Pharmacological Properties of T-Type Ca\textsuperscript{2+} Current in Adult Rat Sensory Neurons: Effects of Anticonvulsant and Anesthetic Agents

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Todorovic, Slobodan M. and Christopher J. Lingle. Pharmacological properties of T-type Ca\textsuperscript{2+} current in adult rat sensory neurons: effects of anticonvulsant and anesthetic agents. J. Neurophysiol. 79: 240–252, 1998. We have used the whole cell patch-clamp method to study pharmacological properties of low-voltage-activated (LVA) Ca\textsuperscript{2+} current in freshly dissociated neurons from dorsal root ganglia of adult rats. Inward barium current [in the presence of internal fluoride to reduce L-type high-voltage-activated (HVA) and external 1 \textmu M \omega-conotoxin GVIA to block N-type HVA current] was evoked from negative holding potentials of \textminus90 mV to test potentials of \textminus25 mV and showed complete inactivation during 200-ms test pulses. Amiloride blocked \textasciitilde90% of current with half-maximal block (EC\textsubscript{50} of 75 \textmu M and a Hill coefficient (n) of 0.99. LVA current was blocked completely by inorganic Ca\textsuperscript{2+} channel blockers: lanthanum (EC\textsubscript{50} = 0.53 \textmu M) > zinc (EC\textsubscript{50} = 11.3 \textmu M) > cadmium (EC\textsubscript{50} = 20 \textmu M) > nickel (EC\textsubscript{50} = 51 \textmu M). The antiepileptics, ethosuximide (EC\textsubscript{50} = 23.7 \textmu M, n = 1.4), phenytoin (EC\textsubscript{50} = 7.3 \textmu M, n = 1.3), \alpha-methyl-\alpha-phenylsuccinimide (EC\textsubscript{50} = 170 \textmu M, n = 2.1), and valproic acid (EC\textsubscript{50} = 330 \textmu M, n = 1.9) maximally blocked \textasciitilde100, 60, 26, and 17% of T current, respectively. Another antiepileptic, carbamazepine (\textasciitilde100 \textmu M), and convulsants such as pentylenetetrazole (1 mM) and tert-butyl-bicyclo[2.2.2]octanol neurons was one of the first T currents to be described (Carbone and Lux 1984), the pharmacological description of this current is incomplete. Specifically, for many blockers of DRG T current, concentration-response information is not available. Available data suggest that considerable diversity not only exists among T currents in terms of fundamental kinetic properties (e.g., Huguenard et al. 1993), but also in terms of pharmacological sensitivities (Huguenard 1996). For example, although the primary target of the clinically used anticonvulsant, ethosuximide, has been proposed to involve T current blockade in thalamic neurons (Coulter et al. 1989a,b), T current in GH\textsubscript{3} cells is relatively resistant to blockade by ethosuximide (Herrington and Lingle 1992).

Although the T-type current in dorsal root ganglion (DRG) neurons was one of the first T currents to be described (Carbone and Lux 1984), the pharmacological description of this current remains incomplete. Specifically, for many blockers of DRG T current, concentration-response information is not available. Given the likelihood that different T currents may exhibit well-defined pharmacological differences, clear definition of those differences is of key importance in the development of agents with selective clinical efficacies. Furthermore, better defined pharmacological tools are essential to the understanding of the roles of different T currents in nervous system function. As part of ongoing work aimed at illuminating similarities and differences among native T currents, here we have examined the pharmacological properties of T current in acutely dissociated adult rat DRG neurons. Particular attention has been directed toward defining complete concentration-response curves to allow detailed comparison with similar data from other T currents.

**METHODS**

*Acutely dissociated DRG neurons*

Male rats (100–250 g; Sprague-Dawley) were anesthetized with halothane and decapitated. DRG (8–10) from thoracic and upper
lumbar regions were dissected out and incubated at 36°C for 60–90 min in Tyrode solution [which contained (in mM) 140 NaCl, 4 KCl, 2 MgCl₂, 10 glucose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)], adjusted to pH 7.4 with NaOH] containing 2 mg/ml collagenase (Sigma type I) and 5 mg/ml dispase II (Boehringer-Manheim). Single neuronal cell bodies were obtained by trituration in Tyrode solution at room temperature. Cells were kept at room temperature and used for electrophysiology within 4 h from dissociation. For recordings, neuronal cell bodies were plated onto a glass coverslip and placed in a culture dish that was perfused with external solution. Most of the data were obtained from smaller diameter neurons (21–27 μm) without visible processes.

**Electrophysiological methods**

Recordings were made with standard whole cell voltage-clamp techniques (Hamill et al. 1981). Electrodes were fabricated from microcapillary tubes (Drummond Scientific, Broomall, PA), coated with silicone elastomer (Sylgard, Dow Corning, Midland, MI), and fire-polished. Pipette resistances were 2–5 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). Data were analyzed using Clampfit (Axon Instruments) and Origin 3.1. Currents were filtered at 5 kHz. Reported series resistance values were taken from the reading of the amplifier. We have confirmed that these values are within 10% of estimates obtained from the time constant of decay and integral of the capacitance transient. Average uncompensated series resistance was 6.8 ± 4.8 MΩ (mean ± SD) and capacitance was 12.6 ± 3.1 pF with n = 245. Series resistance typically was compensated 60–80% without significant oscillations in the current trace. All experiments were done at room temperature (20–23°C). In most experiments, no leakage subtraction was used, although in a limited number of experiments, a P/5 protocol was used for on-line leakage subtraction. For construction of current-voltage curves as in Fig. 1, C and D, leak current was subtracted manually by using the linear portion of current obtained at voltages negative to −90 mV. Error bars indicate standard errors of multiple determinations obtained from at least five different cells.

**Analysis of current blockade**

The percent 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 errors. Mean values on concentration-response curves were fitted to the following function

\[ PB(\text{Drug}) = \frac{PB_{\text{max}}}{1 + (EC_{50}/[\text{Drug}])^n} \]  

(1)

where \( PB_{\text{max}} \) is the maximal percent block of peak T current, the \( EC_{50} \) is the concentration that produces 50% of maximal inhibition, and \( n \) is the apparent Hill coefficient for blockade. In the case of isoflurane and halothane, 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.

The voltage-dependence of steady-state inactivation was described with a Boltzmann distribution:

\[ I(V) = I_{\text{max}}/(1 + \exp[-(V - V_{50})/k]) \]  

(2)

where \( I_{\text{max}} \) represents maximal activatable current, \( V_{50} \) represents the voltage where half of the current is activated, and \( k \) (units of millivolts) represents the voltage dependence of the distribution.

The time course of activation of LVA currents in rat dorsal root ganglion (DRG) neurons. A: currents recorded in response to command steps from a holding potential of −90 mV to test potentials from −60 to −20 mV. Note the significant proportion of sustained current when a test potential of −20 mV was used. \( EC_{50} = 13 \text{ pF} \), \( R_f = 7 \text{ MΩ} \). B: in the same cell, a family of currents were evoked with the same protocol but after application of 1 μM \( \omega \)-conotoxin GVIA to block N-type high-voltage-activated (HVA) current. Note that all currents except one activated at −50 mV completely inactivate during the 200-ms test pulse. C: peak amplitude of inactivating current obtained from A and B are plotted as a function of command potential. Although minimal effect is observed at −50 mV, \( \omega \)-conotoxin GVIA blocked −33% of total peak current at −20 mV. D: peak current activated at different command step potentials is summarized for 12 cells. For comparison across the cells, currents were normalized to current at −20 mV, a voltage at which near-maximal T current was observed.

the voltage where half of the current is inactivated, and \( k \) (units of millivolts) represents the voltage dependence of the distribution.

The time course of activation of LVA currents in rat DRG cells was examined using the following expression from Hodgkin and Huxley (1952):

\[ I(t) = A\times(1 - \exp(-t/\tau_c))^n\times\exp(-t/\tau_s) \]  

(3)

where \( \tau_c \) is the time constant of activation, \( n \) is the number of particles involved in the activation process, and \( \tau_s \) is the time constant of inactivation.

Fitting was done either with Origin 4.0 (Microcal Software, Northampton, MA) or with custom software using a nonlinear least-squares procedure with a Levenberg-Marquardt search algorithm to minimize function parameters.

**Solution exchange procedures**

Glass coverslips with adherent DRG cells were transferred to a standard culture dish with a total volume <1 ml. The solution application system consisted of multiple, independently controlled glass capillary tubes; the solution was removed from the other end of the chamber with the use of constant suction. Switching between solutions was accomplished by manually controlled valves. 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 10–20 s. Switching between separate perfusion syringes, each containing control saline, resulted in no changes in Ca$^{2+}$ current. No dependence on the order of presentation or desensitization with repeated applications was observed for any of the pharmacological agents, making comparison between sequential applications of different concentrations straightforward.

**Solutions and current isolation procedures**

The standard extracellular saline for recording of Ca$^{2+}$ current contained (in mM) 152 tetraethylammonium (TEA)-Cl, 10 HEPES, and 10 BaCl$_2$, adjusted to pH 7.4 with TEA-OH, osmolarity 316 mOsm. Cells generally were maintained in a Tyrode’s solution until seal formation, at which time the bath solution was switched to the Ba$^{2+}$ saline. To facilitate HVA current run down (Akaike et al. 1989; Herrington and Lingle 1992; Kostyuk et al. 1975), the following fluoride (F$^-$)-based intracellular solution was used (in mM): 135–140 tetramethylammonium hydroxide (TMA-OH), 10 ethylene-glycol-bis (β-aminooxy ethyl) N,N,N’,N’-tetraracetic acid, 40 HEPES, and 2 MgCl$_2$, titrated to pH 7.15–7.20 with hydrofluoric acid (HF) (yielding ~80 mM F$^-$); osmolarity was 285–300 mOsm. To block N-type HVA current in these cells, we used 1 μM ω-conotoxin GVIA (ω-CgTX), which blocked most of the remaining sustained current in these cells (see Fig. 1B). Because in control experiments this effect was irreversible for ≥60 min, we routinely preincubated every slide with this toxin and recorded within this time frame. In most cells included in this study, blockade of L- and N-type currents was sufficient to allow investigation of T current in virtual isolation. After 10 min of dialysis with F$^-$-containing internal saline, nifedipine had no effects on inward current (data not shown). However, in some cells, a small residual noninactivating current (typically <40 pA) persisted after F$^-$ and ω-CgTX treatment. Because of the possibility of some residual HVA current contamination, all measurements of T current amplitude 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. After blockade of L- and N-type current, peak T current was stable for ≥1 h. Scroggs and Fox (1992) also have shown that ω-CgTX and nimodipine are sufficient to block most of the HVA current in smaller DRG cells from adult rats.

**Drugs and chemicals**

Thymol-free halothane was a gift of Halocarbon Laboratories (North Augusta, SC), ω-conotoxin GVIA and tert-butyl-bicyclo[2.2.2]phosphorionate (TBPS) were obtained from RBI (Natick, MA), etomidate powder was a gift of Abbott (Abbott Park, IL), and mibebradil (Ro 40-2967) was a kind gift of F. Hoffman-La Roche, Basel. All other chemicals were obtained from Sigma (St. Louis, MO) or Aldrich Chemicals (Milwaukee, WI).

**Drug preparation**

Stock solutions of propofol (100 mM), amiloride (500 mM), etomidade (300 mM), methyl-phenylsuccinimide (1 mM), carbamazepine (100 mM), TBPS (50 mM), and phenytoin (100 mM) were prepared in dimethyl sulfoxide (DMSO) and kept at 4°C until use. The maximal concentration of DMSO used was 0.3% and had no significant effect on T current when tested alone at the same final concentration. All barbiturates were prepared in stock solutions in 0.1 N TEA-OH; the final solution in extracellular saline was pH adjusted with HCl. Volatile anesthetic 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; Stern et al. 1989). 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. Experiments with amiloride were done in dim light. Ethosuximide was added to the extracellular saline without osmotic adjustments. In separate control experiments, application of extracellular saline with an additional 100 mM sucrose had no effect on T current amplitude.

**RESULTS**

**Isolation and physiological properties of DRG T current**

The results described in this paper were obtained from relatively small diameter cells (Cm = 12.6 ± 1.2 pF; n = 245) in which HVA Ca$^{2+}$ current was largely absent after a combination of intracellular F$^-$ and application of ω-conotoxin GVIA (ω-CgTX). Figure 1A shows a family of inward currents evoked from a holding potential of −90 mV to test potentials of −60 to −20 mV before application of 1 μM ω-CgTX. Both inactivating and more sustained inward current components are present. After 1 μM ω-CgTX was applied for 2 min, little residual noninactivating inward current remained in this neuron (Fig. 1B). Figure 1C displays the current-voltage relationship from this experiment before and after blockade of HVA current with ω-CgTX. The example shows that ω-CgTX-sensitive current contributes substantially to inactivating current even at potentials of −40 to −20 mV. Figure 1D summarizes complete current-voltage relationships for 12 cells in which HVA current was removed by the combination of internal F$^-$ and pretreatment with 1 μM ω-CgTX. T current starts to activate at about −60 mV and peak current activation is achieved at around −20 mV. Many of the physiological properties of well-isolated T current in rat DRG neurons have been examined carefully by others (Bossu and Feltz 1986; Carbone and Lux 1987a,b; Lux et al. 1990) and only will be considered briefly here to verify the identity of the current under investigation. Comparable with previous results, the time constant of T current inactivation reached a limiting, voltage-independent value of ~20 ms over the range of −10 through +40 mV (e.g., −10 mV: 21.2 ± 6.1 ms, n = 8; +10 mV: 18.9 ± 3.0 ms, n = 8; +25 mV, 20.8 ± 3.4 ms, n = 6).

Over a limited range of voltages (−60 through −100 mV), the deactivation rates of T current in rat DRG neurons (Carbone and Lux 1987a) and thalamic neurons (Huguenard and Prince 1992) have been reported to exhibit substantial voltage-dependence. However, one T current variant has been reported to exhibit a limiting voltage-independent deactivation rate (Chen and Hess 1990). Figure 2B plots the voltage dependence of the T current deactivation time constant over the range of −60 to −160 mV. No indication of a voltage-independent component of deactivation was observed over this potential range. Figure 2B (○ and □) also plots activation time constants from fits of a Hodgkin-Huxley (1952) activation model to well-isolated T currents.

The complexity of the inactivation process is illustrated by examination of the steady-state inactivation curves obtained after prepulses of different conditioning durations (Fig. 2C).
C only reached after CV voltages of half availability were: 0.25 and 1 s were better described by two Boltzmann distributions. The t with 0: isolated T current was activated by 15-ms steps to 0 mV at 5 and 10 s, respectively.

Huxley activation model to T currents activated at voltages from 6 to 9 cells of activation time constants from a fit of a Hodgkin-Huxley activation model to T currents activated at voltages from 0.25, 1.0, 5, and 10 s, respectively. The slope factors were normalized to the current activated from 0.25, 1.0, 5, and 10 s, respectively. Each point is the mean ± SD of n experiments. Fitted line corresponds to an EC 50 of 75 μM.

Pharmacology of DRG cell T current

A variety of both inorganic and organic agents were tested for ability to block T current in DRG cells. These agents were chosen for several reasons. First, we examined some agents reported to be effective in blocking T current in other cell types. Second, because of the postulated importance of at least some T currents in the action of some anticonvulsant agents, we examined a number of different anticonvulsant drugs. Third, we have examined some agents with anesthetic effects. For each tested compound, we have attempted to define a maximal blocking effect and a half-maximal blocking concentration (EC 50). In most cases, no attempt was made to test systematically for use or voltage dependence in blockade. However, in some cases in which a difference between our results and other studies was observed, experiments were done to examine the effects of an agent on steady-state inactivation, voltage dependence of block, and use dependence.

Using Ni 2+ as an example, Fig. 3 illustrates the general procedure used to test for pharmacological effects and to develop concentration-response relationships. T current was elicited every 10 s (Fig. 3B) or every 20 s (e.g., Fig. 4B) by stepping to −25 mV from a holding potential of −90 mV-containing saline; control saline was flowing through the chamber at all other times. C m: concentration-response curves for inorganic blockers obtained from similar experiments are plotted along with fitted blocking function. Fitted values for EC 50 were as follows: La 3+ : 0.53 ± 0.12 μM (n = 1.1 ± 0.2); Zn 2+ : 11.3 ± 2.2 μM (n = 1.1 ± 0.2); Cd 2+ : 20 ± 4 μM (n = 1.2 ± 0.3); Ni 2+ : 51 ± 10 μM (n = 1.2 ± 0.3). All inorganic blockers produced 100% block of T current with maximal concentrations. D: a concentration-response curve for amiloride is displayed. Each point is an average of n = 5 experiments, and vertical bars represent SE from multiple determinations. Fitted line corresponds to an EC 50 of 75 ± 18 μM (n = 1.0 ± 0.1).
mV. Example currents in control saline and after blockade by 100 μM Ni2+ are shown in Fig. 3A. The time course of blockade and recovery of T current amplitude during the action of Ni2+ also is illustrated (Fig. 3B). In general, agents were applied and currents recorded until steady-state blockade was reached before returning to control saline. These plots also illustrate the stability of the DRG T current under the conditions of these experiments.

INORGANIC BLOCKERS. Figure 3C summarizes the effects of La3+, Cd2+, Ni2+, and Zn2+ on T current in DRG cells. The half-maximal blocking concentrations were in μM: 0.53 ± 0.12 (n = 1.1 ± 0.2), 11.3 ± 2.2 (n = 1.1 ± 0.2), 20 ± 4 (n = 1.2 ± 0.3), and 51 ± 10 (n = 1.2 ± 0.3) for La3+, Zn2+, Cd2+, and Ni2+, respectively. This order of potency resembles that seen in GH3 and hippocampal cells (see Table 1) and is consistent with the reported relative mide, valproate, and blockade and recovery of T current amplitude during the 50% reduction at 30 μM. We observed that in adult rat DRG 50% reduction of current occurred at 75.7 ± 18 μM (n = 1.0 ± 0.1) as shown in Fig. 3D.

ANTIEPILEPTICS. Coulter et al. (1989a) have shown that the antiepileptic, ethosuximide, reduces T current in thalamic neurons. This is believed to contribute to its efficacy in petit mal epilepsy. Because some antiepileptics (e.g., phenytoin) are very effective analgesics when used for treatment of various forms of neuropathic pain in doses similar to those for achieving anticonvulsant effects (McQuay et al. 1995; Swerdlow and Cundill 1981), we have tested a number of these agents for their effect on T current in DRG cells. Figures 4 and 5 summarize effects of phenytoin, ethosuximide, valproate, and α-methyl-α-phenylsuccinimide (MPS). An interesting feature of some of these compounds is that a saturating blocking effect is reached at <100% block.

**TABLE 1. Pharmacological properties of T-type Ca2+ current in rat DRG cells and other cells**

<table>
<thead>
<tr>
<th>Blocker</th>
<th>DRG EC50</th>
<th>GH3 EC50</th>
<th>Other DRG Studies</th>
<th>Thalamus nRT, VB</th>
<th>Hippocampus</th>
<th>Other</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel</td>
<td>EC50: 51 μM max: 100% (1.24)</td>
<td>EC50: 777 μM max: 100% (0.8)</td>
<td>~90% at 100 μM (chick)</td>
<td>nRT: 54% at 200 μM Vb: 66% at 200 μM max: 100%</td>
<td>230 μM</td>
<td>50–200 μM</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>EC50: 20 μM max: 100% (1.18)</td>
<td>EC50: 188 μM max: 100% (0.80)</td>
<td>~80% at 100 μM (rat)</td>
<td>nRT: 61% at 200 μM</td>
<td>EC50: 80 μM</td>
<td>max: 100%</td>
<td></td>
</tr>
<tr>
<td>Lanthanum</td>
<td>EC50: 0.53 μM max: 100% (1.08)</td>
<td>EC50: 2.4 μM max: 100% (2.12)</td>
<td>~50% at 20 μM (chick)</td>
<td>nRT: 97% at 100 μM</td>
<td>EC50: 1 μM</td>
<td>max: 100%</td>
<td></td>
</tr>
<tr>
<td>Amiloride</td>
<td>EC50: 7.5 μM max: &gt;90% (0.99)</td>
<td>EC50: 1.55 μM max: 100% (0.88)</td>
<td>~100% (chick)</td>
<td>nRT: 41% at 500 μM Vb: 38% at 500 μM</td>
<td>30 μM</td>
<td>43% at 300 μM</td>
<td></td>
</tr>
<tr>
<td>MPS</td>
<td>EC50: 170 μM max: 26% (2.1)</td>
<td>EC50: 1.1 μM max: 100% (0.79)</td>
<td>~100% (0.8)</td>
<td>nRT: 53% at 3 mM Vb: 52% at 3 μM</td>
<td>1 μM</td>
<td>50–200 μM</td>
<td></td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>EC50: 23.7 μM max: 100% (1.43)</td>
<td>EC50: 7.3 μM max: 100% (neonatal rat)</td>
<td>~250 μM max: 40% at 1 μM</td>
<td>EC50: ~250 μM</td>
<td>max: 50% at 1 μM</td>
<td>No effect up to 1 mM</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EC50: 7.3 μM max: 60% (1.34)</td>
<td>EC50: 1.5 μM max: 60% (1.34)</td>
<td>~60% at 1 μM</td>
<td>EC50: ~60% at 100 μM</td>
<td>~50% at 30 μM</td>
<td>~50% at 30 μM</td>
<td></td>
</tr>
<tr>
<td>Valproate</td>
<td>EC50: 330 μM max: 17% (1.92)</td>
<td>EC50: 9.5 μM max: 80% (1.2)</td>
<td>No effect up to 1 μM</td>
<td>EC50: ~200 μM max: 16% (rat nodose)</td>
<td>4–8 μM</td>
<td>6–200 μM</td>
<td></td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>EC50: 334 μM max: 100% (1.15)</td>
<td>EC50: 985 μM max: 100% (2.12)</td>
<td>~50% at 500 μM (mouse neonatal DRG)</td>
<td>EC50: 985 μM</td>
<td>max: 100% (1.6)</td>
<td>50 μM</td>
<td></td>
</tr>
<tr>
<td>Octanol</td>
<td>EC50: 122 μM max: 100% (1.14)</td>
<td>EC50: 244 μM max: 100% (2.01)</td>
<td>~75% at 1 μM (rat neonatal DRG)</td>
<td>EC50: 300 μM</td>
<td>max: 100% (1.26)</td>
<td>100 at 20 μM</td>
<td></td>
</tr>
<tr>
<td>Propofol</td>
<td>EC50: 12.9 μM max: 100% (1.26)</td>
<td>EC50: 80 μM max: 100% (2.01) (chick)</td>
<td>~100% (1.26)</td>
<td>100 at 20 μM</td>
<td>max: 100% (2.01)</td>
<td>200 μM</td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>EC50: 303 μM max: 100% (2.3)</td>
<td>EC50: 1.3 μM max: &gt;90% (1.4)</td>
<td>~100% (1.4)</td>
<td>75% at 1 μM</td>
<td>max: &gt;90% (0.4, rat)</td>
<td>22% at 0.65 μM</td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>EC50: 655 μM max: 100% (1.95)</td>
<td>EC50: 1.3 μM max: &gt;90% (1.4)</td>
<td>~100% (1.95)</td>
<td>75% at 1 μM</td>
<td>max: &gt;90% (0.4, rat)</td>
<td>22% at 0.65 μM</td>
<td></td>
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</table>

Values in parentheses in DRG and GH columns are number of neurons. nRT, thalamic reticular neurons; VB, thalamic ventral basal relay neurons. References and charge carrier: 1 This study (10 mM Br−); 2 Herrington and Lingle (1992) (10 mM Ca2+); 3 Coulter et al. (1989) (3 mM Ca2+); 4 Huguier and Prince (1992) (3 mM Ca2+); 5 Takahashi and Akaike (1991) (10 mM Ca2+); 6 Fox et al. (1987) (10 mM Ca2+); 7 Avery and Johnson (1996) (2 mM Ca2+); 8 Study (1994) (3–5 mM Ca2+); 9 Takahashi et al. (1989) (10 mM Ca2+); 10 McDowell et al. (1996) (10 mM Ca2+); 11 Linna (1998) (2 mM Ca2+); 12 Twombly et al. (1988) (50 mM Ba2+); 13 Kelly et al. (1990) (5 mM Ca2+); 14 Takenoshita and Steinbach (1991) (10 mM Ca2+); 15 Kostyk et al. (1992) (1.5 mM Ca2+); 16 Tang et al. (1988) (5 mM Ca2+); 17 Gross and Macdonald (1988) (2 mM Ca2+ and 5 mM Ba2+); 18 Okese et al. (1994) (2 mM Ca2+); 19 White et al. (1989) (2.5 mM Ca2+); 20 Scott et al. (1990) (2.5 mM Ba2+).
and B) of \( \sim 100 \pm 8\% \) with an EC\(_{50} \) of \( 23.7 \pm 0.5 \) mM (\( n = 1.4 \pm 0.4 \)). This is quite different from the reported sensitivity of thalamic relay neurons to ethosuximide (EC\(_{50} \) : 200 \( \mu \)M) (Coulter et al. 1989a,b), in which a maximal inhibition of only 40% was reported with some neurons being insensitive to ethosuximide. Kostyuk et al. (1992) reported maximum block of 100% and an EC\(_{50} \) of 7 \( \mu \)M in neonatal rat DRG. In contrast, Gross et al. (1997) reported an insensitivity of thalamic relay neurons to ethosuximide (EC\(_{50} \) : 1.9 \( \pm 0.4 \) ) . Similarly, thiopental, methohexital, and phenobarbital blocked T current with complete blockade of T current when tested in nine cells at \( \leq 100 \) \( \mu \)M (data not shown).

**Barbiturates.** All tested barbiturates could produce complete blockade of T current in rat DRG cells with apparent Hill coefficients close to 1 (Fig. 6C). An example of the complete inhibition of T current produced by methohexital is shown in Fig. 6A, whereas the amount of inhibition produced by two different concentrations of methohexital is illustrated in Fig. 6B. Pentobarbital, which was reported previously to be ineffective in blocking mouse DRG T current (Gross and Macdonald 1988), blocked rat DRG T with an EC\(_{50} \) of 334 \( \pm 74 \) \( \mu \)M (\( n = 1.2 \pm 0.3 \)).

**Carbamazepine.** Carbamazepine was not effective in blocking rat DRG T current when tested in nine cells at \( \leq 100 \) \( \mu \)M (data not shown).

**Valproic acid.** Valproic acid was reported to have no effect on thalamic neurons (Coulter et al. 1989a), but it produced a maximal block of \( \sim 17\% \) of T current in acutely dissociated rat nodose neurons (Kelly et al. 1990). In rat DRG cells, we observed a small block of DRG T current (Fig. 5A) with a comparable maximum block of 17 \( \pm 2.7\% \) (Fig. 5B), and an EC\(_{50} \) of 330 \( \pm 18 \) \( \mu \)M (\( n = 1.9 \pm 0.2 \); Fig. 5C).

5,5-Diphenylhydantoin. 5,5-diphenylhydantoin (phenytoin) produced maximum block of 60 \( \pm 3.7\% \), an EC\(_{50} \) of 7.3 \( \pm 1.3 \) \( \mu \)M (\( n = 1.3 \pm 0.2 \); Fig. 5C). Phenytoin had little effect on the voltage dependence of activation or voltage of half-availability of steady-state inactivation curves (\( n = 4 \), data not shown). The amount of block produced by phenytoin did not vary whether T current was elicited at 1/20 or 1/5 s in four cells.

**Conclusions.** In summary, the present study showed that 5,5-diphenylhydantoin, phenobarbital, and methohexital blocked LVA current by interacting with the inactivation process of T current. Valproic acid and carbamazepine produced no block of T current. These results suggest that T-type Ca\(^{2+}\) channels have different affinities for these antiepileptic drugs.
ANESTHETICS. All anesthetics or anesthetic-like compounds used in this study produced a complete block of T current and had apparent Hill coefficients close to 1 as shown in Fig. 8.

Octanol. An example of the inhibitory effect of octanol on rat DRG T current is shown in Fig. 8A. The concentration dependence of the octanol effect on one cell is illustrated in Fig. 8B. Octanol, an alcohol previously reported to block completely T currents in inferior olivary neurons at 20 μM (Llinas 1988), produced 50% blockade (Fig. 8C) at 122 ± 14 μM (n = 1.2 ± 0.2).

2.6 diisopropylphenol. 2.6 diisopropylphenol (propofol), an anesthetic, also blocked T current in rat DRG with 50% block at 12.9 ± μM (n = 1.3 ± 0.1; Fig. 8C). Because propofol is known to be a very potent GABAergic agent, we have tested effects of propofol on leak current in DRG cells. In such experiments (data not shown), 100 μM propofol reduced most of the inward current during voltage ramps from −90 to +90 mV without any effect on outward current. Addition of 10 μM La3+ completely blocked inward current, whereas propofol in the presence of this inorganic Ca2+ channel blocker had no apparent effect on outward leak current. Therefore, the apparent inhibition of T current by propofol is not contaminated by

EC50s in μM of 153 ± 25 (n = 1.2 ± 0.1), 502 ± 55 (n = 1.3 ± 0.1) and 1700 ± 252 (n = 1.2 ± 0.1), respectively.

Because of the earlier report that pentobarbital had no effect at ≈0.5 mM on mouse DRG T current (Gross and Macdonald 1988), we examined the voltage and use dependence of pentobarbital block to determine whether the conditions of current activation might influence the apparent block. Figure 7A depicts traces of currents evoked after 1-s prepulses at potentials from −90 to −45 mV. Peak T current amplitude activated from different conditioning potentials is plotted in Fig. 7B along with fits of a single Boltzmann to points obtained before, during, and after drug application. These experiments show very little effect of 300 μM pentobarbital on the voltage dependence of fractional availability of DRG T currents. Similar results were obtained with thiopental, which was the most potent barbiturate in blocking rat DRG T Ca2+ current in this study (data not shown). Furthermore, both the rate of onset of blockade by pentobarbital and the magnitude of block were identical at stimulation frequencies of 1/20 or 1/5 s (Fig. 7C). Thus the magnitude of T current blockade by pentobarbital does not appear to reflect any use-dependent aspect of its action.

FIG. 6. Sensitivity of T current in sensory neurons to barbiturates. A: 5 mM methohexital produces a complete blockade of T current in a rat sensory neuron (Cm = 13 pF, Rm = 5 MΩ). B: time course of blockade is shown for the same cell for 2 concentrations (0.2 and 5 mM) of methohexital. C: concentration-response curves for blockade of T current by several barbiturates are plotted from experiments similar to those shown in A and B. Note that all agents could produce complete block of T current activated at −25 mV. Fitted values for EC50 (in μM) and n values are: thiopental (EC50 = 153 ± 25, n = 1.2 ± 0.1), pentobarbital (EC50 = 334 ± 74, n = 1.2 ± 0.3), methohexital (EC50 = 502 ± 50, n = 1.3 ± 0.1), and pentobarbital (EC50 = 1700 ± 252, n = 1.2 ± 0.1).

FIG. 7. Pentobarbital has little effect on the voltage-dependence of inactivation of T current and blockade exhibits little use-dependence. A: T currents were evoked by test steps to −25 mV after a 1-s prepulse at potentials from −110 to −35 mV before, during, and after application of 300 μM pentobarbital (Cm = 20 pF, Rm = 3 MΩ). B: peak amplitudes from traces in A are plotted as a function of voltage. Fit of a Boltzmann distribution to the points is plotted over each set of points (—). For this cell, half-maximal availability after 1-s prepulses occurred at −60 mV in control saline, −61.8 mV in pentobarbital, and −62.3 mV after wash of pentobarbital with little change in the slope factor. C: T current was elicited by a step to −25 mV from a holding potential of −90 mV either once every 5 s or once every 20 s. Onset and magnitude of block by 300 μM pentobarbital is identical in both cases. Cm = 13 pF, Rm = 9 MΩ.
in order of potency: propofol (EC$_{50}$ = 12.9 ± 4.6 µM, n = 12 ± 0.1), octanol (EC$_{50}$ = 122 ± 10, n = 12.2 ± 0.2), etomidate (EC$_{50}$ = 205 ± 23, n = 13 ± 0.1), and ketamine (EC$_{50}$ = 2,500 ± 383, n = 1.1 ± 0.1)

**Figure 8.** Blockade of rat DRG T current by octanol and parenteral anesthetics. A: traces show T current before, during, and after application of 100 µM octanol (C_m = 22 pF, R_s = 6 MΩ). B: time course of block by 3 different concentrations of octanol in the same cell as in A is shown. T current was activated by steps to −25 mV from a holding potential of −90 mV every 10 s. C: concentration-response curves are plotted for 4 different parenteral general anesthetics. Each agent could produce complete blockade of T current. All points are averages of multiple determinations (5–9 cells) and vertical lines represent SE. Fitted values for EC$_{50}$ (in µM) and n are in order of potency: propofol (EC$_{50}$ = 12.9 ± 4.6, n = 12 ± 0.1), octanol (EC$_{50}$ = 122 ± 10, n = 12.2 ± 0.2), etomidate (EC$_{50}$ = 205 ± 23, n = 13 ± 0.1), and ketamine (EC$_{50}$ = 2,500 ± 383, n = 1.1 ± 0.1).

Any effects of propofol on γ-aminobutyric acid (GABA) receptor activation.

**Ketamine.** Ketamine is an anesthetic agent known to block N-methyl-D-aspartate (NMDA) receptors (Franks and Lieb 1994; MacDonald et al. 1987, 1991). Here, ketamine blocked T current with 50% block at 2.5 ± 0.4 mM (n = 1.1 ± 0.1; Fig. 8C).

**Etomidate.** Etomidate is a general anesthetic reported to potentiate GABA currents (Yang and Uchida 1996) and to have minimal effects on HVA Ca$^{2+}$ currents in canine myocardial cells (Buljubasic et al. 1996). It blocked DRG T currents completely and reversibly with an EC$_{50}$ of 205 ± 23 µM (n = 1.3 ± 0.1; Fig. 8C).

**Halothane and isoflurane.** Two frequently used volatile anesthetics, halothane and isoflurane, previously were reported to block neonatal rat DRG T current at concentrations even below that expected clinically (Takenoshita and Steinbach 1991). Here, under conditions that better isolate T current, we have reexamined the effects of these agents. Figure 9A shows current traces before, during, and after application of 370 µM isoflurane, whereas Fig. 9B shows from another cell the inhibition of T current by nominally 0.45 and 1 mM halothane. Complete concentration-response curves (Fig. 9C) show that both agents can produce 100% blockade with EC$_{50}$s of 303 ± 18 µM (n = 2.3 ± 0.3) for isoflurane and 655 ± 54 µM (n = 2.0 ± 0.2) for halothane (Fig. 9C).

**Convolvulants.** Because thalamic T current has been proposed to play a role in generation of epileptic activity (Huerguena and Prince 1994; Tsakiridou et al. 1995), we have tested two convulsant agents in our experiments. Pentylene-tetrazole is a potent convulsant substance for which the mechanism of action remains largely unknown, although inhibition of GABA current may contribute to its effects (e.g., Coulter et al. 1990b). There was no effect on DRG T current in five cells (1 mM), similar to previous results from thalamic cells (Coulter et al. 1990a). TBPS is a potent GABA$_A$ receptor antagonist presumably interacting with the picrotoxin binding site (Casida et al. 1985; Squires et al. 1983). TBPS (50 µM), when applied for 2–6 min, had no effect on rat DRG T current (9 cells). In four cells, TBPS was applied for ≥6 min without effect.

**Mibebradil.** A new antihypertensive Ca$^{2+}$ channel antagonist, mibebradil, recently has been reported to exhibit selective blocking effects on T-type currents in vascular smooth muscle (Mishra and Hermsmeyer 1994) and guinea pig atrial myocytes (Ertel 1996). Differential effects of mibebradil on cloned Ca$^{2+}$ channel variants, α1A, α1B, α1C, and α1E, are less pronounced (Bezprozvanny and Tsien 1995). However, the clinical effects of mibebradil presumably arise largely from action at peripheral targets because it does not penetrate the blood-brain barrier in vivo (Ertel and Clozel 1997). The ability of mibebradil to inhibit DRG T current is illustrated in Fig. 10A. This effect was only partially re-
versible, similar to experiments on cloned Ca$^{2+}$ channels (Bezprozvanny and Tsien 1995). Therefore escalating concentrations of drug were applied to each cell, as shown in Fig. 10B, to construct concentration-response curves (Fig. 10C). From a holding potential of −90 mV, inhibition of peak T current activated at −25 mV occurred at 3.0 ± 0.35 µM ($n = 1.3 \pm 0.2$). However, this concentration-response curve provides only a qualitative indication of the concentration-dependence of mibefradil action because blockade by this agent exhibits pronounced use- and voltage-dependent features. This aspect of mibefradil action is illustrated in Fig. 10D, in which the steady-state level of block by 1 µM mibefradil is shown to be dependent on the frequency of T-current activation. The effect of mibefradil on availability of T current for activation was also qualitatively examined using 1-s conditioning steps at potentials from −110 to −35 mV (Fig. 10E). The shift in the voltage dependence of fractional availability and change in apparent slope factor produced by mibefradil is consistent with the use-dependent aspects of its action observed in inhibition of cloned Ca$^{2+}$ channel variants (Bezprozvanny and Tsien 1995). Mibefradil more effectively reduces T current availability at more positive holding potentials.

**DISCUSSION**

This paper defines key pharmacological properties of well-isolated T-type Ca$^{2+}$ current in acutely dissociated adult rat DRG cells. Particular attention was given to anesthetic and anticonvulsant compounds, several of which were found to block T current in DRG cells. This work extends earlier observations on the pharmacological sensitivity of DRG T current by providing more complete concentration-effect information.

The present results in conjunction with other results fail to reveal a single identifying pharmacological feature of T current. Some of the pharmacological properties of rat DRG T current are shared with T current in other cell types, but several compounds appear to affect T currents in different cell types differently. However, any conclusions are somewhat limited because, in many cases, full concentration-response curves for blockade of T currents have not been defined. Because some compounds appear to produce only partial blockade of T current, our results emphasize that complete concentration-response information is critical in assessing the potential physiological consequences of a given pharmacological agent.

Table 1 provides a summary of pharmacological properties of different T currents. We have not included a number of compounds that are known to inhibit T currents but that are thought primarily as blockers of the L-type of HVA Ca$^{2+}$ current (Akaike et al. 1989; Ertel and Ertel 1996; Takahashi and Akaike 1990, 1991). In assessing potential pharmacological differences among different T currents, it is also important to realize that estimates of EC$_{50}$ may be influenced both by the identity of the charge carrier and its concentration (Lee and Tsien 1983). Similarly, voltage and use dependence of block may make it difficult to compare estimates obtained from different studies. In spite of these limitations, Table 1 indicates that different T currents have quite different pharmacological sensitivities. Two implications of this are the following. First, it should be noted that in the absence of compelling information about the molecular identity of T channels, the different pharmacological sensitivities may be indicative of a heterogeneous family of channels, each with similar kinetic characteristics. Second, differ-
ent T current blockers may have distinct physiological and/or clinical consequences. Because some compounds studied here may affect T current in clinically used concentrations, it becomes critical to know whether differences in the clinical actions of some agents may reflect, in part, differential effects of compounds on different T current targets.

The discussion that follows focuses on two aspects of our results: first, those compounds that appear to exhibit selectivity among different types of T current and, second, those compounds that may have effects on T current at concentrations used in some clinical situations.

Blockade of T current in DRG neurons by anticonvulsants

SUCINIMIDES. Succinimides are a class of antiepileptics effective against petit mal generalized absence seizures (Macdonald and McLean 1986). Ethosuximide appears to be over an order of magnitude (250 μM vs. 24 mM) less effective at blocking DRG T current than in blocking T current in thalamic neurons (Coulter et al. 1989a,b). Yet, the blockade is complete in DRG cells but only partial (40%) in thalamic neurons. The situation appears to be reversed for MPS, where complete block of thalamic current at high concentrations is reported, whereas block of DRG T current occurs at lower concentrations but only partially. Effects of MPS on DRG cells are within the clinically relevant concentration range (55–220 μM) (Strong et al. 1974). We have no explanation for these differences except that different receptors for these drugs may exist between thalamic and DRG cells. At clinical concentrations, ethosuximide would be expected to have a negligible effect on DRG T current, whereas, at concentrations of MPS effective against thalamic T current, DRG T current should be inhibited partially.

Comparing the results on adult DRG neurons and thalamic neurons suggests that these two categories of T current exhibit quite different sensitivities to the succinimides, whereas the sensitivity of GH3 cell T current to ethosuximide is within the range observed here for DRG neurons. The weak effects of ethosuximide on T current in DRG cells observed here, despite the maximal block of 100%, agree closely with an estimate of ethosuximide block of T current in cultured rat DRG neurons (Gross et al. 1997), but differ markedly (EC50 ~ 7 μM) from results in an earlier study on neonatal rat DRG neurons (Kostyuk et al. 1992).

PHENYTOIN. Phenytoin, also an anticonvulsant, has been reported to inhibit T current in a neuroblastoma line (Twombly et al. 1988) and in rat hippocampal neurons (Yaari et al. 1987). In both cases, 50% inhibition required as much as 30–100 μM phenytoin. Similarly, 100 μM phenytoin reduced T current only 33% in rat thalamic neurons (Coulter et al. 1989b). Because free serum concentrations for anticonvulsant effects of phenytoin are thought to be 4–8 μM (Coulter et al. 1989b), inhibition of T current is not thought to contribute to the anticonvulsant effects of phenytoin. However, the maximal level of inhibition by phenytoin at higher concentrations was not defined in these systems, such that the EC50 for the blockable component of current was not determined. We observed that phenytoin blocked DRG T current with an EC50 of 7.3 μM with maximal block being ~60%. The magnitude of phenytoin block at 100 μM is comparable with that seen in other systems (Coulter et al. 1989a; Twombly et al. 1988). Although block by concentrations as low as 10 μM phenytoin also has been observed on T current in both neuroblastoma cells (Twombly et al. 1988) and in thalamic neurons (Coulter et al. 1989a), it is unclear whether the concentration-dependence of phenytoin block in these different systems is similar. On the basis of the present results, blockade of T current in rat DRG neurons is achieved with concentrations of phenytoin that are within a clinically relevant range.

VALPROATE. Valproic acid is an anticonvulsant the primary effects of which are thought to involve Na+ currents (Van den Berg et al. 1993), but that also affects GABAergic synaptic transmission (Macdonald and McLean 1988). A previous study on rat nodose ganglion neurons (Kelly et al. 1990) reports a small block of T current at 200 μM, comparable with the present results. Our data show that valproate produces only a partial block of T current (maximal block ~17%) with an EC50 of 330 μM. Because therapeutic serum levels of valproate are 17.5–210 μM (Kelly et al. 1990), it is unlikely that blockade of T currents plays a role in its anticonvulsant activity.

The one cautionary remark to this conclusion is that even a partial block of T current may be sufficient for important clinical effects (e.g., ethosuximide).

BARBITURATES. Barbiturates represent a class of drugs used for both their anticonvulsant and anesthetic-hypnotic properties. Pentobarbital (500 μM) has been reported to be without effect on mouse DRG T current but to reduce HVA Ca2+ currents in the same cells (Gross and Macdonald 1988). In contrast, we have found that pentobarbital can produce a complete blockade of T current with an EC50 of 334 μM. Except for the difference in species (mouse vs. rat) and condition of the cells (cultured neonatal mouse DRG vs. acutely dissociated adult rat DRG), we cannot account for the difference in reported effects of pentobarbital between these studies. Furthermore, we have found that all other tested barbiturates could completely block rat DRG T current with EC50 values of 153, 502, and 1,700 μM for thiopental, methohexital, and phenobarbital, respectively. Because clinically relevant anesthetic concentrations of barbiturates generally range from 25 to 100 μM (Franks and Lieb 1994), it is unlikely that effects of barbiturates on T current participate in anesthesia. However, barbiturates (e.g., thiopental and pentobarbital) at 5–20 times higher concentrations than those used to induce anesthesia are used to achieve brain protection during aneurysm clipping (Barash et al. 1992). In such cases, inhibition of T current and a decrease of neuronal excitability may contribute to their neuroprotective properties, the mechanism of which is largely unknown.

Effects of other general anesthetics on T currents

PROPOFOL. Propofol is a frequently used parenteral anesthetic that inhibits DRG T current completely with 50% inhibi-
anesthetic. It was shown previously to block neonatal rat more than an order of magnitude less effective at blocking iso¯urane has been reported to block T current in neonatal cated that mibefradil exhibits substantial selectivity between
DRG T current with an EC50 of 320

In very high concentrations, it also blocks completely rat

m

m

neurons by iso¯urane of 320

for potentiation of GABA currents in cerebellar Purkinje Tsien ( 1995 ) observe qualitatively similar use-dependent
anesthesia also may involve effects on primary afferent The present results identify several compounds that appear
motivated by the lack of concentration-response curves state-dependent aspects of mibefradil action, we would ex-

( McDowell et al. 1996 ) , the present experiments were ( Mishra and Hermsmeyer 1994 ) . Because of the pronounced

Halothane, which is structurally and clinically different from iso¯urane, is another frequently used volatile general
anesthetic. It was shown previously to block neonatal rat DRG T currents with an EC50 of ~100 μM with an extremely

shallow concentration response curve ( n = 0.4 ) ( Takeno

shita and Steinbach 1991 ) . In contrast, in GH3 cells, blockade by halothane was less effective with an EC50 of 1.3 mM
(n = 1.4 ) ( Herrington et al. 1991 ) . The present data (EC50 = 655 μM) suggest that rat DRG T currents are somewhat more sensitive to halothane than neuroendocrine cells. The disparity between our results and previous data on neonatal DRG cells may perhaps be accounted for by a better isolation of T current from HVA current in the present experiments. However, given that neonatal rodent DRG T current also has been reported to exhibit unusual sensitivity to ethosuximide ( Kostyk et al. 1992 ) and iso¯urane ( Scott et al. 1990 ) and neonatal mouse T current may exhibit a weaker sensitivity to pentobarbital ( Gross and Macdonald 1988 ) than reported here, it also remains possible that some unusual T current variant may be present in neonatal rodent sensory neurons.

One MAC for halothane would be ~310 μM, a concentration producing only ~20% block of DRG T current. Thus halothane is ~3.5 times less potent than iso¯urane at equipotent MAC concentrations. Similarly, halothane was less po-
tent than iso¯urane in blocking T current in a thyroid C-cell line ( McDowell et al. 1996 ) . The difference in potency
between iso¯urane and halothane in inhibiting T current may be one factor contributing to observed differences in clinical properties of these anesthetics. In comparison with halo-

thanes, iso¯urane provides somewhat more analgesia ( Levine et al. 1986 ) , more muscle relaxation ( Ali and Savareseánesthetic properties of this agent.

ETOMIDATE. Etomidate is an anesthetic that, in clinically
relevant concentrations of 4 μM, potentiates GABA current-

(KETAMINE. Ketamine is an anesthetic that is a noncompeti-
tive NMDA receptor antagonist ( MacDonald et al. 1991 ) .
In very high concentrations, it also blocks completely rat

ISOFLURANE AND HALOTHANE. Iso¯urane is currently the
most frequently used volatile anesthetic. Although iso¯urane has been reported to block T current in neonatal

rat DRG cells ( Takenoshita and Steinbach 1991 ) , rat hip-

pocampal neurons ( Study 1994 ) and thyroid C cell line ( McDowell et al. 1996 ) , the present experiments were moti-
vated by the lack of concentration-response curves from previous studies and the clinical importance of this
anesthetic. Here, iso¯urane blocked adult rat DRG T current with an EC50 of ~2.5 mM. Effects of ket-

amine on T current appear unlikely to contribute to its clinical effects.

OTANOL. Octanol has been reported to block T current in thalamic relay neurons at low micromolar concentra-
tions ( Llinas and Yarom 1986 ) , and 20 μM octanol has been reported to completely block T current in inferior olivary neurons ( Llinas 1988 ) . Similar sensitivity was reported in
cultured neonatal rat DRG with 1 μM octanol producing almost complete blockade of T type current ( Scott et al. 1990 ) . In contrast, T current in adult rat DRG neurons does not exhibit any unusual sensitivity to octanol. The EC50 for
blockade of adult rat DRG T current by octanol is 120 μM,
which is comparable with the EC50 of 244 μM for inhibition of GH3 T current ( Herrington and Lingle 1992 ) and to
the anesthetic ED50 for tadpoles of ~100 μM ( Franks and Lieb 1984 ) . Additional examination of the effects of octanol and other anesthetics on thalamic and inferior olivary neurons would be of value.

ETOMIDATE. Etomidate is an anesthetic that, in clinically
relevant concentrations of 4 μM, potentiates GABA current-
s ( Yang and Uchida 1996 ) . Blockade of DRG T type

current with EC50 of 205 μM is unlikely to contribute to
anesthetic properties of this agent.

ISOFLURANE AND HALOTHANE. Iso¯urane is currently the
most frequently used volatile anesthetic. Although iso¯urane has been reported to block T current in neonatal
rat DRG cells ( Takenoshita and Steinbach 1991 ) , rat hip-
pocampal neurons ( Study 1994 ) and thyroid C cell line ( McDowell et al. 1996 ) , the present experiments were moti-
vated by the lack of concentration-response curves from previous studies and the clinical importance of this
anesthetic. Here, iso¯urane blocked adult rat DRG T current with an EC50 of 303 μM. This compares with the EC50 for potentiation of GABA currents in cerebellar Purkinje
neurons by iso¯urane of 320 μM ( Hall et al. 1994 ) . Simi-
larly, 1 minimum alveolar concentration ( MAC: produc-
ing anesthesia in 50% of subjects ) for iso¯urane in mam-
als corresponds to an aqueous concentration of ~320 μM ( Franks and Lieb 1994 ) at 37°C. Thus, these results
suggest that at 1 MAC, >50% of T current is blocked in
rat DRG neurons, suggesting that iso¯urane-induced anesthesia also may involve effects on primary afferent
transmission.

Halothane, which is structurally and clinically different
from iso¯urane, is another frequently used volatile general
anesthetic. It was shown previously to block neonatal rat DRG T currents with an EC50 of ~100 μM with an extremely

shallow concentration response curve ( n = 0.4 ) ( Takeno-

shita and Steinbach 1991 ) . In contrast, in GH3 cells, block-
ade by halothane was less effective with an EC50 of 1.3 mM
small differences (about twofold) in effectiveness between rat DRG T current and GH3 T current.

An interesting facet of pharmacological inhibition of T current is that some blockers (e.g., phenytoin, valproate, MPS), although in some cases acting at low concentrations, produce incomplete block when applied even at higher concentrations. Because partial block of any target may be sufficient to produce a clinically important effect, this emphasizes the importance of defining complete concentration-response information for any compound.

Finally, a number of compounds, including phenytoin, MPS, and isoflurane, were identified that do appear to affect DRG T current at concentrations that most likely occur clinically. Although the contribution of DRG T current to the clinical effects of these compounds is unclear, the possibility must be considered that some aspects of the clinical effects of particular compounds may involve partial inhibition of T current in particular target cells.

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