Anesthesiology
74:919–926, 1991

The Effects of Volatile Anesthetics on L- and T-type Calcium Channel Currents in Canine Cardiac Purkinje Cells

Hanna Eskinder, Ph.D.,* Nancy J. Rusch, Ph.D.,† Franjo D. Supan, Ph.D.,‡
John P. Kampine, M.D., Ph.D.,§ Zeljko J. Bosnjak, Ph.D.¶

The effects of halothane (0.45 and 0.9 mM, equivalent to 0.7 and 1.5%, respectively), isoflurane (0.54 and 1.23 mM, equivalent to 0.9 and 2.0%, respectively) and enflurane (0.65 and 1.48 mM, equivalent to 1.2 and 2.5%, respectively) on macroscopic L- and T-type Ca^{2+} channel currents were compared in single canine cardiac Purkinje cells using the whole-cell voltage-clamp technique. Cells were dialyzed with pipette solution containing CsCl and superfused with an external solution containing 10 mM $BaCl_2$ and tetraethylammonium chloride. The long-lasting (L) and transient (T)-type Ca^{2+} channel currents were measured by depolarizing the membrane from different holding potentials (HPs). Voltage steps from an HP of either −80 or −70 mV elicited a low threshold, rapidly inactivating inward current at −40 to −30 mV, which maximally activated at −14 ± 0.9 mV. This current was reduced by Ni^{2+} (100 μM) but not by nifedipine (1 μM), therefore resembling T-type Ca^{2+} channel current. In contrast, depolarizing steps from an HP of −40 mV elicited a sustained inward current that maximally activated at +4.1 ± 0.8 mV and was nifedipine-sensitive, showing the characteristics of an L-type Ca^{2+} channel current. Halothane, isoflurane, and enflurane produced a concentration-dependent suppression of total Ca^{2+} channel current in every cell studied. Separation of Ca^{2+} channel types showed that both L- and T-type Ca^{2+} channel currents were depressed to a similar extent by anesthetic administration. These agents reduced peak L- and T-type current elicited at each pulse potential but did not shift the current–voltage (I–V) relationship for either T- or L-type current activation. There was no significant difference between the sensitivities of either L- or T-type currents to halothane, isoflurane, or enflurane at equianesthetic concentrations. This suppression of L- and T-type currents may help to explain the negative inotropic and chronotropic effects of these agents on the heart. (Key words: Anesthetics, volatile: halothane; isoflurane; enflurane. Current: L-type; T-type; sensitivity. Heart: Purkinje fiber. Ions: calcium.)

TWO DISTINCT TYPES of Ca^{2+} channel currents have been characterized in different cell membranes, including the cardiac sarcolemma.[1–8] The transient (T-type) Ca^{2+} channel current activates at more negative membrane potentials and decays rapidly, whereas Ca^{2+} current through the long-lasting (L-type) channel activates at more positive membrane potentials and decays slowly during maintained depolarization. The L-type Ca^{2+} current is increased by β-adrenoceptor agonists[2,5] as well as by organic Ca^{2+}-channel agonists such as Bay K-8644, and it is decreased by organic Ca^{2+} channel blockers such as nifedipine.[1,2,5] In contrast, the T-type current is insensitive to dihydropyridine Ca^{2+}-channel agonists and antagonists[1–3] and is not affected by β-adrenergic agonists.[1–3,5] It is, however, preferentially blocked by Ni^{2+} at submillimolar concentrations and selectively inhibited by Tetramethrin.[5] Due to these different voltage-dependent and pharmacologic properties, L- and T-type Ca^{2+} channel currents can be measured separately in the same cell membrane.

In cardiac tissues, L- and T-type Ca^{2+} channel currents have been described in atrial[2] and ventricular myocytes,[3,4] sinoatrial node cells,[5] and recently in cardiac Purkinje cells.[1,6] It has been suggested that the two channel types play different functional roles in the heart. L-type current is the major contributor to the slow-inward current in cardiac ventricular cells.[3] Since the main physiologic function of ventricular cells is contraction, L-type channels play a major role in providing external Ca^{2+} required for excitation–contraction coupling. In the depolarized region of the atrioventricular node, L-type current is important also in the generation of and the plateau phase of the slow action potential. The physiologic role of the T-type Ca^{2+} current in cardiac cells is less well understood. The sinoatrial nodal[5] and cardiac Purkinje cells,[1,6] which are important for pacemaking and impulse conduction, have a relatively higher density of T-type channels than do cardiac ventricular cells.[3,6] In these cells, it is postulated that the T-type current plays a role in these two electrophysiologic events and also may participate in the initiation of contraction.

In the current study, experiments were designed to define the action of general anesthetic agents on the L- and T-type Ca^{2+} channel currents in order to understand how anesthetics modulate Ca^{2+} influx at the myocardial membrane. Although it has been demonstrated recently that halothane, isoflurane, and enflurane reduce whole-cell Ca^{2+} current in canine,[7] rat,[8] and guinea pig ventricular myocytes,[9] it is unknown if these agents exert differential effects on the two Ca^{2+} channel types. To examine

* Assistant Professor, Department of Anesthesiology.
† Assistant Professor, Department of Physiology.
‡ Research Associate, Department of Anesthesiology.
§ Professor and Chairman, Department of Anesthesiology; Professor of Physiology.
¶ Professor, Departments of Anesthesiology and Physiology.

Received from the Departments of Anesthesiology and Physiology, The Medical College of Wisconsin, Milwaukee, Wisconsin. Accepted for publication January 24, 1991. Supported in part by National Institutes of Health grants HL 01901 and HL 39776 (ZJB) and Anesthesiology Research Training Grant GM 08377.

Address reprint requests to Dr. Eskinder: Medical College of Wisconsin, MFRC, Room A1000, 8701 West Watertown Plank Road, Milwaukee, Wisconsin 53226.

this possibility, we measured macroscopic L- and T-type Ca^{2+} channel currents in patch-clamped canine cardiac Purkinje cells. The results show that the inward Ca^{2+} current through both L- and T-type Ca^{2+} channels is suppressed reversibly by halothane, isoflurane, and enflurane.

Materials and Methods

These experiments were approved by the Medical College of Wisconsin Animal Care Committee.

Cell Isolation

Adult mongrel dogs weighing 15–25 kg were placed in a Plexiglas® box and anesthetized with halothane. After attainment of surgical anesthesia, the trachea was intubated, and the lungs were ventilated with 1.5% halothane in oxygen. The chest was opened, and the hearts were excised rapidly and rinsed in cold oxygenated Krebs' solution. Free-running Purkinje fibers were removed from the left ventricle, cut into 2–3-mm lengths, and incubated at 37° C for 2.5–3 h in a low-Ca^{2+} solution containing (in millimolar concentrations): NaCl 140, KCl 5.4, $MgCl_2$ 5.0, glucose 5.5, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) 5.0, collagenase 4 mg/ml (Worthington [Type I] 161 units/mg), bovine albumin 2 mg/ml (Sigma), $CaCl_2$ 40 μM, pyruvic acid 5.0, and disodium salt of adenosine triphosphate (Na_2-ATP) 5.5, and the *p*H was adjusted to 6.2 with NaOH. After this incubation, the digested fibers were washed three to four times with K-glutamate solution and triturated with a Pasteur pipette until the tissue was completely dispersed. The K-glutamate solution was of the following composition (millimolar concentrations): K-glutamate 130, $MgCl_2$ 5.7, HEPES 5, glucose 5.5, Na_2-ATP 5, and disodium salt of ethylenediaminetetraacetic acid (Na_2-EDTA) 0.12, and the *p*H was adjusted to 7.4 with NaOH. Dispersed cells were stored in K-glutamate solution at 4° C before use.

Voltage-Clamp Recording

Dispersed cells were placed in a perfusion chamber (22° C) on the stage of an inverted microscope (IMT-2, Olympus Optical, Tokyo, Japan) equipped with modulation contrast. At 500× magnification, a hydraulic micromanipulator (MO-203, Narishige, Tokyo, Japan) was used to position heat-polished borosilicate patch pipettes with tip resistance of 1–5 MΩ on the membranes of single cardiac Purkinje cells. High resistance seals (2–10 GΩ) were formed, after which the pipette patch was removed by negative pressure to give access to the whole cell. Whole-cell Ca^{2+} currents were elicited every 5–10 s by 200–400-ms depolarizing pulses generated by a computerized system (P Clamp Software, Axon Instruments, Burlingame, CA). The currents were amplified by a List EPC-7 patch-clamp amplifier (List-Electronics, Darmstadt-Eberstadt, West Germany), and the amplifier output was low-pass filtered at 1,000 Hz. All data were digitized (sampling rate 10,000/s) and stored on a hard disk to permit analysis at a later time. Leak and capacitive currents were subtracted from each record by linearly summating scaled currents obtained during 10-mV hyperpolarizing pulses.

For recording of Ca^{2+} channel current, barium ion (Ba^{2+}) rather than Ca^{2+} was used as the charge carrier, since it slows the time-dependent decay of L-type current. Substitution of tetraethylammonium chloride (TEA · Cl) for Na^+ in the external solution and cesium ion (Cs^+) for K^+ in the pipette solution eliminated Na^+ and K^+ currents and permitted isolated measurement of Ca^{2+} current. The pipette solution contained (millimolar concentrations): CaCl 120, Na_2-ATP 5, EGTA 10, HEPES 10, $MgCl_2$ 1 (*p*H = 7.2). The external solution contained (millimolar concentrations): $BaCl_2$ 10, TEA · Cl 135, $MgCl_2$ 1, HEPES 10, and glucose 10 (*p*H = 7.4).

Prior to the onset of the experiments, current–voltage (I–V) curves were obtained 5–10 min after rupturing the cell membrane and again 5 min later to monitor time-dependent changes in Ca^{2+} current amplitude. A change of less than 5% in the amplitude of peak Ca^{2+} current between recordings was considered acceptable, and experimental data were obtained after Ca^{2+} current amplitude had stabilized. Cells with unstable Ca^{2+} current amplitudes were discarded. To determine the effect of the anesthetic agents on Ca^{2+} current, the inflow perfusate was changed to one in which a given concentration of anesthetic agent had been equilibrated. To compare halothane, enflurane, and isoflurane at approximately equianesthetic concentrations (1.0 and 2.0 MAC), agents were prepared in final bath concentrations of: halothane (0.45 and 0.90 mM, equivalent to 0.7 and 1.5%, respectively), enflurane (0.65 and 1.48 mM, equivalent to 1.2 and 2.5%, respectively), and isoflurane (0.54 and 1.23 mM, equivalent to 0.9 and 2.0%, respectively). These concentrations are referred to in the text as low and high doses, respectively, for each. Anesthetic content of the perfusate from the perfusion chamber was sampled and verified by gas chromatography. Drug effects were completed within 2–3 min, and the inflow perfusate was then changed to the control solution. A gradual decline ("rundown") in the amplitude of L-type current sometimes occurred, and cells showing more than a 10% reduction in amplitude during the recording period were discarded. The remaining cells in this study demonstrated 94–100% recovery from anesthetic effects.

Ca^{2+} channel current was elicited by depolarizing pulses (200–400-ms duration) from a holding potential of −80, −70, or −40 mV to stepwise (10-mV increments) more positive membrane potentials. A HP of −40 mV was used to elicit L-type current. T-type current was obtained by digital subtraction of currents obtained at an HP of −40

mV from total currents elicited from an HP of −70 or −80 mV in the same cell. In some experiments the effects of anesthetic agents on T-type current were examined in cells pretreated with 1 μM nifedipine. This was done to eliminate any residual L-type current remaining after voltage separation.

Statistics

For the study of anesthetics, all current measurements for I–V curves are expressed as a percent of the control measurement (mean ± SEM). Where appropriate, data are analyzed by the Student *t* test, and differences between anesthetics in suppressing peak currents are analyzed using one-way analysis of variance (ANOVA). Significance was defined as $P < 0.05$.

Results

Two Types of Ca^{2+} Channel Currents in Cardiac Purkinje Cells

Figure 1 shows whole-cell inward currents from a canine cardiac Purkinje cell bathed in a 10 mM $BaCl_2$ solution, illustrating the most commonly encountered current pattern. Current was activated progressively by 200-ms depolarizing pulses from a constant HP of −70 mV (fig. 1A) or −40 mV (fig. 1B) to consecutively more positive membrane potentials. Voltage steps from an HP of −70 mV to more positive membrane potentials elicited a rapidly inactivating, low-threshold current at −40 to −30 mV, which maximally activated at −20 to −10 mV (fig. 1A, top). At more positive membrane potentials (+20 or +10 mV), a long-lasting inward current also was measured (fig. 1A, bottom). In contrast, as shown in fig. 1B, voltage steps from an HP of −40 mV elicited only a sustained inward current that resembled the long-lasting current elicited at positive membrane potentials from an HP of −70 mV. This sustained current activated at −20 to −10 mV, with peak activation at +10 mV. The difference between the activation thresholds and the kinetics of inactivation between the two current types suggested the presence of two Ca^{2+} channel types in canine cardiac Purkinje cells. The I–V relationships of the Ca^{2+} currents shown in figures 1A and 1B are illustrated in figure 1C. Currents elicited from the HP of −70 mV were larger than those elicited from −40 mV. The difference in currents between the two different HPs corresponds to the transient (T-type) Ca^{2+} current.

We confirmed the presence of two types of Ca^{2+} channel currents in these cells by pharmacologic identification. In cardiac tissues, the Ca^{2+} channel blockers with the dihydropyridine structure are selective for the L-type Ca^{2+} channel current, whereas nickel (Ni^{2+}), at lower concentrations, preferentially inhibits the T-type Ca^{2+} channel

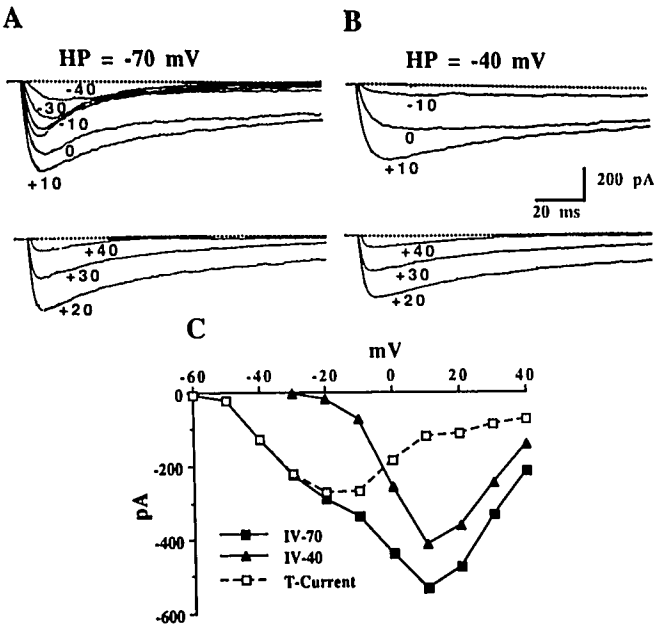


FIG. 1. The two types of Ca^{2+} channel currents in single cardiac Purkinje cells separated by depolarizing pulses from two holding potentials. *A:* Membrane currents in response to stepwise depolarizing pulses from a holding potential (HP) of −70 mV. Voltage steps were increased in 10-mV increments as indicated next to their respective tracings. A rapidly decaying inward current was elicited at −40 to −30 mV, while long-lasting inward current was seen at +10 to +20 mV. *B:* Currents in response to a stepwise depolarizing pulses from a HP of −40 mV. Only the long-lasting type of inward current was recorded. *C:* Peak current–voltage relationships were plotted for total current (HP = −70 mV), for L-type (HP = −40 mV) and T-type current (total current − L-type current).

current.[1,5,6] Figures 2A and 2B illustrate the effects of nifedipine (1 μM) and Ni^{2+} (100 μM) on Ca^{2+} channel currents. The L-type currents were recorded during depolarizing pulses (200-ms duration) from −40 to 0 mV (fig. 2, left), whereas the transient currents were elicited by depolarizing from −70 to −30 mV (fig. 2, right). Recordings in figures 2A left and 2B right show that nifedipine decreased the peak amplitude of the L-type current by 80%, whereas in the same cell it suppressed T-type current by only 11%. However, as shown in figure 2A right and 2B left, Ni^{2+} (100 μM) decreased the maximal T-type Ca^{2+} channel current recorded at −30 mV by 60% whereas it reduced L-type current amplitude only by 10%.

Effects of Volatile Anesthetic Agents on L- and T-type Ca^{2+} Channel Currents

Once we confirmed the two types of Ca^{2+} channel currents in canine cardiac Purkinje cells, we examined the effects of halothane, isoflurane, and enflurane on total L- and T-type Ca^{2+} channel currents. Figure 3 shows the effects of these agents on total whole-cell Ca^{2+} channel

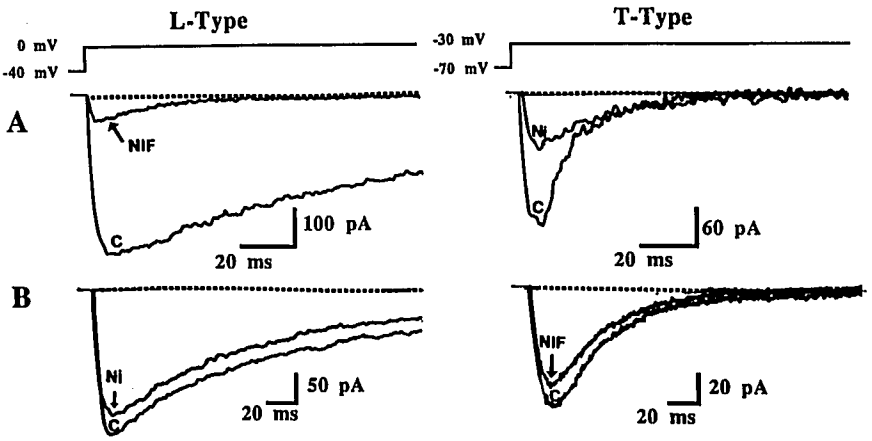


FIG. 2. Superimposed tracings showing the effects of nifedipine (1 μM) and Ni^{2+}, (100 μM) on the transient (T-type) and long-lasting (L-type) Ca^{2+} channel currents in two cardiac Purkinje cells. The L-type currents were elicited by depolarizing the cells from a holding potential of −40 to 0 mV (*left*). T-type currents were elicited by depolarizing the cell from −70 to −30 mV (*right*).

currents. A stepwise depolarization of the cell from an HP of −80 or −70 mV resulted in progressive activation of total Ca^{2+} channel current carried through both L-type and T-type channels. The effects of low and high doses of halothane, isoflurane, and enflurane on the I–V relationship for Ca^{2+} channel activation were quantitated by plotting peak normalized Ca^{2+} current as a function of membrane potential. All three anesthetic agents depressed the amplitude of total Ca^{2+} channel current in a dose-dependent manner in the entire voltage range studied. The voltage-dependency of the I–V relationship for channel activation was unaltered, however, suggesting that both L- and T-type Ca^{2+} channel currents were reduced.

We further examined the effects of anesthetic agents separately on L-type and T-type Ca^{2+} channel currents. A representative experiment demonstrating the experimental protocol is in figure 4. L-type current was elicited by voltage steps from −40 to 0 mV (fig. 4, left), and T-type current elicited by voltage steps was elicited from −80 to −40 mV (fig. 4, right). Currents were recorded before, during, and after exposure to low and high doses of halothane. Exposure of the cell to a low dose (0.7%) of halothane suppressed the amplitude of both L- and T-type Ca^{2+} channel currents, and this suppression was reversed after subsequent washout. At a higher anesthetic concentration (1.5%), the amplitudes of both L- and T-type Ca^{2+} channel currents were depressed further.

Figure 5 shows the effects of low and high doses of halothane, isoflurane, and enflurane on L-type Ca^{2+} channel current in canine cardiac Purkinje cells. Families of Ca^{2+} channel currents were generated by stepwise depolarizing pulses from an HP of −40 mV before and after exposure to anesthetic agents. Representative tracings, showing the effects of halothane, isoflurane, and enflurane on L-type Ca^{2+} channel currents elicited by voltage steps from −40 mV to 0 mV are provided in figure 5, top. All three anesthetic agents at the low dose significantly and reversibly suppressed the maximal L-type current recorded at 0 mV. Figure 5, bottom shows the effects of low and high doses of halothane, isoflurane, and enflurane on the I–V relationship for L-type Ca^{2+} current obtained from canine cardiac Purkinje cells. All three anesthetic agents depressed the amplitude of L-type Ca^{2+} channel current in a dose-dependent manner over the entire voltage range studied without a shift in the voltage dependency of the I–V relationship for channel activation.

Representative tracings showing the effects of low doses of halothane, isoflurane, and enflurane on T-type Ca^{2+} channel currents elicited by depolarizing pulses (200-ms duration for isoflurane and 400-ms duration for halothane and enflurane) from −70 to −30 mV are shown in figure

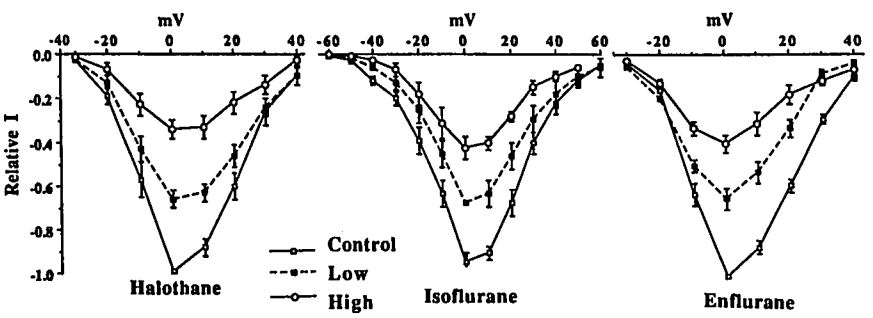


FIG. 3. Modulation of total whole-cell Ca^{2+} channel currents by low and high concentrations of halothane (0.7%, n = 8 and 1.5%, n = 8), isoflurane (0.9%, n = 7 and 2.0%, n = 6) and enflurane (1.2%, n = 10 and 2.5%, n = 9): peak current–voltage relationships obtained before and after exposure to low and high doses of inhalational agents in canine cardiac Purkinje cells. Cells were progressively depolarized from a holding potential of −70 mV to the corresponding potentials indicated on the abscissa. Symbols with error bars represent mean ± SEM.

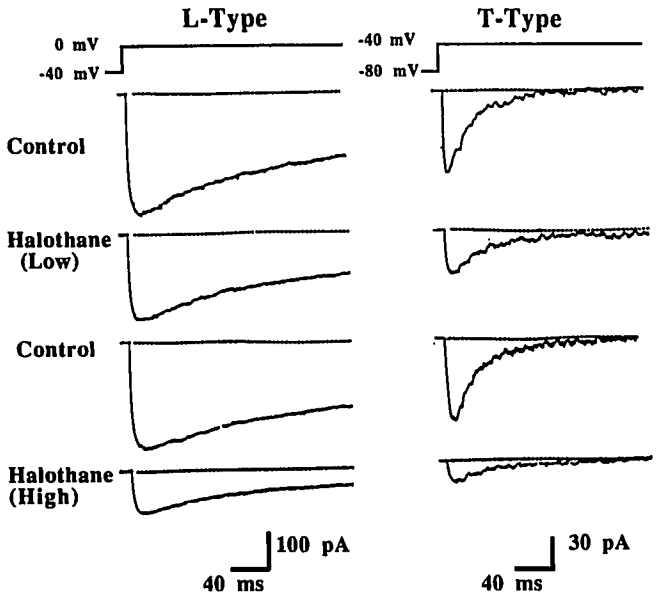


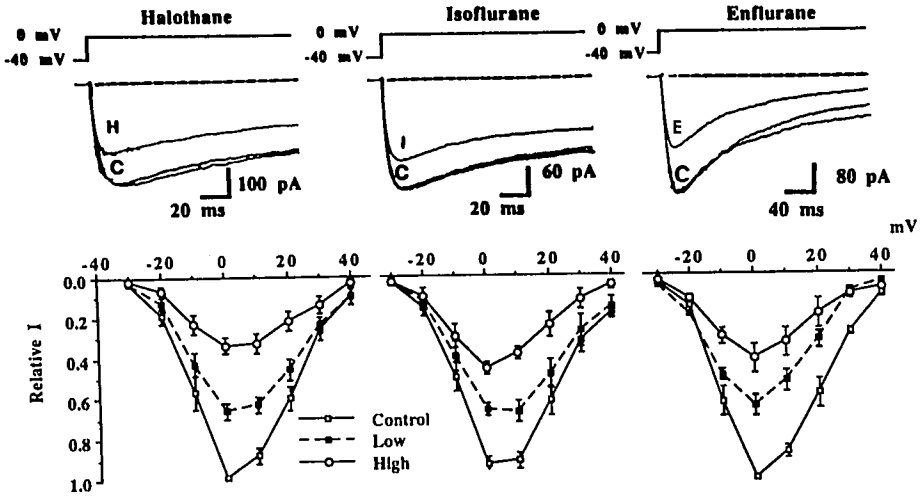
FIG. 4. Concentration-dependent effects of halothane on L- and T-type Ca^{2+} channel currents in a single canine cardiac Purkinje cell. L-type current was elicited by depolarizing the cell from −40 to 0 mV (*left*). T-type currents were elicited by depolarizing the cell from −80 to −40 mV (*right*). Exposure of the cell to 0.7% (low) and 1.5% (high) halothane depressed both L- and T-type currents in a dose-dependent and reversible manner.

6, top. All three anesthetic agents reversibly suppressed T-type Ca^{2+} channel currents recorded at −30 mV. The effects of halothane, isoflurane, and enflurane on the I–V relationship for T-type Ca^{2+} channel activation are shown in figure 6, bottom. Low and high doses of these anesthetic agents produced concentration-dependent suppression of the amplitude of T-type Ca^{2+} channel current over the entire voltage range studied without shifting the voltage dependency of the I–V relationship.

We also examined the effects of low doses of anesthetic agents on T-type current in three cells that were previously exposed to 1 μM nifedipine to eliminate residual L-type current remaining after voltage separation. In the upper panel of figure 7 are representative tracings showing the effect of a low dose of halothane on T-type Ca^{2+} channel current elicited by depolarizing pulses (200-ms duration) from −70 to −30 mV in a nifedipine-pretreated cell. Halothane reversibly suppressed this current. The peak I–V relationship in control solution, in the presence of nifedipine, and after exposure to halothane is shown in figure 7, bottom. Nifedipine selectively suppressed the peak current obtained with larger-amplitude depolarization steps that elicit L-type current, whereas with smaller-amplitude depolarizing steps that elicit T-type current the peak currents were unchanged. The remaining T-type current was subsequently suppressed by halothane.

The suppression of peak L- and T-type Ca^{2+} channel currents by all three anesthetic agents at low and high doses is summarized in figure 8. At low doses, halothane (n = 7), enflurane (n = 10), and isoflurane (n = 7) reduced peak L-type Ca^{2+} channel current to 66 ± 7, 68 ± 4, and 67 ± 4% of control values, respectively. Halothane (n = 6), enflurane (n = 7), and isoflurane (n = 6) at the same concentrations depressed peak T-type Ca^{2+} channel current to 67 ± 9, 70 ± 12, and 63 ± 10%, respectively. At higher concentrations, halothane (n = 6), enflurane (n = 9), and isoflurane (n = 6) further suppressed the L-type Ca^{2+} channel current to 37 ± 6, 41 ± 3, and 46 ± 4% of control, respectively. Higher concentrations of halothane (n = 4), enflurane (n = 5), and isoflurane (n = 4) also further depressed the T-type Ca^{2+} channel cur-

FIG. 5. Modulation of L-type Ca^{2+} channel currents by halothane, isoflurane, and enflurane. *Top:* Superimposed current traces in control solution, after exposure to low doses of the anesthetic agents, and after recovery in control solution. Voltage steps from a holding potential (HP) of −40 to 0 mV elicited L-type currents whose amplitudes were depressed markedly and reversibly by anesthetic agents. *Bottom:* The peak current–voltage relationships in control solution and after exposure to low and high doses of anesthetic agents. The concentrations of anesthetics used were: halothane (0.7%, n = 7 and 1.5%, n = 6), isoflurane (0.9%, n = 7 and 2.0%, n = 6) and enflurane (1.2%, n = 10 and 2.5%, n = 9). Cells were depolarized progressively from a HP of −40 mV to the corresponding potential indicated on the abscissa. Points with error bars represent the mean ± SEM.



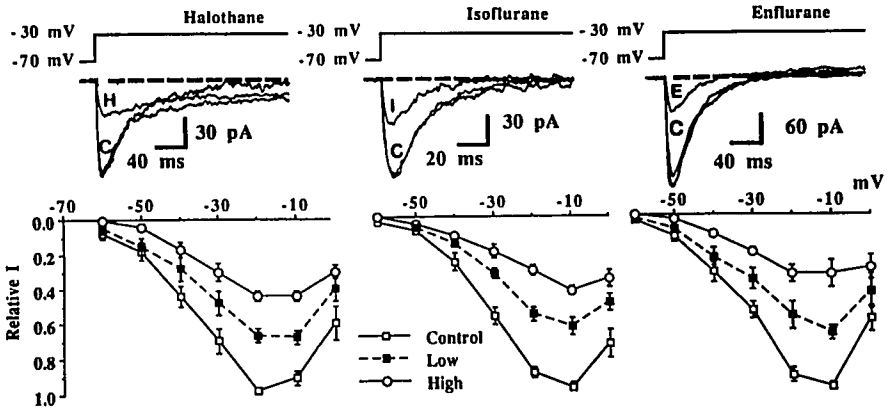


FIG. 6. Modulation of T-type Ca^{2+} channel currents by halothane, isoflurane, and enflurane. *Top:* Superimposed current traces in control solution, during exposure to low doses of the corresponding anesthetic agents, and after recovery in control solution. Voltage steps from a holding potential of −70 to −30 mV elicited T-type currents whose amplitudes were depressed markedly and revesibly by all of the anesthetic agents. *Bottom:* The peak current–voltage relationships in control solution and after exposure to low and high doses of inhalational agents. The concentrations of anesthetics used were: halothane (0.7%, n = 6 and 1.5%, n = 4), isoflurane (0.9%, n = 6 and 2.0%, n = 4) and enflurane (1.2%, n = 7 and 2.5%, n = 5). T-type currents were determined by digital subtraction of currents elicited from −70 and −40 mV. Points represent the means ± SEM.

rent to 44 ± 4, 41 ± 6, and 43 ± 5% of control value. There was no significant difference between the sensitivities of T- *versus* L-type current to the different anesthetics, and there were no significant differences between the anesthetics at equianesthetic concentrations.

The effect of anesthetic agents on the time course of inactivation of T- and L-type currents is shown in figure 9. Amplitude of T-type current was determined during a voltage step from −70 to −30 mV. Analysis in control solution showed that T-type Ca^{2+} channel current decayed to 64 ± 8, 38 ± 4, and 24 ± 12% of initial peak amplitude at 20, 40, and 60 ms, respectively. High concentrations of halothane (n = 4), isoflurane (n = 4), and enflurane (n = 4) did not affect the decay of T-type Ca^{2+} channel current. L-type Ca^{2+} channel current amplitude was determined during a voltage step from −40 to 0 mV. Analysis in control solution showed that L-type current decayed to 74 ± 5 and 55 ± 6% of initial amplitude at 100 and 200 ms, respectively. The decay of L-type current was not affected by lower concentrations of anesthetics (not

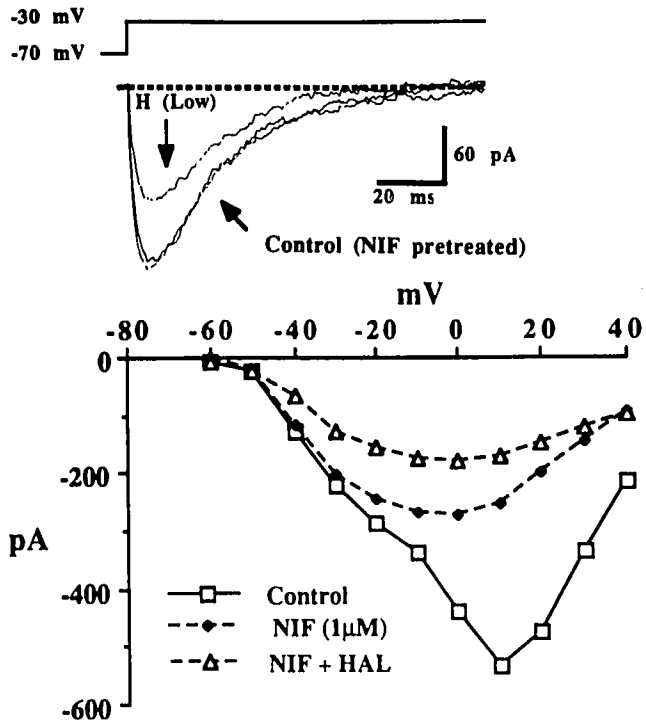


FIG. 7. Modulation of T-type Ca^{2+} channel currents by halothane in a canine cardiac Purkinje cell pretreated with 1 μM nifedipine (NIF). *Top:* Superimposed current traces in the absence and presence of nifedipine and during exposure to a low dose of halothane in nifedipine solution. Voltage steps from a holding potential of −70 to −30 mV elicited a T-type current, whose amplitude was depressed markedly and reversibly by halothane but not by nifedipine. *Bottom:* The peak current–voltage relationships in control solution, in the presence of nifedipine (NIF, 1 μM) and after exposure to a low dose of halothane (HAL, 0.7%). The cell was progressively depolarized from a holding potential of −70 mV to the corresponding potentials indicated on the abscissa.

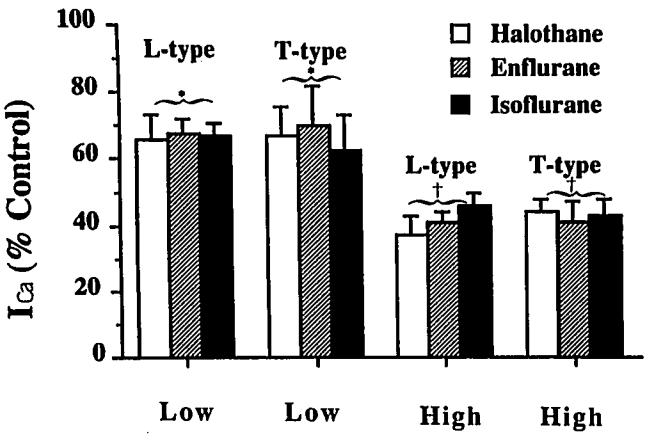


FIG. 8. Concentration-dependent depression of peak L- and T-type Ca^{2+} channel currents by low and high concentrations of halothane (0.7 and 1.5%), isoflurane (0.9 and 2.0%) and enflurane (1.2 and 2.5%) in 28 cardiac Purkinje cells. *Significant difference from control values ($P \leq 0.05$). †Significant difference from values obtained at a low dose of the same inhalational agent.

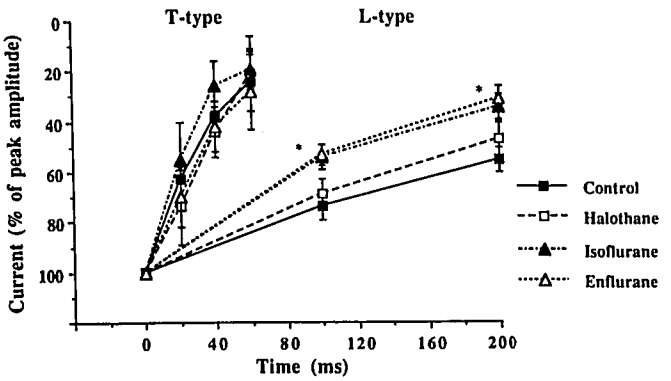


FIG. 9. The effects of halothane (1.5%), isoflurane (2.0%) and enflurane (2.5%) on the time course of inactivation of T- and L-type Ca^{2+} channel currents. The anesthetic agents had no significant effect on the time course of inactivation (measured at 20, 40, and 60 ms) of T-type current elicited by depolarizing pulses from −70 to −30 mV (n = 4 for each anesthetic agent). L-type current was elicited by voltage steps from −40 to 0 mV measured at 100 and 200 ms (n = 4 for each anesthetic agent). Enflurane and isoflurane, but not halothane, significantly enhanced by decay of L-type Ca^{2+} current. *Significant difference (for isoflurance and enflurane) from control values ($P \leq 0.05$).

shown) and also was not altered by the higher concentration of halothane (n = 4). However, higher concentrations of isoflurane (n = 5) and enflurane (n = 5) enhanced L-type current decay, resulting in residual amplitudes at 100- and 200-ms pulse durations of only 54 ± 5 and 34 ± 5% (isoflurane), and 53 ± 4 and 31 ± 5% (enflurane) of initial peak values.

Discussion

The current experiments confirmed the presence of two types of Ca^{2+} channels (L- and T-type) in isolated canine cardiac Purkinje cells.[1] The two channel types were distinguished by their different activation thresholds, kinetics of inactivation, and sensitivities to pharmacologic agents, using a whole-cell voltage-clamp technique. T-type Ca^{2+} channel current was activated at more negative potentials than was L-type current and was rapidly inactivated during sustained depolarization, whereas L-type current was half-maintained during a 200-ms voltage pulse. The L-type current recorded in the current study was effectively blocked by nifedipine (1 μM), whereas Ni^{2+} (100 μM) preferentially reduced T-type current. The two types of channels described here are similar to those found by other investigators in the same tissue.[1,6]

One of the major findings of the current study is that halothane, isoflurane, and enflurane, unlike the classical Ca^{2+} channel blocker nifedipine, nonselectively depressed both L- and T-type Ca^{2+} channel currents in canine cardiac Purkinje cells. All three anesthetic agents at equianesthetic concentrations produced similar depression of peak L- and T-type Ca^{2+} current in the entire voltage range studied, without a shift in the voltage dependency of the I–V relationship for channel activation. There was no significant difference between the sensitivities of L-type *versus* T-type current to all anesthetic agents used in the present study. From the present data, little can be inferred about the molecular mechanisms by which volatile anesthetics lower the amplitude of L- and T-type Ca^{2+} channels. However, isoflurane and enflurane at higher concentrations enhanced the inactivation of L-type but not T-type Ca^{2+} channel currents. Enflurane also has been reported to accelerate the inactivation of L-type current in cardiac ventricular myocytes.[7] These findings suggest that general anesthetics might decrease the amplitude of Ca^{2+} current by reducing the opening probability of single Ca^{2+} channels. However, more precise information on the mechanism of action of general anesthetics on the biophysical properties of Ca^{2+} channels will require single channel recordings.

Several studies have demonstrated that volatile anesthetic agents alter the action potential of cardiac Purkinje fibers. Halothane produces a concentration-dependent decrease in the overshoot and duration of action potentials in sheep Purkinje fibers[10] and shortens the plateau phase of the action potential in canine cardiac Purkinje fibers.[11] In the same preparation, halothane, enflurane and isoflurane shorten the action potential duration and the degree to which it is refractory.[12,13] The current study provides direct evidence that all three volatile anesthetics suppress, in a concentration-dependent manner, the L-type Ca^{2+} channel current in canine cardiac Purkinje cells. Since L-type Ca^{2+} channel current contributes to the slow diastolic depolarization as well as the plateau and repolarization phases of the cardiac action potential, suppression of this inward current may be one of the mechanisms by which inhalational anesthetic agents produce alteration of the action potential in cardiac Purkinje fibers.

Even though the electrical activity of the myocardium is less sensitive to volatile anesthetics than are Purkinje fibers,[10] the three agents in this study produced similar suppression of L-type current amplitude in Purkinje cells, as has been reported previously in ventricular cells.[7–9] The suppression of Ca^{2+} influx across the cardiac sarcolemma by volatile anesthetics most likely contributes to a decrease in Ca^{2+} accumulation by the sarcoplasmic reticulum and subsequent reduction in Ca^{2+} transients and muscle contraction, as has been shown in the presence of halothane in cat papillary muscle.[14] These findings of the current study support the hypothesis that one of the sites of action of volatile anesthetics is at the cardiac sarcolemma. This hypothesis is supported further by evidence that halothane, isoflurane, and enflurane depress the Ca^{2+}-dependent action potential in papillary muscle partially depolarized by high K^+.[15–17] Other possible sites of anesthetic actions include depression of myofibril aden-

osine triphosphatase (ATPase) activity[18] and altered uptake and release of Ca^{2+} from sarcoplasmic reticulum.[19–21] The qualitatively different cardiac effects of isoflurane, halothane, and enflurane may be explained by their differential effects at these other cellular sites.

In the current study as well as in earlier studies,[1] Ni^{2+} (100 μM) preferentially blocked T-type Ca^{2+} channel currents in canine cardiac Purkinje cells. In rabbit sinoatrial node cells, Ni^{2+} (40 μM) also blocked T-type current and caused bradycardia by reducing the rate of depolarization in the late phase of the pacemaker potential; this finding suggests a role for T-type current in generating the slow diastolic depolarization.[5] Since Purkinje cells also may have pacemaker properties, T-type Ca^{2+} channel current may contribute to the pacemaker potential in these cells as well. Volatile anesthetics have been reported to depress slow diastolic depolarization in guinea pig sinoatrial node[22] and canine cardiac Purkinje cells,[11] an effect that may be mediated in part by the suppression of T-type Ca^{2+} channel current.

Since T-type current activates at more negative membrane potentials,[1,6] it also may contribute to abnormal impulse generation such as delayed afterdepolarization. Delayed afterdepolarization often is preceded by membrane hyperpolarization, which would make more T-type Ca^{2+} channels available for subsequent activation. Halothane has been reported to attenuate triggered activity in Purkinje fibers exhibiting delayed afterdepolarization,[12] which may be related to the suppression of T-type Ca^{2+} current. However, halothane depresses not only the Ca^{2+} current but also the fast Na^+ current in isolated rat ventricular cells[23] and the delayed rectifier K^+ current in guinea pig atrial and ventricular myocytes.[24] The lack of specificity of volatile anesthetic effects on different types of ionic channels appears to confirm the existence of a non-receptor-linked general membrane effect of volatile anesthetics at multiple sites. However, further investigations will help to clarify how the action of volatile anesthetic agents on myocardial membrane ionic channels alters the electrical and mechanical properties of the heart.

References

1. Hirano Y, Fozzard HA, January CT: Characteristics of L- and T-type Ca^{2+} currents in canine cardiac Purkinje cells. Am J Physiol 256:H1478–H1492, 1989.
2. Bean BP: Two kinds of calcium channels in canine atrial cells. J Gen Physiol 86:1–30, 1985
3. Nilius B, Hess P, Lansman JB, Tsien RW: A novel type of cardiac calcium channel in ventricular cells. Nature 316:443–446, 1985
4. Mitro R, Morad M: Two types of calcium channels in guinea pig ventricular myocytes. Pro Natl Acad Sci USA 83:5340–5344, 1986
5. Hagiwara N, Irisawa H, Kameyama M: Contribution of two types of calcium currents to the pacemaker potentials of rabbit sinoatrial node cells. J Physiol (Lond) 395:233–253, 1988
6. Tseng GN, Boyden PA: Multiple types of Ca^{2+} currents in single canine Purkinje cells. Circ Res 65:1735–1750, 1989
7. Bosnjak ZJ, Supan FD, Rusch NJ: The effects of halothane, enflurane, and isoflurane on calcium current in isolated canine ventricular cells. ANESTHESIOLOGY 74:340–345, 1991
8. Ikemoto Y, Yatani A, Arimura H, Yoshitake J: Reduction of the slow inward current of isolated rat ventricular cells by thiamylal and halothane. Acta Anaesthesiol Scand 29:583–586, 1985
9. Terrar DA, Victory JGG: Isoflurane depresses membrane currents associated with contraction in myocytes isolated from guinea-pig ventricle. ANESTHESIOLOGY 69:742–749, 1988
10. Hauswirth O: Effects of halothane on single atrial, ventricular, and Purkinje fibers. Circ Res 24:745–750, 1969
11. Reynolds AK, Chiz JF, Pasquet AF: Halothane and methoxyflurane: A comparison of their effects on cardiac pacemaker fibers. ANESTHESIOLOGY 33:602–610, 1970
12. Turner LA, Bosnjak ZJ, Kampine JP: Actions of halothane on the electrical activity of Purkinje fibers derived from normal and infarcted canine heart. ANESTHESIOLOGY 67:619–629, 1987
13. Turner LA, Polic S, Marijic J, Bosnjak ZJ: Actions of halothane, enflurane and isoflurane on the regional repolarization characteristics of canine Purkinje fibers (abstract). ANESTHESIOLOGY 71:A521, 1989
14. Bosnjak ZJ, Kampine JP: Effects of halothane on transmembrane potentials, Ca^{2+} transients and papillary muscle tension in the cat. Am J Physiol 251:H374–H381, 1986
15. Lynch C, Vogel S, Sperelakis N: Halothane depression of myocardial slow action potentials. ANESTHESIOLOGY 55:360–368, 1981
16. Lynch C, Vogel S, Pratila M, Sperelakis N: Enflurane depression of myocardial slow action potentials. J Pharmacol Exp Ther 222:405–409, 1982
17. Lynch C: Differential depression of myocardial contractility by halothane and isoflurane *in vitro*. ANESTHESIOLOGY 64:620–631, 1986
18. Murat I, Ventura-Clapier R, Vassort G: Halothane, enflurane and isoflurane decrease calcium sensitivity and maximum force in detergent-treated rat cardiac fibers. ANESTHESIOLOGY 69:892–899, 1988
19. Su JY, Bell JG: Intracellular mechanism of action of isoflurane and halothane on striated muscle of the rabbit. Anesth Analg 65:457–462, 1986
20. Blanck TJJ, Thompson M: Calcium transport by sarcoplasmic reticulum: Modulation of halothane action by substrate concentration and pH. Anesth Analg 60:390–394, 1981
21. Blanck TJJ, Thompson M: Enflurane and isoflurane stimulate calcium transport by cardiac sarcoplasmic reticulum. Anesth Analg 61:142–145, 1982
22. Bosnjak ZJ, Kampine JP: Effects of halothane, enflurane and isoflurane on the SA node. ANESTHESIOLOGY 58:314–321, 1983
23. Ikemoto Y, Yatani A, Imoto Y, Arimura H: Reduction in the myocardial sodium current by halothane and thiamylal. Jpn J Physiol 36:107–121, 1986
24. Hirota K, Masuda A, Momose Y: Effects of halothane on membrane ionic currents in guinea pig atrial and ventricular myocytes. Acta Anaesthesiol Scand 33:239–244, 1989