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Volatile Anesthetics Reduce Low-voltage-activated Calcium Currents in a Thyroid C-Cell Line

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Background: **Volatile anesthetics may act in part by inhibiting voltage-dependent calcium channels. The effects of several volatile agents on three types of calcium channels in a thyroid C-cell line were examined.**

Methods: **Whole-cell calcium currents were recorded using standard patch clamp techniques. Current-voltage relationships were derived before, during, and after application of isoflurane, enflurane, or halothane. Low-voltage–activated (LVA; T type) calcium currents were isolated based on the voltage range of activation. High-voltage–activated (HVA) calcium currents were separated into L and N types using ω-conotoxin GVIA (ω-CTX) and nicardipine.**

Results: **All three agents reversibly decreased both LVA and HVA currents at clinically relevant concentrations. Isoflurane and enflurane both reduced peak LVA current more than peak HVA current: −33 ± 6% (mean ± SE) *versus* −22 ± 4% for 0.71 mM isoflurane (n = 6), and −46 ± 6% *versus* −35 ± 5% for 1.21 mM enflurane (n = 6). In contrast, halothane depressed LVA and HVA currents to a similar extent: −22 ± 4% *versus* −29 ± 3% for 0.65 mM halothane (n = 6). Isoflurane had no effect on LVA whole-cell current kinetics. Pretreatment with either ω-CTX (400 nM) or nicardipine (1 μM) did not change the sensitivity of HVA current to isoflurane.**

Conclusions: **Isoflurane and enflurane block LVA calcium channels more potently than either L-type or N-type calcium channels, but halothane shows no such preferential effect. These results may have implications for the mechanism action of volatile anesthetics. (Key words: Calcium channels. Volatile anesthetics: enflurane; halothane; isoflurane. Patch clamp. Cells: cell culture. Voltage clamp.)**

VOLATILE anesthetics may act at several sites to produce the clinical manifestations of general anesthesia. Like barbiturates and benzodiazepines, the volatile agents potentiate gamma-aminobutyric $acid_A$-dependent chloride currents,[1-3] which may explain, at least in part, their hypnotic and amnestic effects. Anesthetics also depress voltage-dependent calcium channels in various cell types.[4-15] Certain types of high-voltage-activated (HVA) calcium channels, particularly the N- and P-type calcium channels, are thought to be responsible for the calcium influx required for neurotransmitter release at presynaptic nerve terminals.[16,17] Depression of HVA calcium currents by volatile anesthetics thus could inhibit neuronal synaptic transmission and contribute to some aspects of the anesthetic state.

Another class of calcium channels, known as low-voltage-activated (LVA) or T-type calcium channels, are electrophysiologically and pharmacologically different from HVA calcium channels.[16,17] Low-voltage-activated calcium channels are thought to be responsible for membrane potential oscillations and bursting patterns in some central and peripheral neurons.[16-20] In addition, the excitability of the postsynaptic membrane may be influenced by LVA channels.[21,22] Based on these observations, blockade of LVA calcium channels by volatile anesthetics might be another mechanism by which volatile anesthetics exert their effects.

Our purpose was to examine the effect of volatile anesthetics on both LVA and HVA calcium currents in a model cell, the rat thyroid C-cell line. C cells secrete calcitonin in response to increases in serum calcium. We chose this model for two reasons. First, these cells express both LVA and HVA calcium channels,[23] including the N-type calcium channel that is found primarily in neuronal cells. Second, although they are endocrine cells, their embryologic origin is the neural crest, which also gives rise to neurons of both sympathetic and dorsal root ganglia. Thus thyroid C cells provide a model with which to study anesthetic effects on neuronal calcium channels.

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Materials and Methods

Cell Culture

Thyroid C cells (rat medullary thyroid carcinoma cell line, 6-23, clone 6) were obtained from American Type Culture Collection (Rockville, MD) and grown in Dulbecco's modified Eagle's medium (GIBCO, Gaithersburg, MD) with 10% equine serum (Hyclone, Logan, UT), 50 U/ml penicillin, and 50 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO). Cells were maintained in an incubator at 37°C with 5% carbon dioxide and 95% air. Electrophysiologic recordings were made at room temperature 1 to 4 days after plating cells on glass coverslips coated with poly-D-lysine.

Solutions and Reagents

The patch pipette solution contained 120 mM CsCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 11 mM 1,2 bis-(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid-CsOH, 10 mM N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES), and 5 mM MgATP (pH 7.3 with CsOH). The external solution contained 117 mM tetraethylammonium chloride (TEA-Cl), 5 mM CsCl, 10 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, and 5 mM glucose (pH 7.4 with TEA-OH). Although the absence of extracellular Na^+ appeared to eliminate inward sodium currents, tetrodotoxin (3 μM; Sigma Chemical Co.) was also included in the external solution in some early experiments. Because no difference was noted in the recorded currents regardless of whether tetrodotoxin was present, most experiments were performed without tetrodotoxin. Volatile anesthetics were introduced into the external solution by vigorously bubbling the solution with filtered air passed through agent-specific anesthetic vaporizers. Control solutions were bubbled with filtered air alone. Vaporizers were set to deliver the desired anesthetic doses (in volumes %), which were chosen to represent multiples of the minimum alveolar concentration (MAC) for each agent. Concentrations of anesthetics in solution were determined by gas chromatography as previously described[24] and are presented in table 1. Solution exchanges were accomplished by flushing the recording chamber with approximately five chamber volumes (7.5 ml), which took 30 to 45 s. In some experiments, nickel chloride ($NiCl_2$; Sigma Chemical Co.) and nicardipine (Sigma Chemical Co.) were added to the external solution. In other experiments, cells were incubated with ω-conotoxin GVIA (ω-CTX; Sigma Chemical Co.) for at least 20 min before recordings were made.

Table 1. Aqueous Concentrations of Volatile Anesthetics

Isoflurane		Enflurane		Halothane	
vol %	C_{aq} (mM)	vol %	C_{aq} (mM)	vol %	C_{aq} (mM)
1.25	0.38 ± 0.01	1.5	0.61 ± 0.01	—	—
2.50	0.71 ± 0.06	3.0	1.21 ± 0.02	1.50	0.65 ± 0.03
3.75	1.17 ± 0.09	4.5	2.00 ± 0.08	2.25	1.26 ± 0.04
—	—	—	—	3.00	1.80 ± 0.18

Aqueous concentrations of volatile anesthetics were measured with a gas chromatograph as described previously.[24] Each row represents approximately equipotent concentrations of each anesthetic as determined in humans at 37°C. All values for C_{aq} are means ± SE of three determinations at each anesthetic concentration.

Vol % = volumes percent setting on vaporizer dial; C_{aq} = concentration of anesthetic in aqueous solution (in millimoles/liter).

Whole-cell Patch Clamp Recording

Patch pipettes were made from borosilicate glass using a two-stage pipette puller (Narishige USA, Greenvale, NY) and heat polished on a microforge (ALA Scientific Instruments, Westbury, NY). Whole-cell calcium currents were recorded from individual thyroid C cells according to the technique of Hamill and colleagues[25] using commercial patch clamp amplifiers (model 8900, Dagan Corp., Minneapolis MN; Axopatch 200 and 200A, Axon Instruments, Foster City, CA). Data were acquired using pCLAMP version 5.5.1 (Axon Instruments) with either a 386- or 486-based microcomputer. Test potentials were applied from a holding potential of −80 mV to between −60 and +50 mV in 10-mV steps lasting either 160 or 320 ms each at 0.125 Hz. Currents were filtered at 1 kHz and digitized at 2.5 kHz. Leak and capacitative current subtraction was employed using the -P/4 protocol, in which currents evoked by four hyperpolarizing subpulses, each of amplitude P/4, were added to the current evoked by the depolarizing test potential of amplitude P. Current records were analyzed off-line using a custom program developed in the laboratory.[26]

Separation of LVA and HVA Calcium Currents

Low-voltage-activated current was identified by its lower activation threshold and more complete inactivation compared with HVA current. To ensure that the current measured at more depolarized test potentials

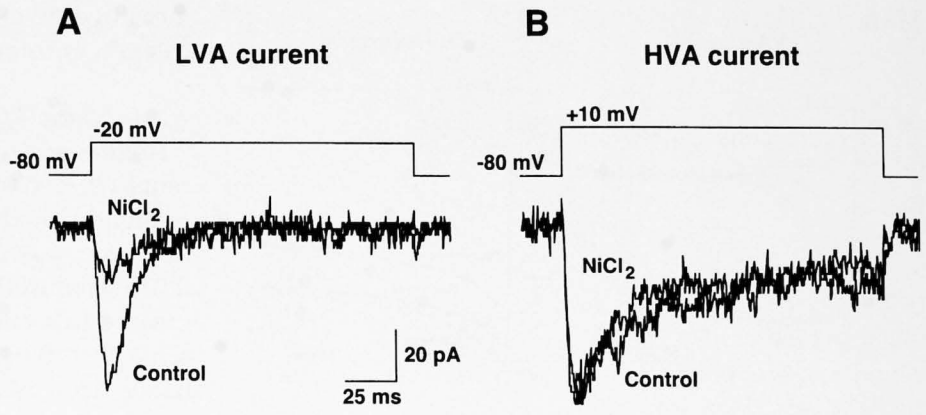

Fig. 1. Whole-cell calcium current traces obtained in a thyroid C cell in response to voltage steps to −20 mV (*A*) and +10 mV (*B*) before and after administration of $NiCl_2$ (100 μM). Low-voltage–activated calcium current (*A*) was markedly reduced by $NiCl_2$ in this cell, whereas high-voltage–activated calcium current (*B*) was not affected.

(more than 0 mV) was primarily HVA current, we used two approaches. In five cells, $NiCl_2$ (100 μM) was added to the external solution. This decreased the LVA current (measured at potentials of −30 or −20 mV) to noise levels, but only decreased the current measured at potentials greater than 0 mV by 2 ± 7% (fig. 1). In five other cells, LVA currents were inactivated by using a conditioning pulse (voltage step to −40 mV for 100 ms) before each test pulse. Current measured at potentials greater than 0 mV after the conditioning pulse was reduced by 22 ± 3%, consistent with a small degree of steady-state inactivation of N-type channels that has been reported previously for these cells.[23] Together these results suggest that the current measured at potentials greater than 0 mV consists primarily of HVA current.

Data Analysis

Calcium current-voltage relationships were derived in the control state, after application of anesthetic-containing solution, and after washout of anesthetic (recovery) for each cell. Anesthetic-induced decreases in peak calcium current amplitude are reported as percentage changes from an average of the control and recovery values to correct for any time-dependent changes in the currents. In 10 of 63 cells (15.8%), calcium current rundown was judged to be excessive (peak current amplitude at end of experiment ≤ 70% of control value), and these cells were excluded from analysis. Three other cells were excluded based on low signal-to-noise ratios. In the remaining 50 cells, the average recovery of peak current after washout of anesthetic was within 90% of the control value.

Changes in LVA current activation were assessed by measuring the time between the beginning of the voltage step and the peak inward current. For analysis of LVA current inactivation, the data were fit to an exponential of the form: $I = A^* \exp - (t/\tau) + C$, where τ is the time constant of the exponential current decay. To test for changes in the voltage dependence of LVA current activation, whole-cell currents measured at test potentials between −60 and −20 mV were converted to whole-cell conductances (g) using the equation

$$g = I/(V - E_{Ca})$$

where E_{Ca} is the reversal potential for calcium, which was approximately 60 mV in these experiments. The conductances were normalized to the maximum conductance and fit to the Boltzmann equation of the form

$$g/g_{max} = 1/[1 + \exp\{-(V - V')/k\}]$$

where V′ is the voltage required for half-maximal actival and k is the slope factor. To analyze the steady-state actival of LVA current, currents were normalized to the maximum current and fit to the Boltzmann equation of the form

$$I/I_{max} = 1/[1 + \exp\{-(V - V')/k\}]$$

Statistical significance was determined by analysis of variance and Scheffe's *post hoc* test where appropriate, and probability values less than 0.05 were considered significant. All values are reported as means and standard errors.

Results

Whole-cell Calcium Currents in Thyroid C Cells

Inward currents recorded from representative thyroid C cells are shown in figures 1 to 4. From a holding

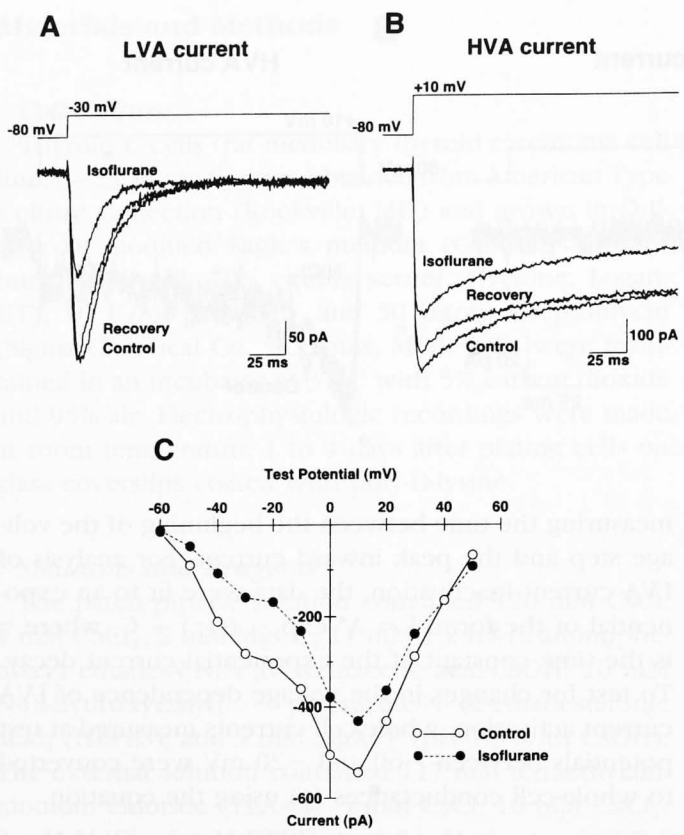

Fig. 2. Whole-cell calcium current traces obtained in a thyroid C cell in response to voltage steps to −30 mV to activate low-voltage–activated calcium (LVA) current (*A*) and +10 mV to activate high-voltage–activated (HVA) calcium current (*B*). Traces were obtained before (control), during (isoflurane), and after (recovery) exposure of the cell to 0.71 mM isoflurane. Isoflurane reduced the peak magnitude of both LVA (*A*) and HVA (*B*) calcium currents, but the reduction in LVA current was greater. There was some rundown of the HVA current in this cell, which was more pronounced at the peak than at the plateau of the current. Only the first 180 ms of the voltage steps are shown. Panel *C* shows the current-voltage relationships with (filled circles) and without (open circles) isoflurane for the same cell as in panels *A* and *B*.

potential of −80 mV, a transient inward current was activated at test potentials greater than −40 mV, with a maximum occurring at either −30 or −20 mV (fig. 2C). This corresponds to the T-type or LVA calcium current previously described in these cells.[23] A more slowly inactivating inward current, representing HVA calcium current,[23] was activated at potentials greater than −20 mV and peaked at test potentials greater than 0 mV (usually +10 mV; fig. 2C). We considered the peak inward current measured at −30 or −20 mV to be LVA calcium current, and the peak inward current measured at more than 0 mV to be exclusively HVA calcium current.

Anesthetic Effects on LVA Calcium Current

Isoflurane decreased both LVA and HVA calcium currents ($P < 0.05$ for both effects; figs. 2 and 5). The reduction of the LVA calcium current by isoflurane, however, was greater than that for HVA current ($P < 0.01$). Like isoflurane, enflurane also preferentially decreased LVA currents compared with HVA calcium currents ($P < 0.01$; figs. 3 and 6). Halothane, on the other hand, decreased LVA and HVA currents to a similar extent ($P > 0.05$; figs. 4 and 7). At clinically equivalent doses, enflurane was the most potent blocker of LVA current. The percentage reduction of LVA current was significantly greater for enflurane (1.21 mM) than for halothane (0.65 mM) at doses corresponding to 2 MAC ($P < 0.05$) and than both halothane (1.26 mM) and isoflurane (1.17 mM) at doses corresponding to 3 MAC (2 mM for enflurane; $P < 0.05$). With all three anesthetics, the decrease in calcium current was rapid (within 1 min of application) and readily reversible on washout of anesthetic.

To compare the relative sensitivity of HVA and LVA currents for each of the volatile anesthetics, we calculated the ratio of the percentage reductions in HVA to LVA current (HVA-to-LVA ratio). Isoflurane demonstrated a marked selectivity for LVA currents, with HVA-to-LVA ratios of 0.61 ± 0.18 (n = 6), 0.65 ± 0.08 (n = 6), and 0.68 ± 0.14 (n = 4) for 1, 2, and 3 MAC doses of isoflurane, respectively. With 1, 2, and 3 MAC doses of enflurane, HVA-to-LVA ratios were 0.76 ± 0.20 (n = 6), 0.78 ± 0.13 (n = 6), and 0.81 ± 0.04 (n = 6), respectively. Halothane, on the other hand, produced similar reductions in HVA and LVA current, with HVA-to-LVA ratios for 2, 3, and 4 MAC doses of halothane of 1.30 ± 0.27 (n = 5), 1.05 ± 0.25 (n = 4), and 1.27 ± 0.20 (n = 5), respectively. Because there were no significant dose-dependent differences in HVA-to-LVA ratios for each agent, the values were combined and found to be significantly greater for halothane than for either isoflurane and enflurane ($P < 0.05$).

Isoflurane Effects on LVA Calcium Current Kinetics

To investigate possible mechanisms for the effect of the volatile anesthetics, we examined the effect of isoflurane on the voltage- and time-dependent activation and inactivation of LVA calcium current. As shown in

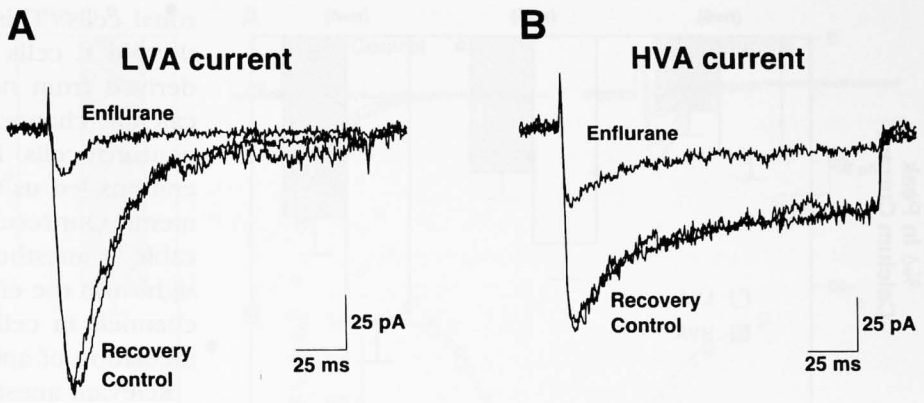

Fig. 3. Whole-cell calcium current traces obtained in response to voltage steps to −20 mV to activate low-voltage–activated (LVA) calcium current (*A*) and +20 mV to activate high-voltage–activated (HVA) calcium current (*B*). Traces were obtained before (control), during (enflurane), and after (recovery) exposure of the cell to 2 mM enflurane. Enflurane reduced the peak magnitude of both LVA (*A*) and HVA (*B*) calcium currents, but the reduction in LVA current was much greater.

panels A and B of figure 8, isoflurane had no effect on voltage-dependent activation or inactivation of LVA calcium current.

In four of five cells, isoflurane (0.71 mM) decreased the time required for peak activation of LVA current, although this was not statistically significant. The time to peak at −30 mV (n = 5) was 9.6 ± 1.0 ms for control *versus* 8.8 ± 1.2 ms for isoflurane ($P > 0.05$; see fig. 8C). The inactivation phase of the LVA current trace was fit to a single exponential as described in Methods (see fig. 8C). Isoflurane had no significant effect on LVA current inactivation at any test potential. At a test potential of −30 mV, for example, τ averaged 17.5 ± 1.3 ms in control *versus* 16.6 ± 3.8 ms for 0.71 mM isoflurane (n = 5; $P > 0.05$).

Anesthetic Effects on HVA Calcium Current

Anesthetic depression of HVA calcium currents was similar regardless of whether LVA current was present. Isoflurane (0.71 mM) decreased the current measured at potentials greater than 0 mV by 22 ± 4% (n = 6, see fig. 5). With the same concentration of isoflurane, the decrease was 17 ± 4% in the presence of $NiCl_2$ (n = 5), and 22 ± 5% after using a conditioning pulse to inactivate LVA current (n = 5).

Thyroid C cells express at least two types of HVA channels, the L-type and N-type calcium channels.[23] To determine whether there was a difference in the sensitivity of either of these HVA channels to isoflurane, we applied isoflurane in the presence of either the L-type calcium channel blocker nicardipine, or the N-type calcium channel blocker ω-CTX. After applying nicardipine (1 μM, n = 7), isoflurane (1.6 mM) reduced LVA current by 51 ± 6% and HVA current by 32 ± 5%, for a HVA-to-LVA sensitivity ratio of 0.64 ± 0.08. After incubation with ω-CTX (400 nM, n = 11), isoflurane (1.6 mM) reduced LVA and HVA currents by 55 ± 2% and 34 ± 3%, respectively, yielding a HVA-to-LVA ratio of 0.63 ± 0.05. These HVA-to-LVA ratios were not significantly different from HVA-to-LVA sensitivity ratios obtained with isoflurane without nicardipine or ω-CTX. This suggests that L-

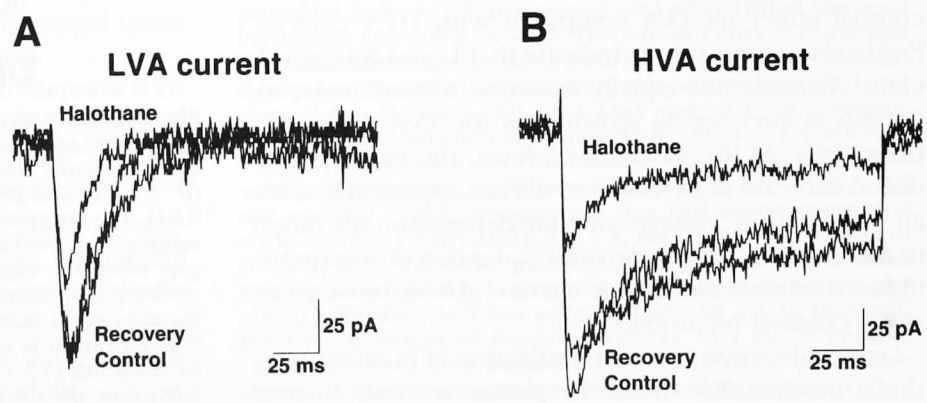

Fig. 4. Whole-cell calcium current traces obtained in response to voltage steps to −30 mV to activate low-voltage–activated (LVA) calcium current (*A*) and +10 mV to activate high-voltage–activated (HVA) calcium current (*B*). Traces were obtained before (control), during (halothane), and after (recovery) exposure of the cell to 1.8 mM halothane. In this cell, halothane reduced the peak magnitude of the HVA (*B*) calcium currents to a greater extent than of the LVA (A) calcium current, although this was not consistently seen.

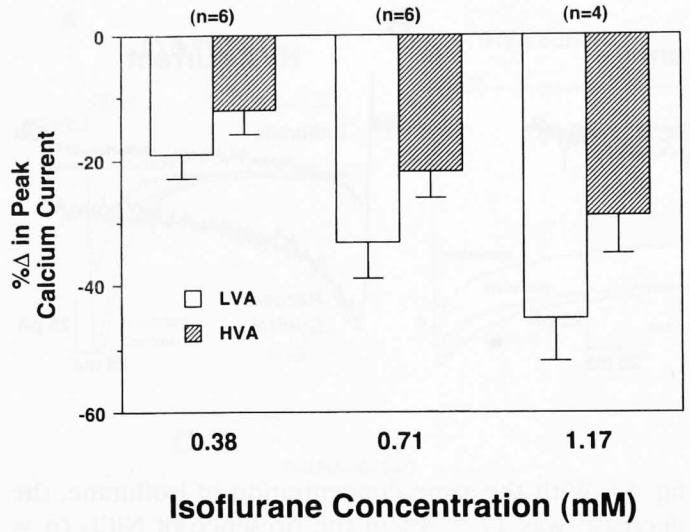

Fig. 5. Isoflurane decreased both low-voltage–activated (LVA; open bars) and high-voltage–activated (HVA; shaded bars) calcium currents. These effects of isoflurane were dose dependent ($P < 0.05$) and greater for LVA than for HVA currents ($P < 0.01$). The aqueous concentrations of isoflurane (expressed in millimoles/Liter) are the values obtained by gas chromatography when the vaporizer was set to deliver isoflurane concentrations (in volumes %) corresponding to 1, 2, and 3 MAC for this agent (table 1). The number of cells studied at each concentration is shown.

and N-type calcium channels are equally sensitive to isoflurane.

Discussion

Isoflurane, enflurane, and halothane decreased whole-cell calcium currents through both LVA and HVA calcium channels in a thyroid C-cell line. The halogenated ethers isoflurane and enflurane were more potent in their inhibition of LVA calcium channels compared with HVA channels. In contrast, halothane had no such preferential effect on LVA compared with HVA current. Furthermore, our results indicate that L- and N-type calcium channels are equally sensitive to isoflurane, although neither are as sensitive as the LVA current in these cells. At the mechanistic level, the isoflurane-induced decrease in LVA current did not appear to involve an alteration of voltage- or time-dependent activation or inactivation. A more detailed evaluation of anesthetic-induced alterations in LVA channel gating must await single-channel recordings.

One could argue that an investigation of possible anesthetic mechanisms should be performed only in neuronal cells. This is a limitation of our study, because thyroid C cells are not neurons. They are, however, derived from neural crest tissue and express N-type calcium channels, which are generally found only in neuronal cells. These and other more technical considerations led us to choose this cell line for our experiments. Our results, although perhaps not directly applicable to anesthetic mechanisms, may provide some insight into the effects of volatile anesthetics on calcium channels in cells that may be more important in the mediation of anesthetic effects.

Relevant anesthetic concentrations for use in experiments performed at subnormal temperatures remain undefined. Franks and Lieb[27] contend that anesthetic requirements do not vary with temperature if they are expressed as aqueous concentrations. An alternative hypothesis is to assume that anesthetic requirements at varying temperatures are correctly expressed as gaseous concentrations (*i.e.*, volumes %). Assuming Franks and Lieb[27] are correct, the relevant aqueous concentrations of isoflurane and enflurane for rat cells at 22°C would be 0.35 mM and 0.68 mM, respectively, similar

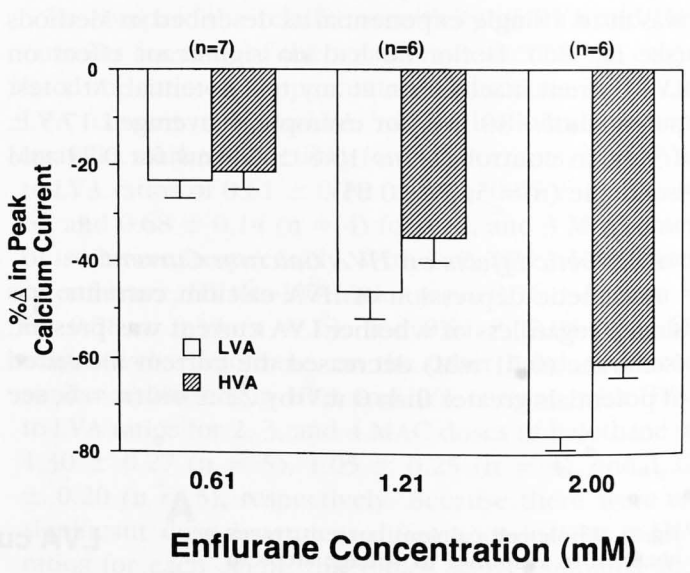

Fig. 6. Enflurane decreased both low-voltage–activated (LVA; open bars) and high-voltage–activated (HVA; shaded bars) calcium currents. These effects of enflurane were dose dependent ($P < 0.01$) and greater for LVA than for HVA currents ($P < 0.01$). The aqueous concentrations of enflurane (expressed in millimoles/Liter) are the values obtained by gas chromatography when the vaporizer was set to deliver enflurane concentrations (in volumes %) corresponding to 1, 2, and 3 MAC for this agent (table 1). The number of cells studied at each concentration is shown. Enflurane was slightly more potent in reducing LVA current than was isoflurane at 3 MAC ($P < 0.05$; note the different scale compared with figure 5).

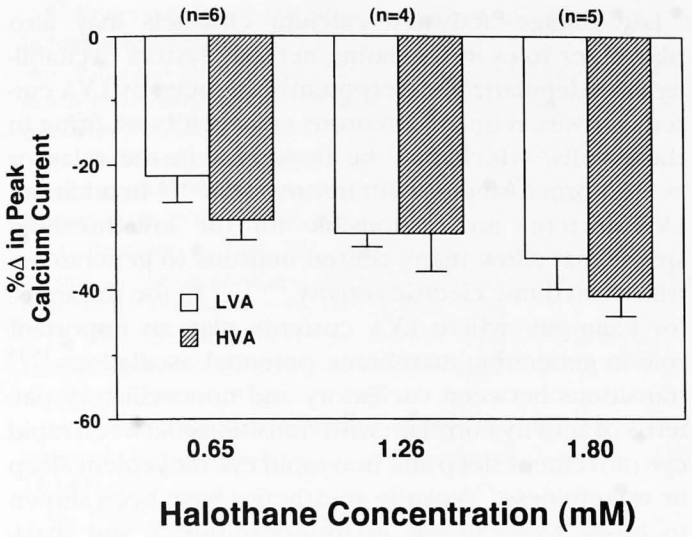

Fig. 7. Halothane decreased both low-voltage–activated (LVA; open bars) and high-voltage–activated (HVA; shaded bars) calcium currents. Unlike isoflurane and enflurane, however, halothane had no preferential effect on LVA compared with HVA currents at any concentration ($P > 0.05$). The aqueous concentrations of halothane (expressed in millimoles/Liter) are the values obtained by gas chromatography when the vaporizer was set to deliver halothane concentrations (in volumes %) corresponding to 2, 3, and 4 MAC for this agent (table 1). The number of cells studied at each concentration is shown.

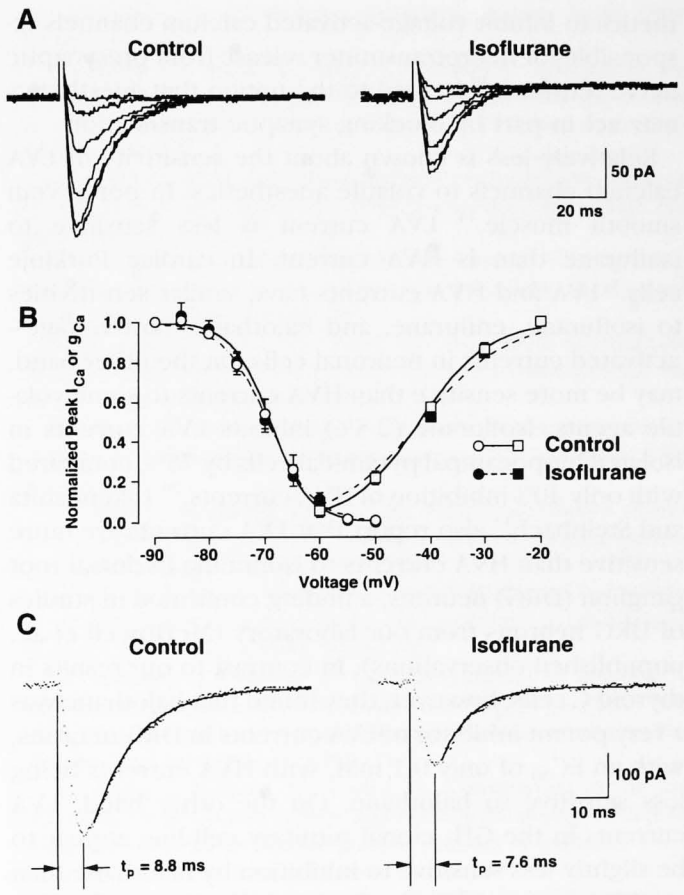

Fig. 8. Effects of isoflurane on low-voltage–activated (LVA) calcium current kinetics. Panel *A* shows inward current traces obtained before and after isoflurane during voltage steps to −20 mV after changing the holding potential from −85 mV (bottom traces) to −60 mV (top traces) in 5-mV increments. Cumulative data are shown in panel *B*, where normalized peak LVA current is plotted against holding potential before (open circles) and after (filled circles) isoflurane (0.71 mM; n = 4). Also shown in panel *B* is a plot of the voltage dependence of LVA current activation, where normalized peak LVA conductance is plotted against test potential before (open squares) and after (filled squares) isoflurane (0.71 mM; n = 4). The smooth curves represent the Boltzmann fits as described in Methods. For voltage-dependent inactivation, the half-maximal voltage (V′) and slope factor (k) were −69.4 ± 1.2 mV and −4.1 ± 0.2, respectively, for isoflurane compared with −69.0 ± 1.4 mV and −3.4 ± 0.1, respectively, for control (n = 4; P > 0.05). For voltage-dependent activation, V′ and k were −41.8 ± 0.7 mV and 7.8 ± 0.8, respectively, for isoflurane compared with −42.0 ± 1.1 mV and 6.3 ± 0.5, respectively, for control (n = 4; $P > 0.05$). In panel *C*, LVA current inactivation was well described by a single exponential both before and after isoflurane. At a test potential of −30 mV in this cell, the time constant of inactivation was 13.4 ms in control compared with 10.3 ms after isoflurane (0.71 mM). Also shown is the time to peak activation of LVA current (t_p).

to the lowest doses of isoflurane and enflurane used in our study, which produced about a 20% depression of LVA currents. According to the alternative hypothesis, the relevant aqueous concentrations for isoflurane and enflurane at 22°C would be about 0.6 mM and 1.16 mM, respectively (calculated aqueous concentrations at 22°C based on MAC values of 1.46% for isoflurane and 2.21% for enflurane[27]), which would produce approximately 30% to 40% depression of LVA currents. Whichever hypothesis is correct, between 20% and 40% depression of LVA channels occurs at "relevant" concentrations of these anesthetics, an effect that probably would cause significant alterations in neuronal function.

Volatile anesthetics have been shown to depress HVA calcium currents in various preparations. L-type calcium channels in smooth muscle from portal vein[14] and trachea,[15] and cardiac Purkinje[5] and ventricular cells[9,28] are all sensitive to volatile anesthetics. High-voltage–activated channels expressed by adrenal chromaffin cells are modestly inhibited by volatile anesthetics in clinically relevant concentrations.[9,10,11] Neuronal cells also express HVA calcium channels that have varying sensitivities to the anesthetics.[6,7,13,29] The ability of anes-

thetics to inhibit voltage-activated calcium channels responsible for neurotransmitter release from presynaptic nerve terminals[30] has led to the notion that anesthetics may act in part by blocking synaptic transmission.

Relatively less is known about the sensitivity of LVA calcium channels to volatile anesthetics. In portal vein smooth muscle,[14] LVA current is less sensitive to isoflurane than is HVA current. In cardiac Purkinje cells,[5] LVA and HVA currents have similar sensitivities to isoflurane, enflurane, and halothane. Low-voltage-activated currents in neuronal cells, on the other hand, may be more sensitive than HVA currents to some volatile agents. Isoflurane (2.5%) inhibits LVA currents in isolated hippocampal pyramidal cells by 75%, compared with only 40% inhibition of HVA currents.[29] Takenoshita and Steinbach[13] also report that LVA currents are more sensitive than HVA currents to isoflurane in dorsal root ganglion (DRG) neurons, a finding confirmed in studies of DRG neurons from our laboratory (McDowell et al., unpublished observations). In contrast to our results in thyroid C cells, however, they found that halothane was a very potent inhibitor of LVA currents in DRG neurons, with an EC_{50} of only 0.1 mM, with HVA currents being less sensitive to halothane. On the other hand, LVA currents in the GH_3 clonal pituitary cell line appear to be slightly less sensitive to inhibition by halothane than are HVA currents.[7] Little else has been reported on the effects of other volatile agents on LVA and HVA currents in neuronal cell types. Interestingly, however, propofol also potently blocks LVA currents in DRG neurons, with less of an effect on N-type HVA channels.[31]

Low-voltage-activated calcium channels are different from HVA channels, not only in biophysical properties but also in functional roles. Whereas HVA channels on presynaptic membranes are thought to function primarily as regulators of transmitter release, LVA channels on the dendrites of hippocampal[22] and neocortical[32] neurons may regulate postsynaptic membrane responsiveness to neurotransmitters. For example, excitatory postsynaptic potentials would be amplified by the inward calcium current elicited by LVA channel activation. In addition, because of the steep voltage dependence of LVA channel inactivation in the range of the normal resting potential of excitable cells, small hyper- or depolarizations induced by inhibitory or excitatory postsynaptic potentials could alter the number of LVA channels available for activation with subsequent depolarizations, causing either enhanced or decreased responsiveness of the dendrite to further stimulation.

Low-voltage-activated calcium channels may also play other roles in regulating nervous system excitability. The depolarizing afterpotential induced by LVA current activation in DRG neurons can elicit burst firing in these cells, which may be important in the relay or central processing of pain information.[20,33] In addition, LVA currents are responsible for the low-threshold spikes that allow many central neurons to generate intrinsic rhythmic electric activity.[18,34-36] In the thalamus, for example, where LVA currents play an important role in generating membrane potential oscillations,[18,35] transitions between oscillatory and nonoscillatory patterns of activity correlate with transitions between rapid eye movement sleep and non-rapid eye movement sleep or wakefulness.[37] Volatile anesthetics have been shown to block LVA current in hippocampus[8,29] and thalamus,[12] and thereby prevent these neurons from exhibiting bursting or oscillatory patterns.[12] Although it is unlikely that the volatile agents alter consciousness solely by blocking thalamic LVA channels, differences in the sensitivity of LVA channels to halothane and isoflurane may partially explain the different electroencephalogram patterns seen when these agents are used.[38,39] Clearly, inhibition of neuronal LVA calcium channels by volatile anesthetics would have widespread effects in the nervous system and could account, at least in part, for many anesthetic actions, both centrally and peripherally.

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