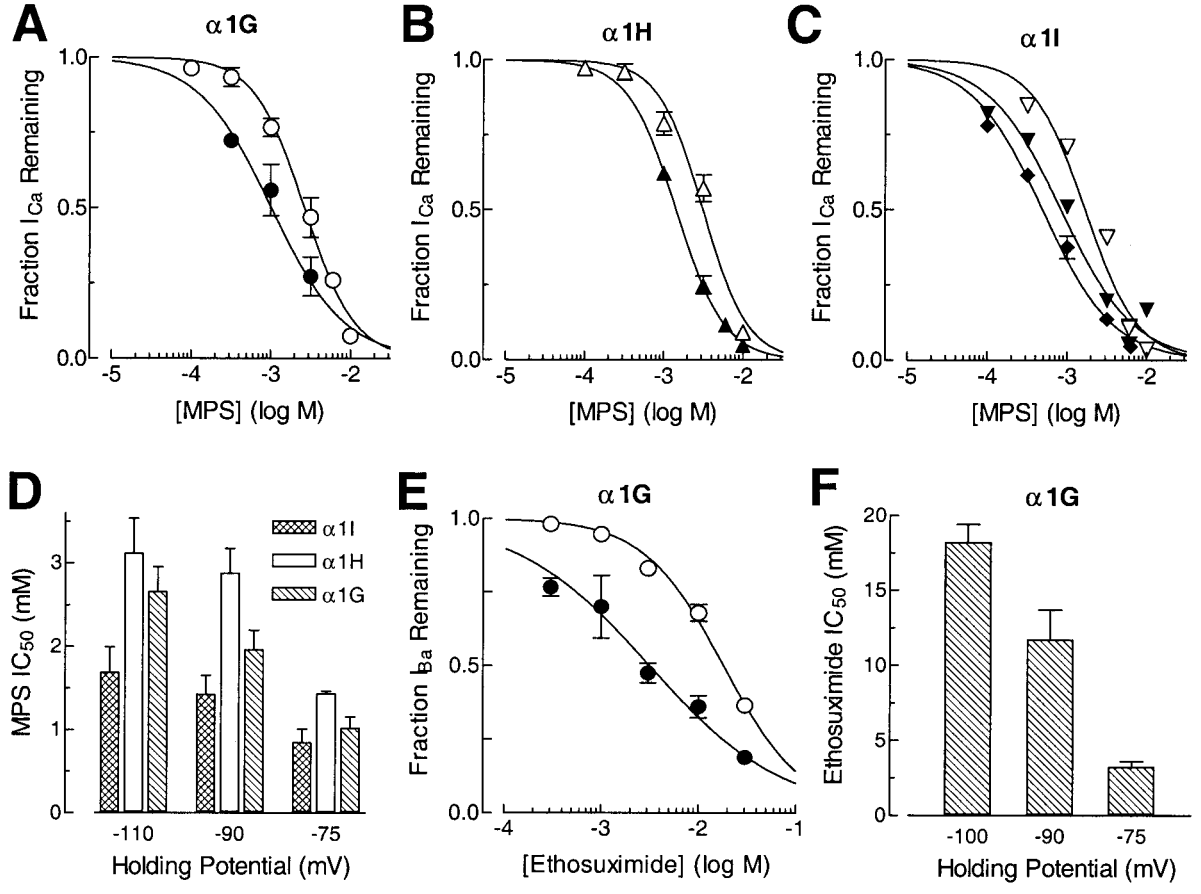

Fig. 7. Block by succinimide antiepileptic drugs is dependent on the holding potential. Dose-response relationship for MPS block of peak Ca^{2+} currents expressed by α1G (A), α1H (B), and α1I (C) channels. Data points were obtained by performing experiments like those described in Fig. 2 (5 mM Ca^{2+} as charge carrier), but using holding potentials of either −110 mV (open symbols) or −75 mV (filled symbols). Smooth lines represent the fit to the data using the Hill equation. The third set of data (♦) and the corresponding fit in C indicate the block of the current measured after 150 ms of depolarization using a holding potential of −75 mV. The calculated IC_{50} for each human T-type channel is plotted against holding potential in D. The corresponding relationship for ethosuximide block of α1G is shown in E; in this case, the holding potentials were −100 mV (○) and −75 mV (●), and the charge carrier was 10 mM Ba^{2+}. F, estimated ethosuximide IC_{50} values from the data shown in E and Fig. 1.

α1G channels obtained in response to the LTS protocol. The voltage protocol and the corresponding currents evoked from an HEK-293 cell expressing the human α1G subunit are shown in Fig. 8B. Ethosuximide reduced the peak LTS current 26 ± 7% (n = 3).

TABLE 2

Apparent affinities of MPS for resting and inactivated channels

Apparent affinities are reported in units of millimolar. K_R was measured as the block of the peak current elicited during pulses to −30 mV from a holding potential of −110 mV (Fig. 7). The shift in the midpoint of the steady-state inactivation curve (Δh) was measured using 30-s prepulses. K_I was calculated using eq. 1. K_{app} was measured as the block of the peak current elicited during pulses to −30 mV from a holding potential of −75 mV (Fig. 7). The value of h was determined for each cell by dividing the baseline current obtained with a holding potential of −75 mV with that obtained previously at −110 mV. K_I* was calculated using eq. 2. Data were obtained using 5 mM Ca^{2+} as charge carrier in the external solution except for Δh of α1H, which was measured in 10 mM Ba^{2+}.

	α1G	α1H	α1I
K_R	2.6	3.1	1.7
K_{app}	1.0	1.4	0.8
Δh	6.7	4.6	7.0
K_I	0.3	0.5	0.3
h	0.41	0.27	0.43
K_I*	0.5	1.2	0.5

Our last series of experiments focused on the structure-activity relationships of succinimide compounds. For this purpose, we investigated the effect of two succinimide analogs that are not antiepileptic drugs, succinimide and trimethylsuccinimide (TMS; α,α-dimethyl-β-methylsuccinimide). Figure 9A shows the effect of these two compounds at the indicated concentrations on the peak current of a cell expressing human α1H channels. Although tested concentrations were as high as 10 mM (in the case of succinimide) the block of the current was considerably weaker in comparison with the effect produced by MPS in these same cells. To illustrate this observation, the fractional block induced by succinimide, TMS, and MPS on the three human T-type Ca^{2+} channels is compared in Fig. 9B. For example, currents expressed for α1G were blocked 7.5 ± 4.7% by 10 mM succinimide, 30 ± 6% by 6 mM TMS, and 87 ± 2% by 3 mM MPS.

Analysis of Drug Block. Both ethosuximide and MPS produced measurable shifts in the h_∞ curve of all three cloned T-type channels. The shift in the mid-point of inactivation (Δh) can be used to calculate the apparent affinity of the drug for the inactivated state, K_I (Bean et al., 1983). Rearrangement of this equation yields

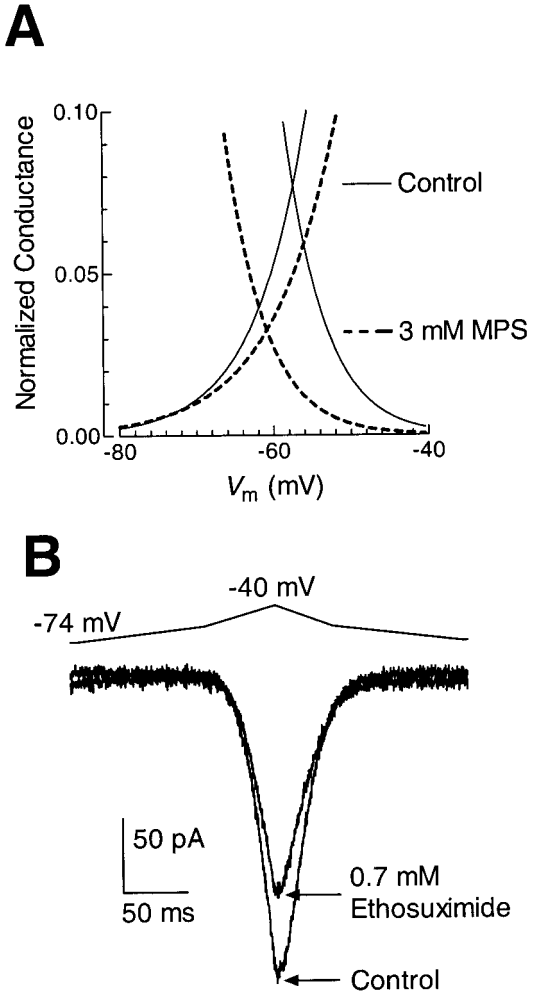

Fig. 8. Window currents are reduced by MPS and ethosuximide. A, overlapping area of voltage-dependent activation (lines originating from −80 mV) and steady-state inactivation curves (lines ending at −40 mV) for α1I channels in the absence (solid lines) and presence (dotted lines) of 3 mM MPS. All lines represent Boltzmann function fits to experimental data, which have been omitted for clarity purposes. MPS shifted the window current region ~5 mV to more negative potentials and reduced the size of the region ~50%. B, effect of 0.7 mM ethosuximide on Ca^{2+} current carried through human α1G channels obtained in response to a mock subthreshold LTS.

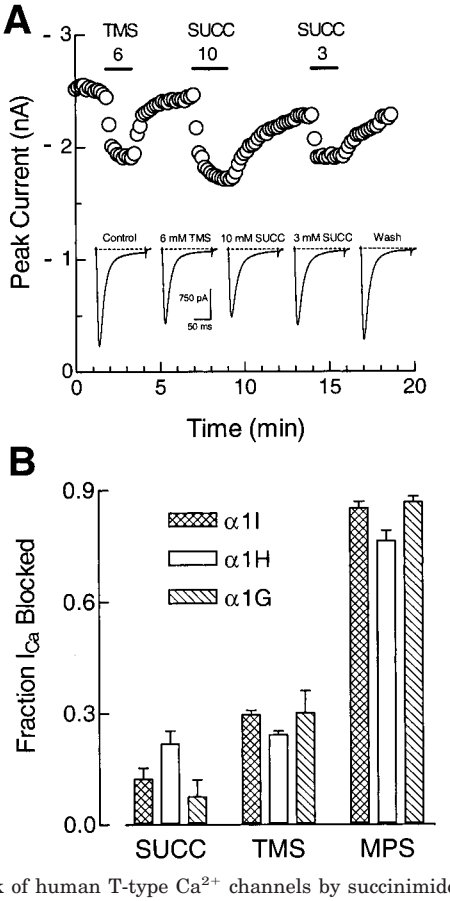

Fig. 9. Block of human T-type Ca^{2+} channels by succinimide nonantiepileptic drugs is much less potent. A, time course of peak current during exposure to trimethylsuccinimide and succinimide recorded from a cell expressing human α1H channels. The insets show representative Ca^{2+} current traces at −30 mV obtained under the indicated experimental conditions. The holding potential was −90 mV, and the charge carrier was 5 mM Ca^{2+}. B, fraction of Ca^{2+} current blocked at −30 mV by succinimide (10 mM), TMS (6 mM), and MPS (6 mM) for the three human T-type Ca^{2+} channels.

$$K_I = D/(((1 + D/K_R)/e^{\Delta h/k}) - 1)$$

where D represents the drug concentration, and k represents the slope of the h_∞ curve estimated from Boltzmann fits to the control data (Fig. 6). As shown in Table 2, the apparent affinity of MPS for inactivated channels is 5- to 6-fold greater than closed/rested channels. Similar results were obtained using the Δh induced by either 1 mM ethosuximide or MPS, as predicted by the model (Bean et al., 1983).

Due to the difficulties in estimating steady-state inactivation, we sought an independent method to estimate the affinity of the drugs for inactivated states. Dose-response relationships were measured using a holding potential that would cause approximately 50% of the channels to inactivate. In comparison with the data obtained using a holding potential of −110 mV (K_R), the potency of MPS to all three cloned T-type channels was increased approximately 2-fold by holding at −75 mV (K_{app}). Rearrangement of the equation derived by Bean, Cohen, and Tsien (Bean et al., 1983) yields

$$K_I^* = (1 - h)/(1/K_{app} - h/K_R)$$

The values for K_I^* using this method were similar to those calculated using eq. 1 (Table 2). The K_I^* of MPS for the inactivated state of α1G and α1I was approximately 0.5 mM, which indicates that MPS has a 4- to 5-fold higher affinity for inactivated channels. The h_∞ data in Table 2 for α1G were measured with 30-s prepulses, however, identical values of K_I^* were obtained using data from 0.3-s prepulses (Fig. 6). The K_I^* of MPS for the inactivated state of α1H was approximately 1.2 mM, which is only 2.6-fold higher than its affinity for closed/rested channels.

Ethosuximide also has different apparent affinities for closed/rested and inactivated/open channel states of the three cloned T-type channels (Figs. 5–7). The potency of ethosuximide to block α1G currents increased 6-fold by changing the holding potential from −100 to −75 mV. Using eq. 2, the K_I^* for the α1G inactivated states was calculated to be 1.9 mM. If a drug has higher affinity for inactivated states, then it may produce biphasic dose-response relationships when measured at holding potentials that induce inactivation (Cai et al., 1997). Since some α1G channels are inactivated at −90 mV, this may partially explain the biphasic response shown in Fig. 1, and the reduced Hill coefficient observed for the data obtained at −70 mV (Fig. 7). Ethosuximide block of α1H was also state-dependent. To calculate K_I for α1H we used eq. 1, Δh (Fig. 6), and the K_R value determined previously (Todorovic et al., 2000). The K_I of ethosuximide for the inactivated state of α1H was approximately 2.5 mM, which is 9-fold higher than its affinity for rested channels.

Discussion

This study describes the block of the cloned human T-type channels α1G, α1H, and α1I by succinimide antiepileptic drugs. Previous studies have used currents from rat or cat neurons (Coulter et al., 1989a, 1990a; Leresche et al., 1998; Todorovic and Lingle, 1998). The amino acid sequences of T-type channels are not identical between rats and humans. For example, the human α1G sequence is only 93% identical to the rat (Cribbs et al., 2000). More importantly, the pharmacology of human α1G also differs from the rat, being 2-fold more sensitive to block by nickel (Lee et al., 1999b; Cribbs et al., 2000). Considering the recent challenge to the hypothesis that the mechanism of action of ethosuximide involves blockade of T-type channels (Leresche et al., 1998), we decided to clone the human T-type channels and to test their sensitivity to these drugs. The main conclusions of this study are that block is observed at clinically relevant concentrations and that block is state-dependent.

The present results indicate that the apparent potency of ethosuximide and MPS is highly dependent on the recording conditions. Block is greater at threshold potentials, if measured at the end of a depolarizing pulse, and if the holding potential is more positive than −90 mV. T-type channels do not need to open to be blocked since there is block of peak currents in the first pulse after drug treatment in the absence of depolarizing pulses (data not shown). This result indicates that there is substantial block of channels in deep rested states. The affinities of the drugs for the closed/rested state were estimated by measuring the dose-response relationship at a holding potential of −110 mV (K_R, Fig. 7).

Open Channel Block. T-type channels, especially those formed by α1I (Fig. 4), do not inactivate completely during a long depolarizing pulse, leading to measurable persistent current, or plateau. This property is difficult to measure with native channels since plateau currents are often contaminated with currents through high voltage-activated Ca^{2+} channels (Sayer et al., 1993). Block of the T-type channel increased during the pulse, such that the plateau current was eliminated at doses that had little effect on the peak current. This had a dramatic effect on the apparent potency of ethosuximide, decreasing the apparent IC_{50} 13-fold to 0.6 mM (Fig. 5). However, at the pulse frequency used (0.1 Hz) there was little evidence of use dependence, indicating that channels recovered from this additional block when the holding potential was −90 mV or more negative. Two possible explanations for the effect on kinetics are 1) antiepileptic drugs accelerate the transition from open to inactivated states and/or 2) that there is additional block of open states.

Two lines of evidence suggest that there was open channel block. 1) Block of the peak current was greatest during test potentials to −50 mV, where inactivation was the slowest (Figs. 3 and 4). If block were only occurring by binding to inactivated states, then one would expect less block at these potentials. 2) At concentrations that blocked Ca^{2+} influx, there was little block of outward currents, which under these experimental conditions are carried by Cs^+ efflux (Fig. 3). Tail currents after pulses that elicited outward currents showed less block than after pulses that elicited inward currents (data not shown). This unidirectional block suggests that the drugs physically plug the pore and that outward currents partially unblock the channel.

Assuming that there is open channel block, then the on-rate of drug can be estimated from the current kinetics. To estimate this rate, the currents measured in the presence of MPS were fit with three exponentials, with two of the τ values fixed to that observed in control. Thus, the third τ corresponded to the difference between control and MPS and reflected the on-rate of drug during the pulse. From the data shown in Fig. 4 with α1I, this rate was calculated to be 25 ms, and this rate did not vary with the test potential between −30 and 20 mV. Similarly, we estimated the on-rate for 30 mM ethosuximide to be 15 ms during pulses to −30 mV. This

on-rate may explain the apparent voltage dependence of block, the effect on the voltage dependence of activation, and their effect on inactivation rate. Since cloned α1I channels activate very slowly during test pulses in the range of −60 and −30 mV (e.g., at −50 mV, τ_{act} = 44 ± 6 ms), MPS block is nearly complete before the peak is reached. At more positive potentials, the peak is reached before block is complete (e.g., at +10 mV, τ_{act} = 4.0 ± 0.3 ms), and block occurs during the inactivating phase, causing an apparent increase in the rate of inactivation. Therefore, block of the peak current is greatest at negative potentials and decreases at more positive test potentials, causing an apparent shift in the position of *I-V* curve. Alternatively, MPS block may be inherently voltage-dependent [even though it is uncharged at physiological pH (Huffman, 1982)]; it may alter the voltage dependence of gating; and it may affect the transition rate between open and closed channels. The observation that block is greatest at −50 mV where MPS does not affect the inactivation rate (Fig. 4) favors the simpler hypothesis that block occurs on channels that are near or in the open state.

Preferential Binding to Inactivated States. Two experiments indicated that succinimide antiepileptic drugs preferentially blocked inactivated states of the T-type channel: 1) the drugs shifted the steady-state inactivation curve, and 2) potency was enhanced by depolarizing shifts in the holding potential. Analysis of these experiments allowed independent calculations of the drug affinity for inactivated states (Table 2). For most channel subtypes these two methods produced similar estimates for K_I. The selectivity for inactivated states over rested states of the channel was modest, ranging from 3- to 9-fold. Changing the holding potential also increases the potency of ethosuximide to block a mouse α1G (Lacinova et al., 2000).

Ethosuximide Sensitivity of T-Type Channels. Studies on the dose response of human α1G indicated that therapeutically relevant concentrations of ethosuximide were capable of producing modest block (~15%). Since a small degree of run-down could complicate the measurement of such a small effect, the experiment was repeated using a variety of set-ups, external solutions (10 mM Ba^{2+} or 5 mM Ca^{2+}), and flow rates. In each case, there was evidence for a plateau in the response to concentrations ranging from 0.1 to 1 mM. The original observation that ethosuximide could inhibit T-type channels in isolated rat thalamic neurons also had similar results; block was partial (32%) and appeared to plateau at 2 mM (Coulter et al., 1989a). Higher concentrations were not reported in that study. One study using isolated rat dorsal root ganglion (DRG) neurons also found evidence for ethosuximide block at low concentrations; block was complete and had an apparent IC_{50} of 0.007 mM (Kostyuk et al., 1992). Subsequent studies on rat DRG neurons failed to confirm block at concentrations below 1 mM, but did find block at higher concentrations (Todorovic and Lingle, 1998). The apparent IC_{50} was 23 mM. Recent studies on isolated and slice preparations of rat thalamic neurons found no effect of 0.75 mM ethosuximide on the T-type current (Leresche et al., 1998). Although there are many differences in the recording conditions used in these studies, one notable difference is the holding potential. Since studies that reported no effect used very negative holding potentials (−110 mV), we suggest these experiments tested block of rested channels, which are less sensitive to block. Preferential block of inactivated states can produce biphasic dose-response curves (Cai et al., 1997). Therefore, the partial block originally observed in thalamic neurons (Coulter et al., 1989a) may have corresponded to block of inactivated channels.

Sensitivity of T-Type Channels to MPS. In contrast to the discrepant findings with ethosuximide, there is general agreement that MPS is a potent blocker of rat T-type currents (Coulter et al., 1990a; Huguenard and Prince, 1992; Todorovic and Lingle, 1998). The present results indicate that MPS is also a potent blocker of all three cloned human T-type channels. Block was complete and had apparent IC_{50} values between 2 and 3 mM. Similar results were obtained previously with the rat α1G and human α1H (Todorovic et al., 2000). Block of thalamic T-type currents was also complete and displayed a similar IC_{50} of 1.1 mM (Coulter et al., 1990a). Three millimolar MPS blocked 45% of the T-type currents recorded from thalamic neurons isolated from either the ventrobasal or the reticular nuclei (Huguenard and Prince, 1992). In situ hybridization studies indicated that α1G is the predominant isoform expressed in the rat ventrobasal nucleus, whereas α1H and α1I were expressed in the reticular nucleus (Talley et al., 1999). Therefore, MPS blocks both native and cloned channels with similar potency. In contrast, MPS block of DRG T-type currents was only partial (~30%), but occurred at lower doses (IC_{50} ~ 0.18 mM; Todorovic and Lingle, 1998; Todorovic et al., 2000). It is unclear why MPS produces only partial block of DRG currents, but complete block of currents from either thalamic neurons or cloned channels.

Structure-Activity Relationships of Succinimides. Tetramethylsuccinimide has been reported to induce seizures in mice (Klunk et al., 1982). It was found to have no effect on either thalamic T-type currents (1 mM) or their block by MPS (Coulter et al., 1990a). This result rules out a single binding site where compounds have agonist or antagonist properties (Klunk et al., 1982). Evidence indicates that this second site is on γ-aminobutyric acid receptors (Coulter et al., 1990b). We were unable to test its effect because it is no longer commercially available. In contrast to ethosuximide, the trimethylsuccinimide analog (α,α-dimethyl-β-methylsuccinimide) has been shown to be ineffective at reducing spontaneous epileptiform discharges in rat thalamocortical slices (1 mM; Zhang et al., 1996). The present study shows that trimethylsuccinimide is similarly ineffective at blocking T-type currents. The base molecule, succinimide, has been reported to be devoid of anticonvulsant activity (Ferrendelli and Kupferberg, 1980). It is also ineffective at blocking rat thalamic T-type currents (0.5 mM; Coulter et al., 1989b), and at blocking epileptiform discharges in thalamocortical slices (1 mM; Zhang et al., 1996). The present results indicate it was also ineffective in blocking the cloned human T-type channels. These results confirm the correlation that only succinimides that are capable of blocking T-type channels are effective anticonvulsants.

The present studies also show that MPS is 10-fold more potent than ethosuximide. If the mechanism of action of these drugs involves blockade of T-type channels, then MPS should be more potent. Therapeutic plasma levels are consistent with this hypothesis; MPS concentrations are approximately 0.1 mM, whereas ethosuximide concentrations range between 0.3 and 0.7 mM (Strong et al., 1974; Browne et al., 1975; Porter et al., 1979; Wilder and Buchanan, 1981). Sim-

ilar differences in potency were noted in the prevention of pentylenetetrazol-induced seizures in rats (Chen et al., 1963). More striking were the differences in potency observed in the prevention of maximal electroshock seizures in mice, where MPS was 25-fold more potent than ethosuximide (Chen et al., 1963). This result suggested that methsuximide might be effective in a wider range of epilepsies. Studies have shown that it was also effective in the treatment of complex partial seizures (Wilder and Buchanan, 1981; Browne et al., 1983). Unfortunately this drug had a small therapeutic window, producing too many undesirable side effects at concentrations just above those required for seizure prevention (Browne et al., 1983). Therefore, drugs that block T-type channels may be useful in a wide variety of seizures. Evidence exists for the corollary of this hypothesis, drugs such as phenytoin and zonisamide, which are useful in the treatment of generalized tonic-clonic and complex partial seizures, also block T-type channels (Twombly et al., 1988; Kito et al., 1996; Todorovic et al., 2000).

Therapeutic Implications of State-Dependent Block. T-type Ca^{2+} channels mediate low threshold Ca^{2+} spikes in a number of neurons (Crunelli et al., 1989; Suzuki and Rogawski, 1989). Such spikes play an important pacemaker role in the initiation of burst firing of thalamic neurons, and such firing underlies the spike-wave activity observed in the EEG during absence seizures (McCormick and Bal, 1997; Huguenard, 1999). Similar synchronized oscillations occur during slow wave sleep. However, they are not observed during rapid eye movement sleep or in wakeful states. In these states, the thalamus fires single Na^+-dependent spikes (tonic mode), because the membrane potential of the neuron is depolarized to such an extent that T-type channels are inactivated (Steriade and Llinas, 1988). Therefore, clinically relevant targets for antiepileptic drugs include T-type channels in inactivated states, and blocking these states may prevent the transition from tonic to burst firing. The present results show that both ethosuximide and MPS blocks are enhanced by holding the membrane at a potential that is similar to the resting potential of many neurons. We also found that block was greatest at threshold potentials. These two effects combine such that therapeutically relevant concentrations can cause significant block of current elicited with mock LTS waveforms (Fig. 8).

The conclusion that a drug blocks at therapeutically relevant concentrations is usually based on comparisons of the apparent IC_{50} for block observed in vitro with the plasma concentrations reached in vivo. However, since T-type channels are pacing Na^+ channels that fire in an all-or-nothing manner, even 10% block of the T-type current may lead to a pronounced effect on firing (Huguenard and Prince, 1994; Narahashi, 2000).

T-type window currents are thought to play an important role in determining neuronal excitability (Williams et al., 1997). Due to their ability to shift inactivation to more negative potentials, we found that ethosuximide and MPS reduced the window current region. The window region is close to the resting membrane potential of many resting neurons. Mutations in Na^+ channels that affect window currents have profound effects on excitability, leading to a number of diseases including epilepsy (for review, see Lehmann-Horn and Jurkat-Rott, 1999). Due to the similarities in T-type channel gating, it is interesting to speculate that mutations in human T-type channel genes may also produce hereditary disease. Over-activity of T-type channels in the thalamic reticular nucleus has been observed in a rat model of absence epilepsy (Tsakiridou et al., 1995), and this has been attributed to an increase in expression of α1H mRNA (Talley et al., 2000). It should be noted that persistent currents are particularly difficult to measure with native channels, since neurons contain other Ca^{2+} channels that are capable of producing plateau currents and that can activate even during relatively negative test potentials (−30 mV; Avery et al., 1996). Although α1G and α1H display persistent currents, these currents are much more prominent in α1I channels (Figs. 2 and 4). Ethosuximide was 13-fold more effective in blocking these persistent currents than block of the peak current, displaying an apparent IC_{50} of 0.6 mM. Due to the expression of α1I in the reticular nucleus of the thalamus (Talley et al., 1999) and the role of this nucleus in controlling thalamic oscillations (Steriade and Llinas, 1988), the present results are consistent with the hypothesis that blockade of T-type channels may underlie the therapeutic usefulness of succinimide antiepileptics. T-type channel blockade may also be useful in a wide variety of neurological disorders that are caused by thalamocortical dysrhythmias, such as neuropathic pain (Llinas et al., 1999).

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