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Anticonvulsants But Not General Anesthetics Have Differential Blocking Effects on Different T-Type Current Variants

SLOBODAN M. TODOROVIC, EDWARD PEREZ-REYES, and CHRISTOPHER J. LINGLE

Washington University School of Medicine, Department of Anesthesiology, St. Louis, Missouri (S.M.T., C.J.L.) and Department of Pharmacology, University of Virginia, Charlottesville, Virginia (E.P.-R.)

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ABSTRACT

The sensitivity to anticonvulsants and anesthetics of Ca^{2+} currents arising from α1G and α1H subunits was examined in stably transfected HEK293 cells. For comparison, in some cases blocking effects on dorsal root ganglion (DRG) T currents were also examined under identical ionic conditions. The anticonvulsant, phenytoin, which partially blocks DRG T current, blocked α1G current completely but with weaker affinity (~140 μM). Among different cells, α1H current exhibited either of two responses to phenytoin. In one subpopulation of cells, phenytoin produced a partial, higher affinity block (IC_{50} ~7.2 μM, maximum block ~43%) similar to that in DRG neurons. In other cells, phenytoin produced complete, but lower affinity, blockade (IC_{50} ~138 μM, maximum block ~89%). Another anticonvulsant, α-methyl-α-phenylsuccinimide (MPS), blocked DRG current partially, but blocked both α1G and α1H currents completely with weaker affinity (~1.7 mM). These data suggest that higher affinity blockade of T-type currents by phenytoin and MPS may require additional regulatory factors that can contribute to native T-type channels. In contrast, anesthetics blocked all T current variants similarly and completely. Block of α1G current by anesthetics had the following order of potency: propofol (IC_{50} ~20.5 μM) > etomidate (~161 μM) = octanol (~160 μM) > isoflurane (~277 μM) > ketamine (~1.2 mM), comparable with results on DRG T currents. Barbiturates completly blocked α1G currents with potency [thiopental (~280 μM), pentobarbital (~310 μM), phenobarbital (~1.54 mM)] similar to that in DRG cells. The effects of propofol, octanol, and pentobarbital on α1H currents were indistinguishable from effects on α1G currents.

Among voltage-dependent Ca^{2+} channels, T-type channels, by virtue of their activation at relatively negative potentials, are believed to play a crucial role in the control of cell firing rates (Llinas, 1988; Huguenard, 1996). Major proposed roles for T-type channels in neurons include promotion of Ca^{2+}-dependent burst firing, low-amplitude intrinsic neuronal oscillations, promotion of Ca^{2+} entry, and boosting of synaptic signals (Huguenard, 1996). Furthermore, T-type currents appear to play a role in seizure susceptibility and initiation (Chung et al., 1993; Huguenard and Prince, 1994; Tsakiridou et al., 1995).

T currents in different native tissues appear to share a number of common features that help distinguish them from high-voltage-activated Ca^{2+} currents. These include relative permeation by Ca^{2+} and Ba^{2+} (Carbone and Lux, 1987a,b), more negative activation voltages (Carbone and Lux, 1984), similarity in deactivation and inactivation kinetics, and generally a greater sensitivity to blockade by Ni^{2+} over Cd^{2+} (Fox et al., 1987). However, despite these similarities, differences in both kinetic behavior of some native T currents (Huguenard and Prince, 1992; Huguenard et al., 1993) and pharmacological properties (e.g., Herrington and Lingle, 1992; review by Huguenard, 1996; Todorovic and Lingle, 1998) have been observed. Diversity in native T-type Ca^{2+} channel function might arise from tissue-specific expression of the α1G, α1H, and α1I subunits now known to encode T-type Ca^{2+} channels (Perez-Reyes et al., 1998; Cribbs et al., 1998). For example, the α1H subunit appears to be the primary contributor to T-type current in sensory neurons (Talley et al., 1999). In addition, T-like Ca^{2+} currents in native cells might also arise from other mechanisms. For example, Meir and Dolphin (1998) reported that multiple subunits of high-voltage-activated (HVA) channels can under certain conditions give rise to T-type currents.

One difficulty in evaluating pharmacological studies of T currents in native cells is that for some agents the conditions under which a compound has been investigated have not been identical. Similarly, in many cases, full concentration-response curves for effects on T currents have not been defined. As a consequence, it has not been possible to determine

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ABBREVIATIONS: HVA, high-voltage-activated; MPS, methyl-phenyl-succinimide; ω-CgTx-GVIA, ω-conotoxin GVIA; ω-CgTx-MVIIC, ω-conotoxin MVIIC; DMSO, dimethyl sulfoxide; DRG, dorsal root ganglion; phenytoin, 5,5-diphenylhydantoin; ES, ethosuximide; VPA, valproic acid.

the extent to which different T currents share a particular pharmacological profile or represent distinct current variants. Incomplete concentration-response information also makes it unclear whether effects seen in vitro correlate with the concentrations of a particular drug in plasma necessary to achieve therapeutic effects. In those cases where more complete information about the sensitivity of a T current to particular pharmacological agents is available, some clear differences in pharmacological properties are revealed. Two sets of agents for which quite disparate blocking effects on T currents among different cells have been reported include divalent cations, including Ni^{2+} and Cd^{2+} (Kaneda and Akaike, 1989; Herrington and Lingle, 1992; Ye and Akaike, 1993; Todorovic and Lingle, 1998) and anticonvulsants (Herrington and Lingle, 1992; Gross et al., 1997; Todorovic and Lingle, 1998). With the availability of the cloned α1G, α1H, and α1I subunits encoding T-type currents, it is now possible to assess directly the pharmacological sensitivities of the α subunits.

Toward this end, we examine here the sensitivity of α1G and α1H currents to anesthetics and anticonvulsants. Where the blocking effect of a compound is found to differ from previously described effects on T currents in dorsal root ganglion (DRG) neurons (Todorovic and Lingle, 1998), we have re-examined the blocking effect of that compound on DRG T current under identical ionic and recording conditions. We observed that, for the anesthetics examined, blocking effects are essentially identical for α1G, α1H, and native DRG T currents. However, for the anticonvulsants, phenytoin and α-methyl-α-phenylsuccinimide (MPS), blockade of α1G current differed from blockade of native DRG T current. Specifically, in contrast to blockade of DRG T current where block was of higher affinity, but only partial, these agents blocked α1G current completely but with lower affinity. Furthermore, for α1H current, phenytoin produced two different kinds of responses, one similar to its effects on DRG T current and one similar to the effects on α1G current. No physiological differences in the α1H currents underlying these responses were observed. This raises the possibility that either post-translational modification of α1H subunits or an additional accessory subunit may contribute to the pharmacological sensitivity of native T-type currents.

Experimental Procedures

Cell Preparation. HEK 293 cells were stably transfected with either α1G or α1H constructs as described previously (Lee et al., 1999). Cells were typically used 1 to 3 days after plating. Average cell capacitance (C_m) was 20.9 ± 5.7 pF, and the average series resistance (R_s) was 5.29 ± 2.08 (n = 34). For DRG neurons, 100- to 300-g male rats (Sprague-Dawley) were used as we described elsewhere (Todorovic and Lingle, 1998). Eight to ten DRG from thoracic and upper lumbar regions were dissected out and incubated at 36°C for 60 to 90 min in Tyrode's solution (140 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, adjusted to pH 7.4 with NaOH) containing 5 mg/ml collagenase (type I, Sigma Chemical Co., St. Louis, MO) and 5 mg/ml dispase II (Boehringer-Manheim, Indianapolis, IN). Single neuronal cell bodies were obtained by trituration in Tyrode's solution at room temperature. Cells were kept at room temperature and used for electrophysiology within 4 to 6 h from dissociation. For recordings, neuronal cell bodies were plated onto a glass cover-slip and placed in a culture dish that was perfused with external solution. All data were obtained from smaller diameter DRG neurons (21–27 μm) without visible processes. The average C_m for DRG cells was 14.6 ± 2.5 pF, and the average R_s was 6.4 ± 1 (n = 12).

Electrophysiological Methods. Recordings were made with the standard whole-cell voltage-clamp technique (Hamill et al., 1981). Electrodes were prepared from microcapillary tubes (Drummond Scientific Company, Broomall, PA), coated with Sylgard (Dow Corning, Midland, MI) and fire-polished. Pipette resistances were 2 to 5 MΩ. Voltage commands and digitization of membrane currents were done with Clampex 5.5 of the pClamp software package (Axon Instruments, Foster City, CA) running on an IBM-compatible computer. Membrane currents were recorded with an Axopatch 200A patch-clamp amplifier (Axon Instruments). Typically, cells were held at −90 mV and depolarized to −35 mV every 20 s to evoke inward currents. Data were analyzed using Clampfit (Axon Instruments). Currents were filtered at 5 kHz. Reported series resistance values were taken from the reading of the amplifier. In all experiments, series resistance was compensated 60 to 80%. All reported membrane potentials are nominal values. All experiments were done at room temperature (20–23°C). In most experiments, leakage subtraction was achieved with a P/5 on-line subtraction protocol. Error bars indicate standard deviations of multiple determinations obtained from at least five different cells.

Analysis of Current Blockade. The percentage reduction in peak T current at a given blocker concentration was used to generate concentration-response curves. For each concentration-response curve, all points are averages of multiple determinations obtained from at least five different cells. On all plots, vertical bars indicate standard deviation. Mean values on concentration-response curves were fit to the following function:

$$PB([\text{drug}]) = PB_{max}/(1 + (IC_{50}/[\text{drug}])^h) \quad (1)$$

where PB_{max} is the maximal percent block of peak T current, the IC_{50} is the concentration that produces 50% of maximal inhibition, and h is the apparent Hill coefficient for blockade. In the case of isoflurane, because each application of blocker involved a separate determination of anesthetic concentration, all data points were fit with the above function. Fitted values are typically reported with 95% linear confidence limits. Fitting was done with Origin 3.7 (Microcal Software, Northhampton, MA).

Solution Exchange Procedures. The solution application system consisted of multiple, independently controlled glass capillary tubes, whereas solution was removed from the other end of the chamber with the use of constant suction. Manually controlled valves accomplished switching between solutions. Test solutions were maintained in closed, weighted all-glass syringes (to avoid saline evaporation and loss of volatile drugs) and allowed to fall by gravity. Changes in Ca^{2+} current amplitude in response to rapidly acting drugs or ionic changes were typically complete in 10 to 20 s. Switching between separate perfusion syringes, each containing control saline, was without effect on Ca^{2+} current amplitude. No dependence on the order of presentation or desensitization with repeated applications was observed for any of the pharmacological agents.

Solutions and Current Isolation Procedures. The standard extracellular saline for recording of Ca^{2+} current contained (in millimolar): 160 TEA-Cl, 10 HEPES, 2 $CaCl_2$, adjusted to pH 7.4 with TEA-OH, osmolarity 316 mOsm. Cells were generally maintained in a Tyrode's solution until seal formation, at which time the bath solution was switched to the Ca^{2+} saline. Internal solution consisted of (in millimolar) 110 Cs-methane sulfonate, 14 phosphocreatine, 10 HEPES, 9 EGTA, 5 Mg-ATP, and 0.3 Tris-GTP, pH adjusted to 7.15 to 7.20 with CsOH (standard osmolarity: 300 mOsm). When this internal saline was used for recording of T current in DRG cells, most of the HVA current in these cells was blocked by preincubating cells with 1 μM ω-CgTx-GVIA, 2 μM ω-CgTx-MVIIC and by including 5 μM nifedipine in the external solution to block N, P, Q, and L types of HVA current. Because in control experiments this effect was irreversible for up to 60 min, we routinely preincubated every slide

with these toxins and recorded within this time frame. In most cells included in this study, blockade of L-, N-, P-, and Q-type currents was sufficient to allow investigation of T current in virtual isolation. Because of the possibility of some residual HVA current contamination, all measurements of T current amplitude in DRG cells were made from the peak of the inward current to the current remaining at the end of a 200-ms test step. Typically, the residual current at 200 ms was indistinguishable from leak current.

Drugs and Chemicals. ω-Conotoxin GVIA (ω-CgTx-GVIA), ω-conotoxin MVIIC (ω-CgTx-MVIIC) and *tert*-butylbicyclo[2.2.2]phosphorothionate (TBPS) were obtained from RBI (Natick, MA), and etomidate powder and isoflurane were obtained from Abbott (Abbott Park, IL). All other chemicals were obtained from Sigma or Aldrich Chemicals (Milwaukee, WI).

Drug Preparation. Stock solutions of propofol (50 mM), etomidate (300 mM), MPS (1 M), TBPS (50 mM), and phenytoin (100 mM) were prepared in dimethyl sulfoxide (DMSO) and kept at 4°C until use. 0.1% DMSO had no effect when tested alone in DRG cells or HEK cells with either α1G or α1H constructs.

The maximal DMSO concentration used in experiments with α1G currents and DRG T currents was 0.6%, and this did not have any effect on inward currents in these cells ($n = 4$). In HEK cells with α1H constructs, 0.3% DMSO reduced current amplitude by 8 ± 3.3% ($n = 11$), and 0.6% DMSO reduced current amplitudes by 16.8 ± 8% ($n = 9$). Therefore, we did not use concentrations higher than 0.3% DMSO in our experiments with α1H currents. Ethosuximide (ES) and octanol were prepared in extracellular solutions and sonicated. All barbiturates were prepared in stock solutions in 0.1 N TEA-OH; the pH of the final extracellular solution was adjusted with HCl to 7.4. Isoflurane solutions were prepared from saturated saline solutions, and the final concentration in the bath was determined with gas chromatography for each experiment (Evers et al., 1986). Stocks of other drugs were made by dissolving the compound in either the extracellular saline or distilled water. All test solutions were prepared the day of the experiment by diluting the stock solutions with the appropriate amount of extracellular saline. ES was added to the extracellular saline without osmotic adjustments. In separate control experiments, application of extracellular saline with additional sucrose (100 mM) had no effect on α1G-based current amplitude (4.3 ± 1.5% block; mean ± S.D., three cells).

Results

Here, we have studied the effects of anesthetic and anticonvulsant compounds on α1G and α1H currents in HEK cells. All of the compounds have been previously systematically examined for effects on native T currents in adult rat DRG neurons (Todorovic and Lingle, 1998) and, to some extent, on T current in GH3 cells (Herrington and Lingle, 1992). Some information on the blocking effects of these agents on high-voltage-activated Ca^{2+} currents is also available, in particular, for blockade of α1E current (Nakashima et al., 1998). For MPS and phenytoin, we describe differences between effects on α1G current and previous results on DRG cells (Todorovic and Lingle, 1998). Because we were concerned that the observed differences could be due to the different charge carriers used (10 mM Ba^{2+} versus 2 mM Ca^{2+} in this study), we have also repeated pharmacological experiments on DRG neurons under ionic conditions identical with those used to study α1G and α1H currents.

Effects of Anticonvulsants on α1G and α1H Current. A number of studies have indicated that various anticonvulsants, including succinimides (Coulter et al., 1989, 1990) and phenytoin (Twombly et al., 1988) have blocking effects on T-type Ca^{2+} current. Work on thalamic relay neurons (Coulter et al., 1989, 1990) suggested that the anticonvulsant drug, ES, was a blocker of T current within clinically effective concentrations. This effect was believed to contribute to its efficacy in petit mal seizures. More recent work now suggests that T current in thalamic neurons is not blocked by ES (Leresche et al., 1998), and other studies have shown that effects of ES on various T currents only occur at concentrations well beyond those obtained clinically (Herrington and Lingle, 1992; Todorovic and Lingle, 1998). However, the anticonvulsants, phenytoin and MPS, do have some blocking effects on DRG T currents at clinically relevant concentrations (Todorovic and Lingle, 1998). Furthermore, because of the proposed role of thalamic T currents in the initiation of epileptic activity (Chung et al., 1993; Huguenard and Prince, 1994; Tsakiridou et al., 1995), the effects of possible effects of anticonvulsants on T current variants remains of interest. Here, we have therefore examined the effects of phenytoin, MPS, and ES on α1G and α1H currents.

Phenytoin Differentially Affects Native DRG T Current and α1G and α1H Current. The effect of phenytoin on α1G current is illustrated in Fig. 1. 10 μM phenytoin produced a small reduction in α1G current (left traces in Fig. 1A), whereas 300 μM phenytoin produced a more complete blockade (right traces in Fig. 1A). The effects of three different phenytoin concentrations on peak α1G current are illustrated in Fig. 1B. The concentration dependence of phenytoin block of α1G current is summarized in Fig. 1E, indicating that with sufficiently high concentrations of phenytoin a nearly complete block of α1G current can be achieved.

The ability of higher phenytoin concentrations to produce nearly complete block of α1G current (Fig. 1E) differs from the effect of phenytoin on DRG currents described in our recent work (Todorovic and Lingle, 1998). In the earlier study 10 mM Ba^{2+} was used as the extracellular permeant ion, whereas here we have used 2 mM Ca^{2+} to study α1G current. Therefore, we re-examined the blocking effects of phenytoin on DRG T current using 2 mM extracellular Ca^{2+}. We found that the effects of phenytoin on DRG T current with 2 mM Ca^{2+} as the extracellular permeant cation are essentially identical with previous results with 10 mM Ba^{2+} (Todorovic and Lingle, 1998). The effect of 10 and 300 μM phenytoin on DRG T current is shown in Fig. 1C. 10 μM phenytoin produces a larger fractional reduction of DRG T current than of α1G current. However, 300 μM phenytoin is less effective at blocking DRG T current than α1G current (compare Fig. 1C with 1A). The similarity in the blocking effect of 300 and 600 μM phenytoin on DRG T current is shown in Fig. 1D. The concentration dependence of the blocking effect of phenytoin on either DRG T current or α1G current is shown in Fig. 1E. The DRG T current clearly differs from the α1G current in its sensitivity to phenytoin: the DRG T current is inhibited at lower concentrations but is incompletely blocked at the highest concentrations. For DRG T current, the IC_{50} was 7.8 ± 1.4 μM with a Hill coefficient (h) of 1.9 ± 0.5 and maximal block of 58 ± 3%. For α1G current, the IC_{50} was 140 ± 65 μM (h = 1.2 ± 0.4) with a fitted maximal block of 100 ± 18%. The higher affinity, partial block by phenytoin is similar to its effect on N1E-115 neuroblastoma cells (Twombly et al., 1988).

Because the α1H subunit appears to be the primary T-type current subunit in sensory ganglia (Talley et al., 1999), we were interested in whether the observed discrepancy in pharmacological sensitivities between DRG T currents and α1G

currents might reflect the predominance of α1H current in DRG cells. Surprisingly, when we examined the effects of phenytoin on α1H current in the stably transfected HEK293 cells, phenytoin produced two distinct types of blocking effects on α1H current.

Figure 2A shows an example of the effect of 10 and 300 μM phenytoin on α1H currents in a cell (13 of 23 cells) in which increases in phenytoin concentration from 100 to 300 μM produced an increase in block (Fig. 2B), whereas 10 μM phenytoin had negligible blocking effects. In contrast, in 10 of 23 cells 10 μM phenytoin produced a distinct blocking effect on α1H current (Fig. 2C), whereas blockade by phenytoin appeared to reach a limiting value over the range of 30 to 300 μM (Fig. 2D).

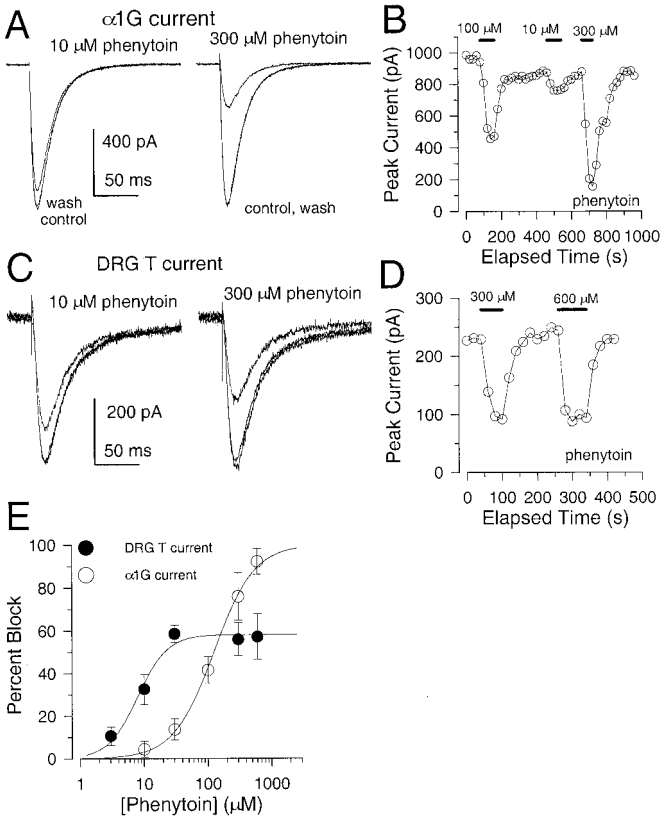

Fig. 1. The anticonvulsant phenytoin has differential effects on α1G current in HEK cells and T currents in native DRG cells. A, traces show block by 10 μM phenytoin (left) and 300 μM phenytoin (right) of α1G current in HEK293 cells. B, plot of a temporal record of the reduction of peak α1G current by multiple applications of phenytoin, indicating the substantial difference in block produced by 100 and 300 μM phenytoin. Note that 300 μM phenytoin almost completely blocks the α1G currents. C, traces show block of T currents in DRG currents under ionic conditions identical with those used in A. Left traces show effect of 10 μM phenytoin, whereas right trace shows blocking effect of 300 μM phenytoin from the same cell. Note that 10 μM phenytoin blocked a larger percentage of DRG T current, and 300 μM phenytoin blocked less, in comparison to the α1G current. D, peak T current amplitude during the course of an experiment on a DRG neuron shows that 300 and 600 μM phenytoin produce a similar and partial block. Horizontal bars indicate time of application. E, the concentration dependencies of phenytoin inhibition of DRG T current (filled symbols) and α1G current (open symbols) are displayed. Vertical lines indicate SE and solid lines are best fits of eq. 1. For DRG T current, the fitted maximal block is 58% with little difference observed between 30 and 600 μM phenytoin. For α1G current, nearly 100% block is achieved with 600 μM phenytoin with an IC_{50} of 124.2 $\pm$ 9.1 μM (h = 1.4; 7 cells). However, the IC_{50} for block of DRG T current (IC_{50} of 8.0 $\pm$ 2.9 μM; h =1.9; 8 neurons) is more than 10-fold less than for block of α1G current.

Because phenytoin qualitatively appeared to produce different types of blockade in the two types of cells, we sought criteria that might justify separation of the cells into groups. In one set of experiments, the blocking effects of 10, 30, 100, and 300 μM phenytoin were examined. In a subsequent set of experiments, we tested 100, 300, and 600 μM. For the first set of cells, for each cell we determined the fold increase in block resulting from increasing the phenytoin concentration from 30 to 300 μM. In five of these cells, the increase to 300 μM resulted in an increase of 1.2- $\pm$ 0.3-fold (mean $\pm$ S.D.; maximum increase in any cell: 1.5-fold). In eight other cells, the increase to 300 μM resulted in a fold increase of 4.0 $\pm$ 1.9 (minimum increase in any cell: 2.7-fold). In a second set of cells, the fold increase block that resulted from raising the phenytoin concentration from 100 to 600 μM was determined. For five cells, the fold increase was 1.1 $\pm$ 0.3 (maxi-

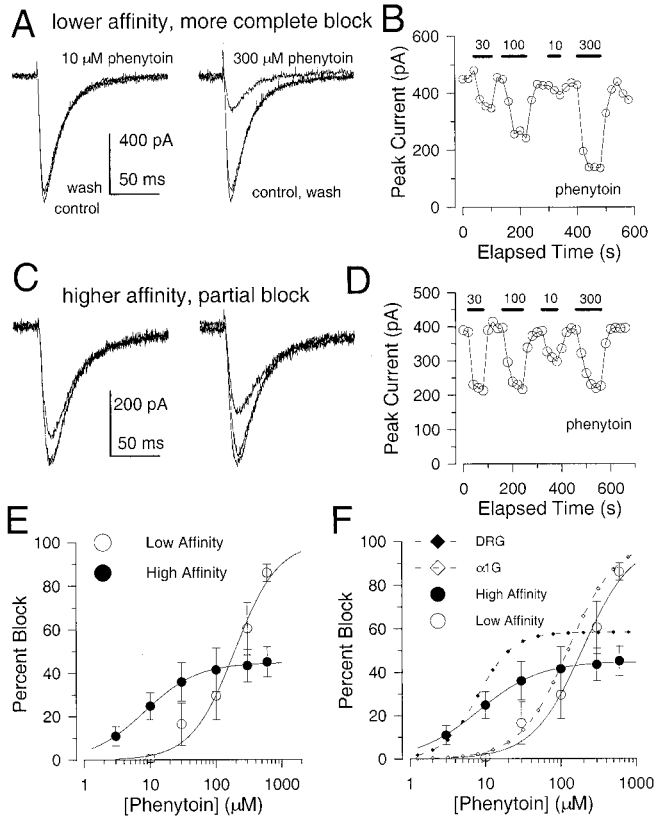

Fig. 2. α1H currents in HEK293 cells exhibit either of two types of sensitivities to phenytoin. A, traces show effects of 10 μM (left) and 300 μM (right) phenytoin on α1H current in a cell in which blockade by phenytoin increased substantially over the range of 100 to 300 μM. B, the temporal record of the inhibition of peak α1H current by phenytoin illustrates the progressive increase in block with increasing concentrations. Horizontal bars indicate time of drug application. C, current traces show effects of 10 (left) and 300 (right) μM phenytoin, respectively, on inward α1H current in another HEK cell. Note that 10 μM phenytoin produced more block (about 25%) and 300 μM phenytoin blocked less block (only about 40%) than for the cell in A. D, the temporal record of the effects of phenytoin on peak current for the cell in C shows that for this cell there is little difference in inhibition by phenytoin over the range of 30 to 300 μM with maximal block occurring at less than 50%. E, concentration dependencies for block by phenytoin is displayed for the two categories of cells: those in which phenytoin produces a partial, but higher affinity, block (IC_{50} = 8.3 μM; maximum = 44.8%; n = 10 cells) and those in which phenytoin produces a complete, but lower affinity, block (IC_{50} = 192 μM; n = 13 cells). F, concentration dependencies of block of α1H current by phenytoin are compared with block of DRG current and α1G current by phenytoin (from Fig. 1E).

mum increase: 1.6-fold), whereas for another five cells the fold increase was 4.3- ± 1.2-fold (minimum increase: 3.1-fold). Although we cannot completely exclude that, in individual cells, there may be mixtures of pharmacological sensitivities, the groupings just outlined strongly suggest that the α1H current in HEK cells exhibits either of two types of sensitivity to phenytoin.

The concentration-response curves for phenytoin for both types of block are shown in Fig. 2E. For the lower affinity, but complete, block produced by phenytoin (open symbols), the IC_{50} was 192.2 ± 47 μM (h = 1.3 ± 0.3). For the partial block of higher affinity, the IC_{50} was 8.3 ± 0.4 μM (h = 1.1 ± 0.1) with a fitted maximal block of only 44.8 ± 0.5%. The lower affinity, but complete block, by phenytoin closely mirrors the results obtained for block of α1G current by phenytoin, whereas the higher affinity, but partial block, is similar to results obtained from native DRG T currents (Fig. 2F).

Both types of response to phenytoin were observed in the same culture dishes with the same test solutions, and no obvious differences in current properties were noted. For seven cells with a partial block by phenytoin, peak current density (−35 mV from a holding potential of −90 mV) was 44.5 ± 8.6 pA/pF (mean ± S.D.), whereas for seven cells with complete block by phenytoin peak current density was 47.5 ± 13 pA/pF. Similarly, there was no difference in the 10 to 90% activation time (7.7 ± 2.5 ms for five cells with partial block, and 7.7 ± 2.7 for five cells exhibiting complete block) or inactivation time constant at −35 mV (25.4 ± 8.7 ms for five cells with partial phenytoin block and 23.3 ± 4.5 ms for five cells with complete block). Because a different amount of maximal block was observed in the two cases, the results can not be explained as a simple shift in the concentration-response curve due to inadequate exchange of solutions. These results minimally suggest that there is something different about the molecular composition of the T current channels among different α1H cells. Given the pharmacological similarity of one subset of α1H cells to the DRG neurons and the other α1H subset to α1G cells, it is suggestive that there is some regulatory factor or additional type of accessory subunit that can associate with α1H channels that may be present in DRG cells and also some, but not all, HEK cells.

Effects of Succinimides on α1G and α1H Current. MPS is another anticonvulsant reported to have only partial blocking effects on DRG T current (Todorovic and Lingle, 1998), although it has been reported to produce complete block of thalamic neuron T current (Coulter et al., 1990). The effects of 300 μM and 3 mM MPS on α1G current are shown in Fig. 3A, whereas the effects of four different MPS concentrations on peak α1G current are summarized in Fig. 3B. MPS, like phenytoin, appears able to produce a nearly complete block of α1G current with sufficiently high concentrations. The IC_{50} for block of α1G current by MPS was 1.7 ± 0.3 mM (n = 1.7 ± 0.3) with maximal block near 100%.

The effect of MPS on α1G current just described using 2 mM Ca^{2+} as the permeant ion differs from our previous results with MPS on DRG neurons in which 10 mM Ba^{2+} was employed (Todorovic and Lingle, 1998). Therefore, we re-examined the effects of MPS on DRG T current using 2 mM extracellular Ca^{2+}. In contrast to the effects of MPS on α1G current, there was little difference in the blocking effect of 300 μM and 3 mM MPS on DRG T current. The similarity in blocking effectiveness of 1 mM and 3 mM MPS on DRG T current is also summarized in Fig. 3D. For DRG T current, the IC_{50} was 190 ± 20 μM (h = 1.2 ± 0.5) with a maximal block of only 37 ± 4% similar to our previous measurements (170 μM with a maximum of 26%).

We next tested the effects of MPS on α1H current. In a set of 19 cells in which the effects of up to 6 mM MPS were examined (Fig. 3E), MPS produced an almost complete block in all cells with an IC_{50} of 2.3 ± 0.4 mM (h = 1.5 ± 0.3). This complete block by MPS was observed both in cells in which phenytoin produced only a partial block and in cells in which phenytoin produced a complete block. A comparison of the concentration dependence of MPS block of the three currents (Fig. 3F) indicates that MPS produces a higher affinity, partial block of DRG T current. This is similar to our previous observations with 10 mM Ba^{2+} (Todorovic and Lingle, 1998), whereas MPS produces a weaker, more complete block of both α1G and α1H current.

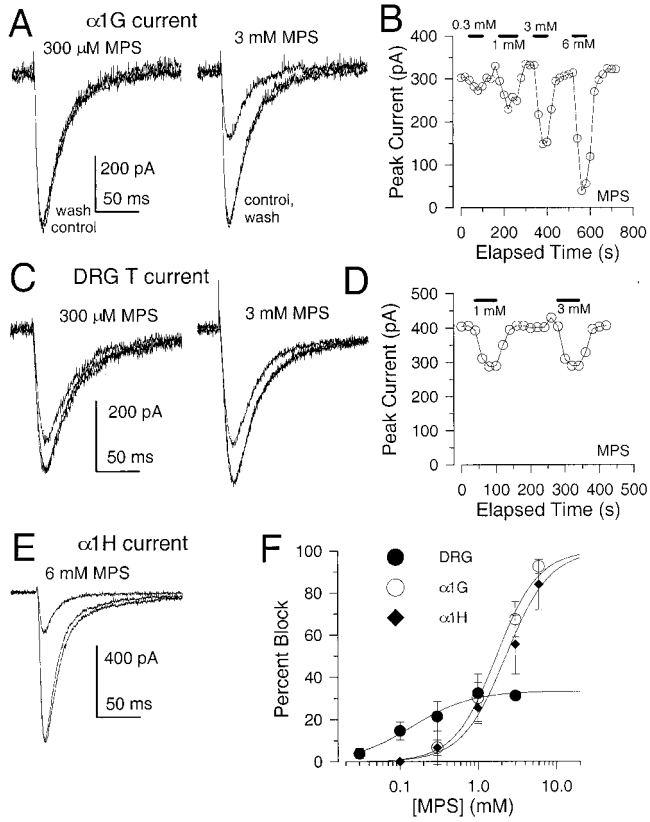

Fig. 3. MPS produces a low affinity, complete block of α1G and α1H current, which differs from blockade of DRG T current. A, left and right panels display α1G currents blocked by 0.3 and 3 mM MPS, respectively. B, temporal record of the peak current amplitude over the course of an experiment illustrates the concentration dependence of MPS inhibition of α1G current. 6 mM MPS produces an almost complete block of α1G current. C, left and right panels show examples of the inhibition of DRG T current by 0.3 and 3 mM MPS, respectively. Note that 3 mM MPS produces little additional inhibition over that achieved with 0.3 mM MPS. D, a temporal record of the inhibition of peak DRG T current by 1 and 3 mM MPS shows that MPS maximally blocks only about 30% of the peak current in this case. E, blockade of α1H current by 6 mM MPS is illustrated. F, the concentration dependencies of MPS block of DRG T current (filled circles), α1G current (open circles), and α1H current (diamonds) are displayed. Vertical lines represent S.D. Solid lines are best fits of eq. 1. For MPS inhibition of DRG T current (n = 6 cells), the IC_{50} was 0.14 ± 0.1 mM (h = 1.2) with a maximal block of 33%. For inhibition of α1G current (n = 5 cells), the IC_{50} was 1.7 ± 0.3 mM (h = 1.6) with maximal block constrained to 100%. For α1H current (n = 19 cells), the IC_{50} was 2.3 ± 0.4 mM (h = 1.5) with maximal block constrained to 100%.

We also examined the effects of ES. As shown in Fig. 4A, concentrations of ES well in excess of 1 mM are necessary to produce blockade of α1G current, with sufficiently high concentrations of ES producing complete block. Blockade does not result from the osmotic effects of such high concentrations, because equivalent osmotic additions of sucrose have no effects on α1G current (not shown). Blockade by ES was readily reversible (Fig. 4B); the concentration dependence of the block (Fig. 8E) yielded an IC_{50} of 14 ± 3.7 mM (h = 0.9 ± 0.2). ES produced very similar effects on α1H current (Fig. 4C, D) with an IC_{50} of 22.4 + 3.0 mM (h = 1.5 ± 0.3). Thus, both α1G and α1H currents are relatively insensitive to ES, similar to other reports of a lack of ES on T current in DRG neurons (Gross et al., 1997; Todorovic and Lingle, 1998) and in thalamic neurons (Leresche et al., 1998).

Valproic acid (VPA) is an anticonvulsant that produced a 17% reduction of DRG T current at 3 mM in a previous study (see Table 1 of Todorovic and Lingle, 1998). On α1G currents, 1 mM VPA had no effect, and 3 mM VPA blocked only 4.3 ± 3.2% (n = 4 cells). On α1H currents, 1 mM VPA reduced peak current by 8.6 ± 2% (n = 3 cells).

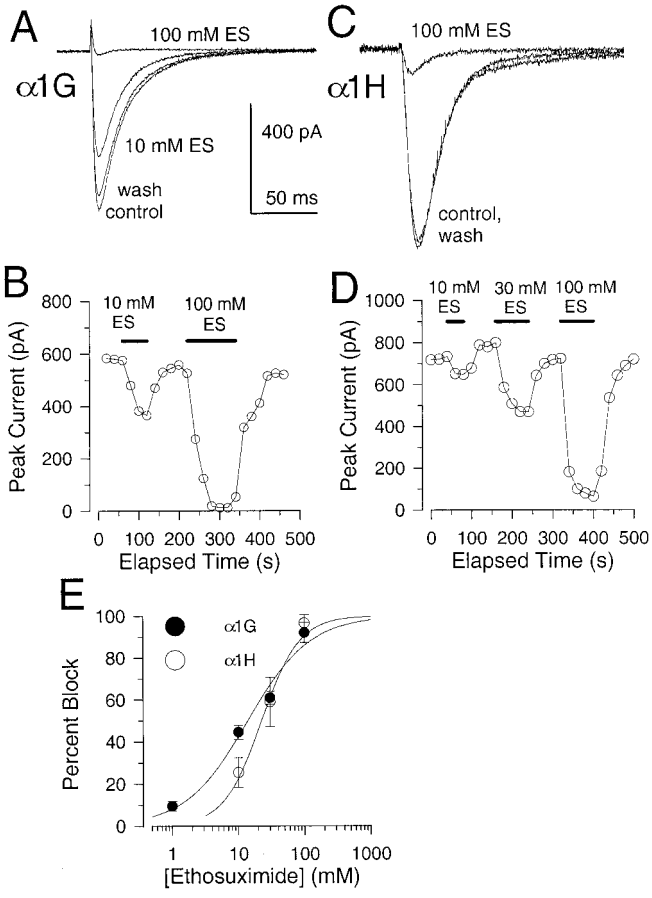

Fig. 4. The anticonvulsant, ES, blocks α1G and α1H current similarly. A, traces show block of α1G current produced by either 10 and 100 mM ES. B, peak inward current activated every 20 s from the experiment shown in A is plotted as a function of elapsed time. C, traces show block of α1H current produced by 100 mM ES. D, peak inward current activated every 20 s from the experiment shown in C is plotted as a function of elapsed time. E, the concentration dependence of α1G and α1H current reduction by ES is plotted. Each point is the average of the block produced by ES applications to at least five different cells, with vertical lines indicating S.D. Solid lines are best fit of eq. 1, yielding for α1G current, an IC_{50} of 14 ± 3.7 mM (h = 0.9) with the fit constrained to a maximal block of 100% and, for α1H current, an IC_{50} = 22.4 ± 3.0 mM (h = 1.5) with maximal block constrained to 100%.

Lack of Effect of Convulsants on α1G Current. Because thalamic T currents have been proposed to play an important role in the initiation of epileptic activity (Chung et al., 1993; Huguenard and Prince, 1994; Tsakiridou et al., 1995), we tested two convulsant compounds on α1G current. Pentylenetetrazol at 1 mM had no effect on α1G current in five cells (4.4 ± 6% inhibition). This was similar to the reported insensitivity to this agent of T current in DRG cells (Todorovic and Lingle, 1998) and thalamic cells (Coulter et al., 1990). TBPS is a potent $GABA_A$ receptor antagonist (Casida et al., 1985). In four HEK cells, 50 μM TBPS had no effect on α1G current.

Blockade of α1G Current by Parenteral Anesthetics and Related Compounds. Various anesthetics have also been reported to inhibit T-type currents in various cells, in some cases at concentrations that overlap their clinical usage. All parental anesthetics used in this study did produce complete block of α1G currents and, when tested, complete block of α1H currents. Blocking potency was, in general, quite comparable with previous results on T current in DRG neurons.

Octanol is an anesthetic-like agent of interest primarily because of early reports that it potently blocks T currents in inferior olivary neurons (Llinas 1988) and neonatal DRG cells (Scott et al., 1990). Later reports indicated weaker blocking effects on T currents in GH3 cells (Herrington and Lingle, 1992) and DRG neurons (Todorovic and Lingle, 1998). Figure 5A shows examples of α1G currents elicited by voltage steps to −35 mV before and during application of 0.3 and 1 mM octanol. The peak inward current amplitude during one experiment in which four different concentrations of octanol were applied to a cell is plotted in Fig. 6B, showing the rapid onset of block and complete recovery from block. A characteristic of blockade by octanol was an increase in the apparent rate of current inactivation (Fig. 5C). Octanol blocked α1G current completely with 50% inhibition (IC_{50}) at 160 ± 13 μM (h = 1.3 ± 0.1) (Fig. 4C).

Very similar effects of octanol were observed on α1H current (Fig. 6A, B), including the increase in apparent current inactivation rate in the presence of octanol (Fig. 6A). Figure 6D compares the effects of octanol on both α1G and α1H current. For inhibition of α1H current, the IC_{50} for blockade by octanol was 218.6 ± 44 μM (h = 1.4 ± 0.3).

Propofol (2,6-diisopropylphenol) is a frequently used i.v. anesthetic. It blocked α1G currents completely with an IC_{50} at 20.5 ± 2.0 μM (h = 1.4 ± 0.2) (Fig. 5D). Similarly, propofol blocked α1H currents in a reversible fashion (Fig. 6C) with the IC_{50} for block of α1H current being 27 ± 3 μM (h = 1.2 ± 0.2) (Fig. 6D). Propofol produced no effect on the rate of current inactivation.

Etomidate is also an i.v. general anesthetic. It was also able to inhibit α1G currents completely with an IC_{50} of 161 ± 46 μM (h = 1.7 ± 0.5). Finally, ketamine, an i.v. anesthetic and N-methyl-D-aspartate antagonist, was least potent in blocking α1G currents with an IC_{50} of 1.2 ± 0.1 mM (h = 1.4 ± 0.1). Effects of etomidate and ketamine on α1H current were not determined.

Concentration-response curves for effects of these anesthetics on α1G current are summarized in Fig. 5D. The IC_{50} values for block of both α1G and α1H currents are compara-

ble (e.g., Fig. 6D) and similar to previously reported values for blockade of DRG T currents as presented in Table 1.

Effects of Isoflurane on α1G Current. Volatile anesthetics, like isoflurane and halothane, are reported to block T currents in DRG cells in clinically relevant concentrations (Todorovic and Lingle 1998). Of the volatile anesthetics that have been tested on T currents, isoflurane appears to have the most pronounced blocking effects at concentrations likely to occur during clinical use (Todorovic and Lingle, 1998). Examples of the effects of isoflurane on α1G current are shown in Fig. 7A. Blockade was rapid and readily reversible (Fig. 7B). At sufficiently high concentrations, isoflurane blocked α1G current essentially completely. In all experiments, actual concentrations of isoflurane in the bath were measured using gas chromatographic analysis. Like octanol, but not propofol, isoflurane increased the apparent rate of inactivation of α1G current (Fig. 7C). The concentration-response curve for blockade of α1G current by isoflurane is shown in Fig. 7D, yielding an IC_{50} of 277 ± 24 μM and h of 2.2 ± 0.4. This compares to an IC_{50} for blockade of DRG T current by isoflurane of 303 μM (Todorovic and Lingle, 1998).

Blockade of α1G and α1H Current by Barbiturates. Barbiturates are interesting compounds, because they are both general anesthetics and antiepileptics. Pentobarbital, methohexital, thiopental, and phenobarbital block DRG T currents in adult rats, although at concentrations higher than those achieved during clinical use (Todorovic and Lingle, 1998).

Examples of the blocking effects of thiopental and phenobarbital on α1G currents are shown in Figs. 8A and 8B, respectively, whereas Fig. 8C shows the temporal summary of the effects of multiple barbiturate applications on peak α1G current amplitude. Of the barbiturates, thiopental and pentobarbital blocked with similar potency with phenobarbital being less effective. For pentobarbital, blockade occurred with an IC_{50} of 310 ± 40 μM (h = 1.4 ± 0.2). For thiopental, the IC_{50} was 280 ± 40 μM (h = 1.2 ± 0.2). For phenobarbital, the IC_{50} was 1.54 ± 0.2 mM (h = 1.2 ± 0.2). The concentration-response curves for these three compounds are displayed in Fig. 8D. These values are similar to those previously reported for blockade of DRG T current (see Table 1). The barbiturates had no effect on the time constant of inactivation of α1G current.

To evaluate the sensitivity of α1H current to barbiturates, the ability of pentobarbital to inhibit α1H current was examined. Pentobarbital blocked α1H current in a manner similar to its effects on α1G current (Fig. 8E) with an IC_{50} of 345 ± 94 μM (h = 1.1 ± 0.2). As with α1G current, pentobarbital had no effect on the time constant of α1H current inactivation. The similarity of the blocking effect of pentobarbital on both α1G and α1H current is summarized in Fig. 8F.

It must be noted that, for most compounds, we did not test for voltage or use dependence of block. Substantial voltage or use dependence of block can affect estimated IC_{50} values. However, there was no indication of slow block by any of the anesthetic-like compounds we examined.

Discussion

The low threshold for activation of T type Ca^{2+} currents allows them to play a critical role in the regulation of cellular excitability, both in neurons and other cell types (Huguenard, 1996). Despite general similarity among various T-type currents in different cells, some differences in kinetic behaviors and pharmacological sensitivities have been observed (Huguenard, 1996). With the availability of cloned T-type current variants (Cribbs et al., 1998; Perez-Reyes et al., 1998; Lee et al., 1999a), it is now possible to examine physiological and pharmacological properties of T currents of defined molecular components. Here, we have examined the sensitivity of cloned T-type current variants to anticonvulsants and anesthetics. The results suggest that, although anesthetic sensitivity does not vary among DRG, α1H, and α1G currents, blocking effects of some anticonvulsants may depend on specific subunits contributing to the T channel.

Blockade of T Current by Anticonvulsants. Interest in the possible role of T current inhibition in the action of anticonvulsants arose from reports that ES and MPS may inhibit T current in thalamic relay neurons (Coulter et al., 1989, 1990). These succinimide compounds belong to a class

TABLE 1
A comparison of sensitivities of native T currents in DRG cells and α1G, α1H, and α1E currents in HEK cells

	Summary of Effects of Anesthetics and Anticonvulsants				
	DRG[a]	α1G[b]	α1H[b]	α1H	α1E[c]
Propofol	IC_{50} 12.9 μM; max. 100%	IC_{50} 20 μM; max. 100%	IC_{50} 27 μM; max. 100%		
Octanol	IC_{50} 122 μM; max. 100%	IC_{50} 160 μM; max. 100%	IC_{50} 218.6 μM; max. 100%		IC_{50} 206 μM; max. 100%
Isoflurane	IC_{50} 303 μM; max. 100%	IC_{50} 271 μM; max. 100%			
Pentobarbital	IC_{50} 334 μM; max. 100%	IC_{50} 310 μM; max. 100%	IC_{50} 345 μM; max. 100%		IC_{50} 600 μM; max. 100%
Phenobarbital	IC_{50} 1.7 mM; max. 100%	IC_{50} 1.54 mM; max. 100%			IC_{50} 2.7 mM; max. 100%
ES	IC_{50} 23.7 mM; max. 100%	IC_{50} 14.3 mM; max. 100%	IC_{50} 22.4 mM; max. 100%		IC_{50} 20 mM; max. 100%
MPS	IC_{50} 145 μM; max. 42%	IC_{50} 1.67 mM; max. 100%	IC_{50} 2.3 mM; max. ~100%		IC_{50} 2.3 mM; max. 100%
Phenytoin	IC_{50} 7.8 μM; max. 58%	IC_{50} 140 μM; max. 100%	IC_{50} 8.3 μM; max. 44.8% (10 of 23 cells)	IC_{50} 192 μM; max. 100% (13 of 23 cells)	IC_{50} 360 μM; max. 100%

[a] Data for MPS and phenytoin from this study with 2 mM Ca^{2+}, other data from Todorovic and Lingle (1998) with 10 mM Ba^{2+}.
[b] This study with 2 mM Ca^{2+}.
[c] From Nakashima et al. (1998) with 10 mM Ba^{2+}.

of anticonvulsants used to treat petit mal-generalized absence seizures (Macdonald and McLean, 1986). However, although some results support a potential contributory role of T current in experimental models of epilepsy (Chung et al., 1993; Tsakiridou at el., 1995), more recent work indicates that 1 mM ES does not inhibit T current on thalamic cells (Leresche et al., 1998). The lack of effect of ES on thalamic T currents is comparable with the relative insensitivity (IC_{50} values greater than 10 mM) of native T currents (Herrington and Lingle, 1992; Todorovic and Lingle, 1998) and α1G and α1H current that we observe. The results would suggest that the anticonvulsant action of ES is unlikely to arise from effects on T-type currents.

In contrast to ES, some blockade by MPS of both native and cloned T-type channels is expected at therapeutically relevant concentrations [~50–200 μM: Strong et al. (1974)]. Studies in thalamic neurons reported that MPS blocked T currents completely with an IC_{50} of 1.1 mM (Coulter et al., 1990). In agreement with these results, we find that MPS blocked α1G currents completely with similar potency, consistent with the view that α1G is the predominant isoform expressed in thalamic relay nuclei (Talley et al., 1999).

The most interesting aspect of our results is the selectivity of phenytoin among different T current variants and the selectivity of MPS between the cloned T current variants and the native DRG T current. Specifically, DRG T current exhibits a higher affinity, partial block by both phenytoin and MPS, whereas α1G current is completely blocked by both anticonvulsants, although with lower affinity. Thus, DRG T current is pharmacologically distinct from α1G current. Furthermore, α1H current exhibits a mixed sensitivity to phenytoin with both partial, higher affinity block in some cells, and complete, lower affinity block in others.

Because the α1H channel variant is thought to be more abundant in sensory ganglia than either the α1G or α1I variants (Lee et al., 1999a), a difference in the pharmacological sensitivities of α1G and native DRG T currents might be attributable to a greater abundance of the α1H channel in DRG cells. In support of this hypothesis, Ni^{2+} was found to block cloned α1H currents (Lee et al., 1999b) at similar concentrations as it blocks DRG T currents (Todorovic and Lingle, 1998), whereas 40-fold higher concentrations are required to block α1G and α1I currents. However, the present results show that the pharmacological properties of neither α1G nor α1H currents correspond fully with the properties of DRG T current. Specifically, blockade of DRG T current by

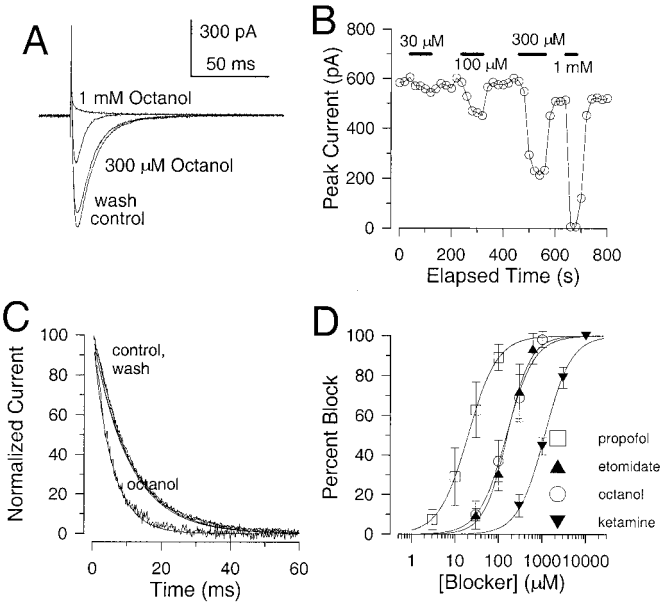

Fig. 5. Parenteral general anesthetics block α1G currents in HEK cells. A, traces display inward currents activated by a 200-ms voltage step from −90 mV to −35 mV before, during, and after application of 0.3 and 1 mM octanol. Note the faster rate of inactivation in the presence of 300 μM octanol. B, peak inward current over the course of the experiment for the cell shown in A is displayed to illustrate the concentration dependence and reversible nature of block by octanol. Currents were activated every 20 s. Horizontal bars indicate time of application of the indicated concentrations of octanol. C, the decay phase of normalized currents before, during, and after application of 300 μM octanol are shown to illustrate the effect of octanol on current inactivation rate. Inactivation time constants were 10.7, 5.5, and 10.4 ms before, during, and after octanol application, respectively, from single exponential fits to the current decay phase. D, average concentration-response curves for blockade of peak α1G current by propofol, etomidate, ketamine, and octanol. All points are averages of at least five different cells. Vertical bars indicate S.D. of multiple determinations, and solid lines are best fits of the Hill equation (eq. 1, *Materials and Methods*). Fits were constrained to 100% block with IC_{50} values of 20.0 ± 2.0 μM (h = 1.3), 158.9 ± 25.5 μM (h = 1.6), 152.3 ± 29.7 μM (h = 1.4), and 1.14 ± 0.17 mM (h = 1.4), for propofol, etomidate, octanol, and ketamine, respectively.

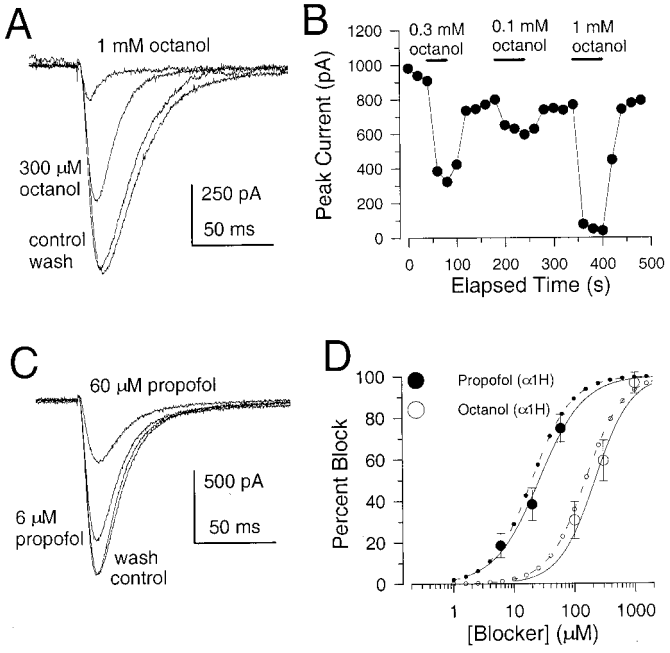

Fig. 6. Octanol and propofol block α1H and α1G current similarly. A, traces display α1H currents activated by 200-ms voltage steps from −90 mV to −35 mV before, during, and after application of 0.3 and 1 mM octanol. Note the faster rate of inactivation in the presence of 300 μM octanol. B, peak inward current over the course of the experiment for the cell shown in A is displayed to illustrate the concentration dependence and reversible nature of block by octanol. C, traces show the effects of 6 and 60 μM propofol on α1H current activated as in A. D, average concentration-response curves are shown for blockade of peak α1H current by propofol and octanol. All points are averages of at least five different cells. Solid lines are best fits of the Hill equation (eq. 1, *Materials and Methods*). Fits were constrained to 100% block with an IC_{50} for propofol of 27 ± 3 μM (h = 1.2) and an IC_{50} for octanol of 218.6 ± 44.5 μM (h = 1.4). Lines with smaller symbols are the fitted concentration-response curves for block of α1G current by propofol (filled circles) and octanol (open circles) from Fig. 5.

MPS differs from block of both α1G and α1H current. Similarly, the results with phenytoin do not demonstrate a simple equivalence of either the α1G or α1H currents with the DRG currents. Thus, our results lead us to suggest that DRG neurons may contain either additional regulatory subunits that alter the pharmacological properties of α1G or α1H channels or that native α1G or α1H subunits may undergo post-translational modifications that distinguish them from their properties in expression systems.

The pharmacological heterogeneity of α1H currents to phenytoin may also be most easily explained by this hypothesis. The correspondence of the DRG T current to one type of α1H subtype and the correspondence of the α1G current to the other α1H subtype might result from some additional molecular component, perhaps an accessory subunit, present in DRG cells and some HEK293 cells. The pharmacological uniformity of the α1G currents, in contrast to that of the α1H currents, would suggest that any such accessory subunit may selectively influence only the α1H subunit.

Although the subunit composition of native T-type channels has not been determined, studies with cloned subunits indicate that $\alpha2\delta$-1 can increase the expression of α1G in COS cells and *Xenopus* oocytes (Dolphin et al., 1999). The effect of $\alpha2\delta$-1 was to increase the expression of α1G immunoreactivity at the plasma membrane and to increase the α1G-mediated currents. No effect was observed on the biophysical properties of the currents. Many studies with HVA channels have also reported that $\alpha2\delta$ simply scales the expressed current, although in some cases it appears to affect inactivation kinetics (reviewed in Walker and De Waard, 1998). In addition, $\alpha2\delta$ has been shown to modulate the pharmacology of cloned L-type Ca^{2+} channels (Wei et al., 1995). Therefore, it is possible that endogenous $\alpha2\delta$ subunits modulate the apparent pharmacological properties of α1H in a subpopulation of the stably transfected cell line. Recent work with the α1I subunit indicates that physiological properties of this T-type channel differ markedly dependent on whether the subunit is expressed in HEK293 cells or *Xenopus* oocytes (Lee et al., 1999a). This result was used to argue for the possible existence of an unknown accessory subunit,

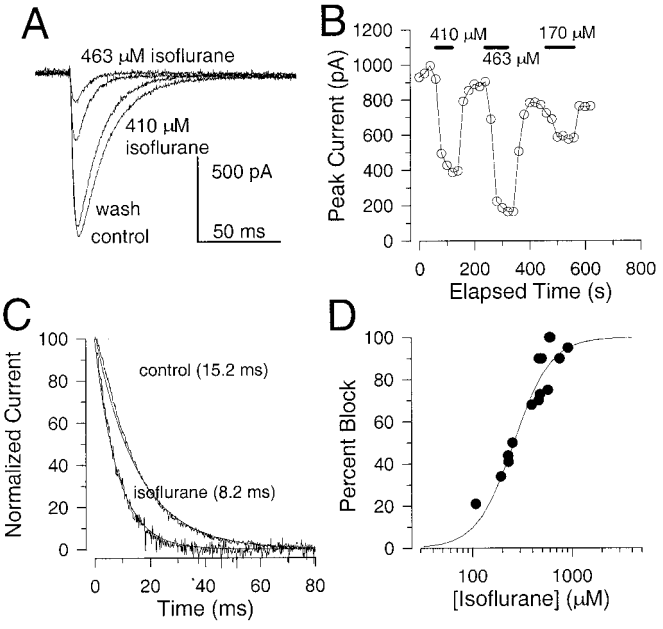

Fig. 7. Isoflurane blocks α1G currents at concentrations likely to occur in clinical usage. A, traces show currents activated by voltage steps to −35 mV in the presence and absence of isoflurane. Concentrations were determined by gas chromatography (Evers et al., 1986). Similar to octanol, isoflurane increased the current inactivation rate. B, a temporal record of the effects of three concentrations of isoflurane on peak α1G current for the cell shown in A is plotted. Open circles represent peak current evoked every 20 s. C, the effect of 410 μM isoflurane on the normalized time course of current inactivation is shown. τ_i was 17.7, 8.2, and 15.2 ms for control, 410 μM isoflurane, and following wash. D, percent blockade of peak α1G current is plotted as a function of isoflurane concentration for isoflurane applications to six cells. All isoflurane values were determined by gas chromatography. The solid line is the best fit of eq. 1 yielding an IC_{50} of 262 ± 16.6 μM and h of 2.3 ± 0.3 with maximal block constrained to 100%.

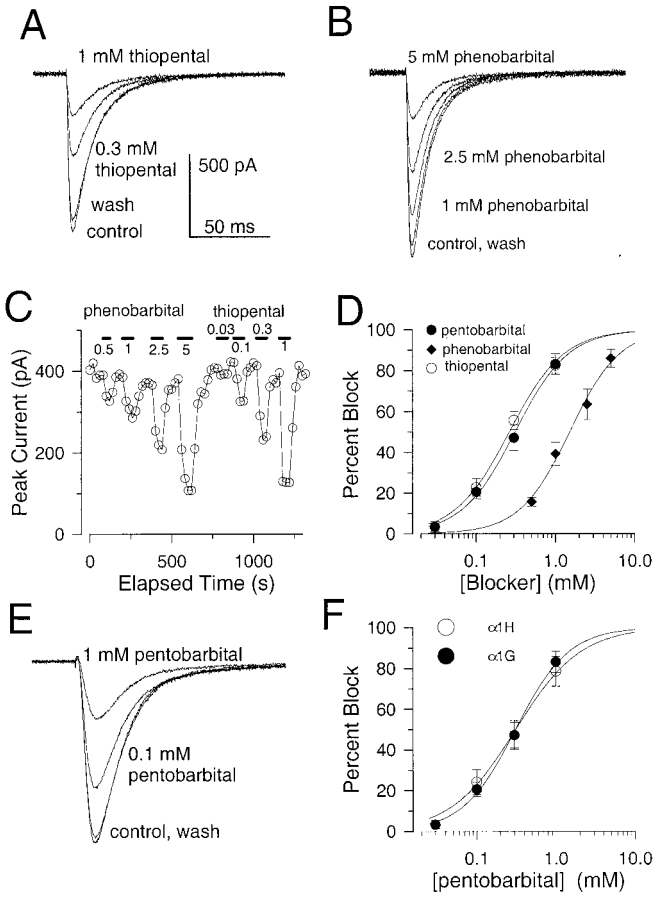

Fig. 8. Blockade of α1G current by barbiturates. A, traces show α1G currents activated by voltage steps to −35 mV in the presence and absence of thiopental applied at the concentrations indicated on the figure. B, traces show α1G currents activated as in A before, during, and after the application of the indicated concentrations of phenobarbital. In contrast to the effects of octanol and isoflurane shown in Figs. 5 and 6, the barbiturates did not affect the time course of current inactivation. In A, in control saline, τ_i = 14.4 ms; with 1 mM thiopental, τ_i = 15.5 ms. In B, in control saline, τ_i = 12.0 ms, whereas with 5 mM phenobarbital, τ_i = 12.2 ms. C, the time course of block for multiple concentrations of phenobarbital and thiopental is plotted from the same cell as in A and B. Bars indicate time of application. D, concentration-response curves for three barbiturates are shown, with each point being the average of at least five different cells. Vertical lines are the S.D. The solid lines are best fits of eq. 1. IC_{50} values for block of α1G current were 263 ± 31 μM (h = 1.3) for thiopental, 311 ± 31 μM (h = 1.3) for pentobarbital, and 1.5 ± 0.2 mM (h = 1.4) for phenobarbital. E, traces show α1H currents activated as in A in the presence and absence of the indicated concentrations of pentobarbital. F, the concentration dependence for block of both α1H and α1G current by pentobarbital is compared. The IC_{50} for block of α1H current was 345 ± 94 μM (h = 1.1).

present in one or the other of the two expression systems, which affects the gating properties of the α1I subunit. A similar phenomenon may account for the differences in sensitivity of α1H currents to phenytoin.

The ability of anticonvulsants to differentiate among T current variants may have important clinical implications. Phenytoin is used for treatment of generalized tonic-clonic seizures (Macdonald and McLean, 1986) and for treatment of neuropathic pain (McQuay et al., 1995). The higher affinity, partial blockade of DRG, and α1H T current occurs within clinically relevant concentrations (Todorovic and Lingle, 1998). In contrast, the lower affinity effect of phenytoin on α1G current suggests that some native T currents may be unaffected by this compound during clinical usage. The clinical consequences of phenytoin may, in part, involve an ability to selectively affect particular T current subtypes. Similar arguments apply to MPS.

Blockade of T currents Arising from α1G and α1H Subunits by General Anesthetics. Anesthetics and anesthetic-like compounds blocked all three T-type currents similarly and are of limited utility in distinguishing among T-type currents. Table 1 shows the sensitivity of cloned α1E currents to these same compounds (Nakashima et al., 1998), indicating that anesthetics are relatively ineffective in distinguishing between T-type current and at least one type of HVA Ca^{2+} current.

Some general anesthetics, e.g., isoflurane and, to a lesser extent, halothane, block native DRG T currents at concentrations that probably occur during anesthesia (Todorovic and Lingle, 1998). Similar to its effects on DRG T current, isoflurane could completely block α1G current with an IC_{50} of 271 μM. This concentration is less than the reported MAC (minimum alveolar concentration producing anesthesia in 50% of subjects) value of 400 μM for this anesthetic (Franks and Lieb, 1994). This argues that some inhibition of DRG T current and also any T current-containing α1G subunits will certainly occur during isoflurane-induced anesthesia. However, except for isoflurane, it appears unlikely that blockade of native T-type currents participates in the clinical effects of other anesthetic agents examined here.

An interesting feature of the anesthetic-like compounds is that two categories of blocking mechanism may occur. Isoflurane and octanol share an ability to alter the rate of α1G or α1H current inactivation. Halothane and octanol produce similar increases in current inactivation rate on native GH3 T currents (Herrington et al., 1991; Herrington and Lingle, 1992). In contrast, other anesthetics, including the barbiturates and propofol, produce no alteration in the time course of α1G, α1H, or DRG T current. This suggests that blockade of T current by the anesthetic compounds can occur by either of two types of mechanism, either of which can produce complete inhibition of T current.

In summary, we have examined the sensitivity of α1G and α1H T-type currents to a number of anesthetic and anticonvulsant agents. The results indicate that blockade of some T currents may participate in the clinical actions of some agents (isoflurane, MPS, phenytoin). The differential blocking effects of phenytoin and MPS between α1G current and native DRG T current, which for phenytoin was also mirrored between subsets of α1H-expressing HEK293 cells, argue that some additional factor, perhaps an unknown accessory subunit, may influence the sensitivity of T-type channels to pharmacological agents.

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Send reprint requests to: Christopher Lingle, Department of Anesthesiology, Washington University School of Medicine, Box 8054, St. Louis, MO 63110. E-mail: clingle@morpheus.wustl.edu