

Effects of Halothane and Isoflurane on Calcium and Potassium Channel Currents in Canine Coronary Arterial Cells

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The effects of halothane (0.75% and 1.5%) and isoflurane (2.6%) on macroscopic Ca^{2+} and K^+ channel currents (I_{Ca} and I_{K} , respectively) were investigated in voltage-clamped vascular muscle cells from the canine coronary artery. Single coronary arterial cells were dialyzed with K^+ glutamate solution and superfused with Tyrode's solution for measurement of I_{K} ($n = 45$). Stepwise depolarization from a holding potential of -60 mV to beyond -30 mV elicited an outward, slowly inactivating I_{K} that had a macroscopic slope conductance of 18 nS. I_{K} was reduced 75% by 10 mM 4-aminopyridine, a K^+ channel antagonist. Compared to 4-aminopyridine, halothane at 0.75% and 1.5% reduced peak I_{K} amplitude only by $14 \pm 2\%$ and $36 \pm 3\%$, respectively. At approximately equianesthetic concentrations, 2.6% isoflurane suppressed I_{K} less than did 1.5% halothane, reducing peak amplitude by $15 \pm 3\%$. In other sets of experiments, cells were dialyzed with 120 Cs^+ -glutamate solution and superfused with 10 mM BaCl_2 or CaCl_2 solutions to isolate I_{Ca} ($n = 39$) pharmacologically. Under these conditions, progressive depolarizing steps from -60 mV elicited a small inward current, which was potentiated 3.4-fold by equimolar substitution of Ba^{2+} for Ca^{2+} in the external solution and was blocked by 1 μM nifedipine. This inward current, which resembled L-type I_{Ca} , was blocked $37 \pm 4\%$ and $70 \pm 4\%$ in the presence of 0.75% and 1.5% halothane, respectively. Isoflurane (2.6%) also decreased I_{Ca} by $55 \pm 5\%$. It appears that while halothane and isoflurane suppress both I_{K} and I_{Ca} , these anesthetics preferentially reduce I_{Ca} . Also, at equianesthetic concentrations, halothane (1.5%) appears more potent than isoflurane (2.6%) in suppressing ionic currents in the coronary arterial cells. The significantly greater suppression of I_{K} and I_{Ca} by 1.5% halothane than 2.6% isoflurane may contribute to the more predominant halothane-induced coronary dilation by halothane. Thus, in the coronary artery, which relies on Ca^{2+} entry for vascular muscle activation, the reduced Ca^{2+} influx may represent one of the mechanisms by which volatile anesthetics induce vasodilation. (Key words: Anesthetics, volatile: halothane; isoflurane. Animal: dog. Artery: coronary. Current: calcium; potassium. Muscle: Vascular smooth.)

THE EFFECTS of halothane and isoflurane on vascular muscle have been examined in a number of studies. The general conclusion is that these anesthetics cause vasodi-

lation in specific vascular beds either by a direct depressant action on vascular smooth muscle^{1,2} or by an indirect attenuation of vasoconstrictor activity.³⁻⁶ Although it is frequently contended that isoflurane is a more potent coronary vasodilator than halothane in the isolated perfused heart, isoflurane was a less potent dilator of isolated coronary arterial segments.⁷ This suggests that in the intact system the direct effect of volatile anesthetics on coronary arterial muscle likely interact with other mechanisms to determine the final level of vascular activation.

In support of this, the mechanisms of volatile anesthetic-induced changes in the vasculature appear to be diverse. Volatile anesthetics reportedly alter sympathetic neurotransmission, thus changing the central vasoconstricting influence on the vasculature.⁸ At the vascular neuromuscular junction, receptor-mediated contractile responses are reportedly suppressed.^{4,6,9} The known effects of halothane on sarcoplasmic reticulum include increasing Ca^{2+} release and decreasing Ca^{2+} accumulation,¹⁰ possibly by inhibiting Ca^{2+} ATPase activity, as has been shown in isolated cardiac sarcoplasmic reticulum.¹¹ Halothane also may directly decrease Ca^{2+} activation of the contractile proteins¹⁰ and increase intracellular levels of cAMP^{1,12} and cGMP.¹³ Involvement of the endothelium in volatile anesthetic-induced changes in the vasculature is controversial at present.¹⁴⁻¹⁶

Despite the available literature, the actions of volatile anesthetics on the electrical properties of vascular muscle are largely unknown. Ionic transsarcolemmal fluxes, a potentially very important site of anesthetic action, have not been intensively studied in vascular smooth muscle. It appears, however, that the dilation induced by halothane of vital vascular beds, such as the cerebral circulation, involves a direct action of halothane on the vascular muscle membrane.¹⁷ In isolated cerebral arteries, relaxation by halothane is associated with concentration-dependent membrane depolarization.¹⁷ The mechanism by which halothane induces this apparent electromechanical uncoupling, *i.e.*, causes membrane depolarization concurrent with vasodilation of vascular muscle cells, is not known.

It was shown recently that halothane and isoflurane suppress membrane Ca^{2+} and K^+ channel currents in cardiac myocytes.^{18,19} However, no reports are available on the effects of inhalational anesthetics on the above currents in vascular muscle cells. Thus, the purpose of this

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study was to use the voltage-clamp method to directly measure the effects of halothane and isoflurane on K^+ and Ca^{2+} channel currents (I_K and I_{Ca} , respectively) in canine coronary arterial cells. Our results show that halothane and isoflurane decrease the amplitudes of both I_K and I_{Ca} but preferentially suppress I_{Ca} amplitude. Halothane was a more potent depressor of these currents than was isoflurane. Because K^+ channel block would favor membrane depolarization, whereas I_{Ca} suppression would induce vascular dilation, these anesthetic-induced effects on the vascular muscle membrane may help to explain the electromechanical uncoupling of vascular smooth muscle by inhalational anesthetic agents. The preferential depression by halothane and isoflurane of I_{Ca} amplitude also may contribute to coronary dilatation.

Materials and Methods

PREPARATION OF SINGLE VASCULAR MUSCLE CELLS

After the approval by the Institutional Animal Care Committee, 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 placed in cold oxygenated Krebs' solution. The left anterior descending and/or left circumflex coronary arteries were dissected free from the epicardial surface and placed in a chamber containing Krebs' solution. Any remaining blood was cleared from the arteries, excess fat and adventitia removed, and the arteries cut into 2-mm rings. These were placed in an enzyme solution containing (in millimolar concentration): NaCl 136, KCl 6, $MgCl_2$ 1, HEPES 10, $CaCl_2$ 0.5, glucose 10, collagenase CLS II 500 U/ml (Worthington Co., Freehold, NJ), dithiothreitol 4 mM (Sigma Chemical Co., St. Louis, MO), and papain 2 U/ml (Worthington Co.).

The vascular rings were placed in a vial containing the enzyme solution, maintained at 37° C in a water bath, and stirred at 5 rpm by a microstirrer (Micro-Stir, Wheaton Instruments, Millville, NJ). After 1–2 h, the vascular pieces were disrupted mechanically by forcing them repeatedly through a Pasteur's pipette. The cell suspension was pelleted by centrifugation at 120 × g for 8 min. The pellet was resuspended in enzyme-free solution and placed on ice. Experiments were initiated immediately.

VOLTAGE-CLAMP RECORDING

A drop of dispersed single coronary arterial cells was placed in a perfusion chamber (22° C) on the stage of an inverted microscope (Olympus IMT-2, Leeds Instruments, Minneapolis, MN) equipped with modulation con-

trast. At 500× magnification, a hydraulic micromanipulator (Narishige, Tokyo, Japan) was used to position heat-polished borosilicate patch pipettes with tip resistances of 4–6 MΩ on the membrane of arterial cells. High resistance seals (3–30 GΩ) were formed, after which the pipette patch was removed by negative pressure to obtain the electrical access to the whole cell as previously described.²⁰ Whole-cell currents were elicited by 200-ms depolarizing pulses generated by a computerized system (Pclamp software, Axon Instruments, Burlingame, CA) every 5–10 s. The currents were amplified by a List EPC-7 patch-clamp amplifier (Adams & List Assoc., Great Neck, NY), and the amplifier output was low-pass-filtered at 500 Hz. All data were digitized (sampling rate 10,000 per second) and stored on a hard disk to permit analysis at a later time. Leak and capacitative currents were subtracted from each record by linearly summing scaled currents obtained during 10-mV hyperpolarizing pulses.

RECORDING SOLUTIONS

The external solution used for the measurement of I_K contained (in millimolar concentration): $CaCl_2$ 2, NaCl 135, KCl 4.7, $MgCl_2$ 1, glucose 10, and HEPES 5 (pH = 7.4). The pipette solution contained (in millimolar concentration): K-glutamate 130, $MgCl_2$ 1, EGTA 1, HEPES 10, and Na_2ATP 3 (pH = 7.1–7.2). The external solution used for I_{Ca} measurement consisted of (in millimolar concentration): $CaCl_2$ or $BaCl_2$ 10, tetraethylammonium chloride 135, $MgCl_2$ 1, glucose 10, and HEPES 10 (pH = 7.4). In the pipette solution, Cs^+ was substituted for K^+ to eliminate I_K and permit isolated measurement of I_{Ca} .

DRUG EFFECTS

Repetitive current–voltage curves were obtained in control solutions to monitor time-dependent changes in I_K . After steady-state current amplitude was established by repetitive measurements, cells were exposed to 1 MAC (0.75%) of halothane, 2 MAC (1.5%) of halothane, or 2 MAC (2.6%) of isoflurane by changing the inflow perfusate to one containing anesthetic. Measurements using other drugs were performed similarly. Effects of halothane and isoflurane were complete within 2 min and were reversible during I_K measurements. This was verified by obtaining similar measurements following removal of anesthetic from the perfusate. Contrary to the effects on I_K , anesthetic-induced changes of I_{Ca} were longer-lasting. Thus, after two control measurements of I_{Ca} amplitude, a third measurement was obtained after a 3-min exposure to halothane or isoflurane. Parallel time control measurements were taken over the same time period in the absence of anesthetics. Halothane was solubilized at final bath concentrations of 0.4 ± 0.01 mM and 0.8 ± 0.01

mM, which were calculated to correspond to 0.75% and 1.5% halothane, respectively. The isoflurane concentration of 1.0 ± 0.02 mM was calculated to correspond to 2.6%. Solution was sampled from the perfusion chamber and analyzed by gas chromatography to verify the anesthetic concentration at the cell proximity.

Nifedipine (Sigma, St. Louis, MO) was dissolved in 70% ethanol to make a 1 mM stock solution. The final concentration of solvent in the recording solution was 0.07% and by itself did not have any effect of the current. 4-Aminopyridine (4-AP, Sigma, St. Louis, MO) was dissolved directly in the recording solutions.

STATISTICAL ANALYSIS

All currents are expressed as mean \pm SEM and were analyzed by two-way analysis of variance. If the *F* test showed significance, Fisher's test for least significant difference was performed, with the level of significance at $P \leq 0.05$.

Results

POTASSIUM CURRENT

Cells dialyzed with high K^+ solution showed large outward currents during 200-ms depolarizing steps from a holding potential of -60 mV to potentials beyond -30 mV (fig. 1A). Activation of peak outward current was linear between -20 and $+40$ mV, with an average macroscopic slope conductance of 18 nS (fig. 1B). This current

was eliminated when Cs^+ was substituted for K^+ in the pipette solution, indicating that K^+ was the mandatory charge carrier for this current. During 200-ms depolarizing pulses from -60 mV to consecutively less negative voltages, the amplitude of outward K^+ current was largely sustained over time. This is illustrated by comparing the current-voltage relationship for peak I_K , with I_K amplitude measured at the end of the 200-ms depolarizing pulse (fig. 1B). At any given voltage, the mean peak and remaining I_K values were the same, suggesting that I_K inactivation over the 200-ms pulse period was insignificant. For example, peak I_K elicited by depolarization to $+40$ mV from a holding potential of -60 mV averaged 1169 ± 202 pA, of which 1058 ± 196 pA (91%) remained at the end of a 200-ms pulse. Outward current never showed the transient, rapidly inactivating component that has been seen in other smooth muscle preparations.^{21,22} Instead, when the duration of the depolarizing pulse was increased to 5 s to discern better the time course of I_K decay, only a single slow-decay phase was observed (fig. 1C).

4-AP was a potent antagonist of the voltage-dependent I_K . Concentrations of 0.1, 1, and 10 mM 4-AP caused dose-dependent depression of macroscopic I_K , as illustrated in one coronary arterial cell (fig. 2A). Figure 2B illustrates the average effect of 4-AP on the current-voltage relationship for K^+ channel activation in six cells. In a dose-dependent manner, 4-AP reduced I_K amplitude elicited by 10-mV steps from -60 mV to $+40$ mV. Peak I_K elicited by a voltage step to $+40$ mV was reduced to

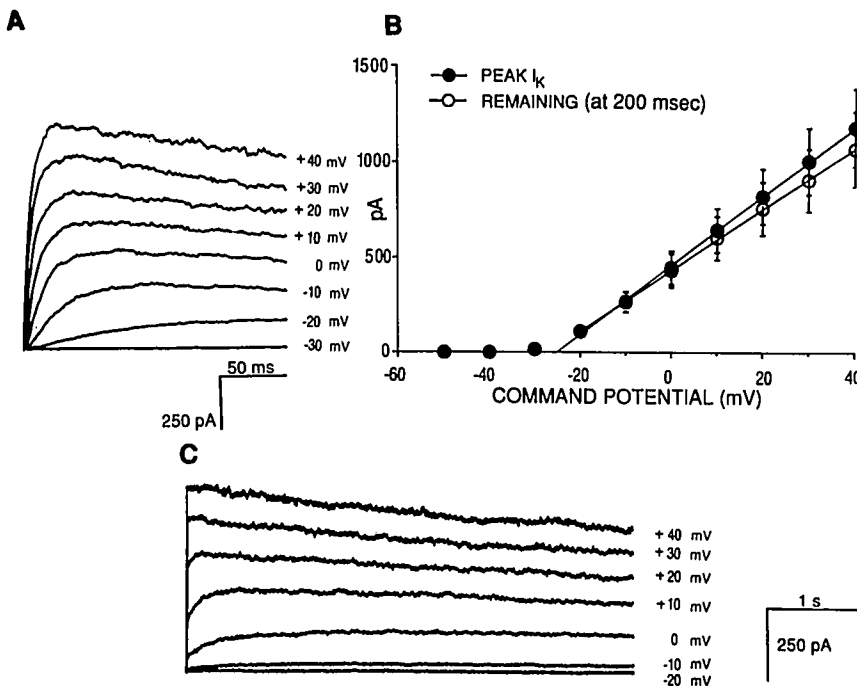
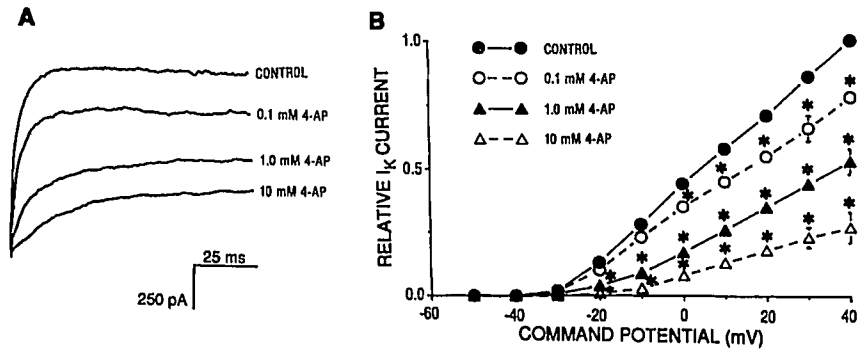


FIG. 1. Voltage and time-dependent activation and decay of outward K^+ current (I_K) in a single cell (A). The cell was depolarized in 10-mV increments from holding potential of -60 mV to voltages of up to $+40$ mV. In this cell, I_K was activated at command potentials positive to -30 mV. The current-voltage plot of I_K in coronary smooth muscle cells compares peak I_K and I_K remaining at 200 ms against command potential in 5 cells (B). Time-dependent decay in I_K in a single cell depolarized to different command potentials for 5 s is also shown (C).

FIG. 2. In a dose-dependent manner, increasing concentrations of 4-aminopyridine (4-AP) (0.1, 1, and 10 mM) decreased outward K^+ current (I_K) elicited by depolarizing pulses from a holding potential of -60 to $+40$ mV in one cell (A). The control current-voltage relationship ($n = 6$) for activation of I_K (expressed as fraction of control maximal I_K obtained by depolarization to $+40$ mV) was reduced in a dose-dependent manner by 0.1, 1, and 10 mM 4-AP throughout the entire voltage range studied (B). Standard error bars are less than symbol size for some measurements. $*P \leq 0.05$ versus control.



$73 \pm 6\%$, $49 \pm 5\%$, and $25 \pm 6\%$ of control amplitude by 0.1, 1, and 10 mM 4-AP, respectively.

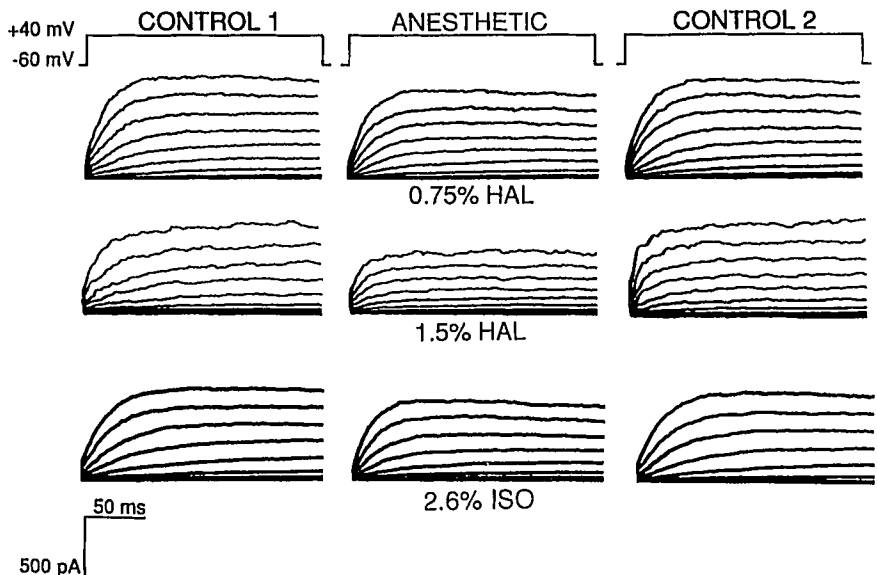
The effects of halothane and isoflurane on whole-cell I_K were determined by applying a series of stepwise (10 mV) depolarizing test pulses from a holding potential of -60 mV to positive potentials as high as $+40$ mV. Figure 3 shows actual traces of patch-clamp recordings illustrating the depressant effect of 0.75% and 1.5% halothane and 2.6% isoflurane on I_K amplitude. Halothane caused a reversible, dose-dependent reduction in I_K at all voltages. Isoflurane similarly induced a reversible suppression of I_K . Peak I_K was plotted as a function of membrane potential to analyze the mean effect of 0.75% and 1.5% halothane (figs. 4A and 4B, respectively) and 2.6% isoflurane (fig. 4C) on I_K amplitude. Halothane at concentrations of 0.75% and 1.5% depressed peak I_K amplitude obtained at $+40$ mV by $14.4 \pm 2\%$ and $36.4 \pm 3\%$, respectively ($n = 7$). Isoflurane (2.6%) reduced I_K amplitude by $15 \pm 3\%$ ($n = 8$). At approximately equianesthetic concentrations, I_K suppression by 1.5% halothane was

significantly greater than by 2.6% isoflurane at voltages positive to $+10$ mV.

CALCIUM CURRENT

Whole-cell I_{Ca} was pharmacologically isolated for measurement by substituting Cs^+ for K^+ in the pipette solution and tetraethylammonium chloride for Na^+ and K^+ in the external solution, as described previously.²³ I_{Ca} was generated by stepwise depolarizing pulses (10 mV; 200 ms) from a holding potential of -60 mV to command potentials as high as $+40$ mV. The concentration of extracellular Ca^{2+} was increased from 2 to 10 mM to enhance I_{Ca} amplitude and facilitate the accurate measurement of otherwise relatively small inward current. Furthermore, substitution of 10 mM barium (Ba^{2+}) for 10 mM Ca^{2+} in the external solution increased peak I_{Ca} at $+40$ mV by 3.4 ± 0.6 -fold, as shown in an example (fig. 5A). All further experiments were done with 10 mM Ba^{2+} in the external solution. In 33 cells, threshold activation occurred

FIG. 3. Actual recordings showing the effect in different cells of 0.75% halothane (top), 1.5% halothane (middle), and 2.6% isoflurane (bottom) on K^+ current elicited by progressive 10-mV test pulses from -60 mV to $+40$ mV. Recordings were obtained before (control 1), during (anesthetic), and after (control 2) exposure to volatile anesthetics. Both anesthetics produced significant depression of K^+ current, which was completely reversible on washout (control 2). Effects of halothane were dose-dependent.



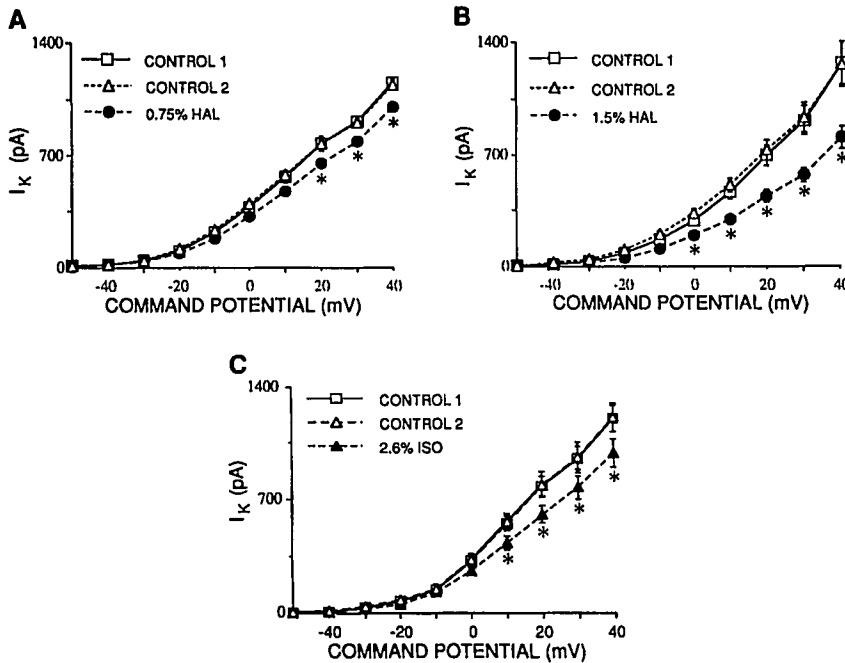


FIG. 4. The current-voltage relationship for K^+ current (I_K) activation in control solution and in the presence of 0.75% halothane (A, $n = 7$), 1.5% halothane (B, $n = 7$), and 2.6% isoflurane (C, $n = 8$). Halothane depressed I_K amplitude in a dose-dependent fashion throughout the entire voltage range studied. Isoflurane also depressed I_K amplitude at all potentials, with the magnitude of depression being similar to that of 0.75% halothane. * $P \leq 0.05$ versus control 1 and control 2.

at -10 mV, and the maximal activation was reached at $+20$ mV (fig. 5B). Maximal I_{Ca} varied greatly between cells (range 15–170 pA) and averaged 106 ± 20 pA.

The effect of nifedipine on I_{Ca} was measured using the same pulse protocol to further characterize the channel pathway for inward current. As illustrated in the recording in figure 5C, addition of $1 \mu M$ nifedipine to bath solution containing 10 mM Ba^{2+} reduced I_{Ca} to $12 \pm 1\%$ of original amplitude ($n = 5$). Both Ba^{2+} and nifedipine are more selective for the long-lasting (L-type) Ca^{2+} channel, the former being a preferential charge carrier and the latter a specific blocker of this channel.²⁴

Figure 6 shows four examples of three consecutive recordings, 3 min apart, of I_{Ca} elicited from a holding potential of -60 mV. Traces at the top show three sequential control recordings in the absence of anesthetic agents. Two initial control recordings (controls 1 and 2) at 3-min intervals preceded subsequent administration of 0.75% and 1.5% halothane, respectively (figs. 6B and 6C). Figure 6D shows the effect of 2.6% isoflurane on I_{Ca} elicited by the same pulse protocol. Halothane (0.75% and 1.5%) depressed I_{Ca} in a dose-dependent manner throughout the voltage range studied, and isoflurane had a qualitatively similar action. The effects of halothane, and to a lesser degree isoflurane, on I_{Ca} were long-lasting, and I_{Ca} amplitude did not recover even after 15 min. Because of the slow recovery of I_{Ca} after exposure to volatile anesthetics, a series of control measurements was taken in a separate group of cells during the same time period to quantify time-dependent changes in I_{Ca} . As shown by the single recording in figure 6A, and for mean values in

figure 7A, the current-voltage relationship for the activation of I_{Ca} was the same for three consecutive controls (control 1 = 107 ± 32 pA, control 2 = 113 ± 37 pA, and control 3 = 101 ± 29 pA; $n = 8$). However, figures 7B and 7C show that 0.75% halothane decreased I_{Ca} by $37 \pm 4\%$ from a control peak current of 100 ± 24 pA ($n = 8$), whereas 1.5% halothane produced $70 \pm 4\%$ reduction from a control peak current of 153 ± 27 pA ($n = 7$). Isoflurane (2.6%) also produced $55 \pm 5\%$ depression of I_{Ca} amplitude from the control peak current of 105 ± 14 pA. At approximately equianesthetic concentrations, I_{Ca} suppression by 1.5% halothane was significantly greater than by 2.6% isoflurane at voltages positive to $+10$ mV. The effects of volatile anesthetics were not voltage-dependent; *i.e.*, there was no shift in the current-voltage relationship.

Discussion

Halothane and isoflurane have direct coronary vasodilatory effects *in vitro* and *in vivo*.^{25,26} In *in vitro* preparations, halothane directly relaxes isolated coronary arterial rings previously constricted with K^+ or prostanoid to a greater degree than does isoflurane.⁷ This would suggest that halothane is a more potent suppressor than isoflurane of voltage-dependent Ca^{2+} entry, since voltage-gated Ca^{2+} influx is the mechanism underlying K^+ -induced contractions.²⁷ In the *in vivo* setting, the mechanism for coronary flow increase during anesthesia likely involves complex interactions among endothelium, vascular smooth muscle, myocardium, and the autonomic nervous

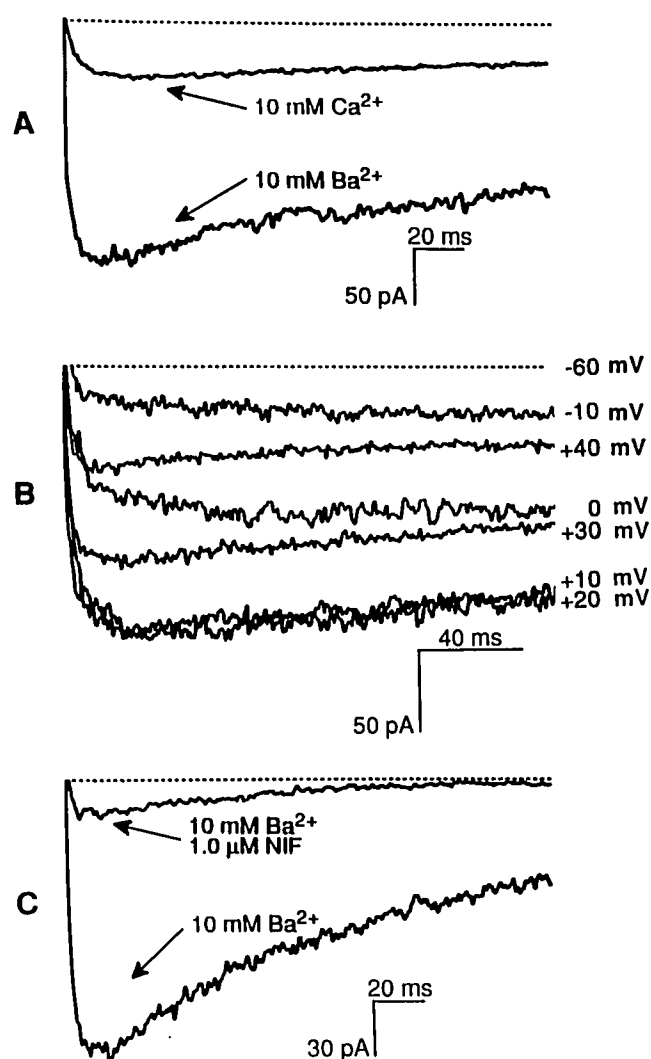


FIG. 5. Recordings of inward current in three arterial cells. *A*: The potentiating effect of 10 mM Ba^{2+} substitution for 10 mM Ca^{2+} on peak Ca^{2+} current (I_{Ca}) elicited by a depolarizing pulse from -60 mV to $+20$ mV in an individual cell. *B*: Traces showing small inward current, voltage, and time-dependent inactivation of I_{Ca} in a single cell superfused with 10 mM Ba^{2+} . The cell was depolarized in 10-mV increments from holding potential of -60 mV to voltages of up to $+40$ mV. In this cell, I_{Ca} was activated at command potentials positive to -20 mV. *C*: Effect of 1 μM nifedipine (NIF) on peak I_{Ca} elicited by a depolarizing pulse from -60 mV to $+20$ mV. Peak I_{Ca} was almost completely blocked by 1 μM nifedipine.

system. For example, in the intact circulation the direct effects of volatile anesthetics on arterial muscle^{7,25,26} are superimposed on their dominant depressant action on the myocardium, namely a reduced cardiac work and oxygen demand.²⁸ In addition to these complex interactions, the effects of anesthetic agents on the coronary endothelium may regulate arterial diameter by modulating the synthesis and release of constricting and relaxing factors.^{14,15} Muldoon and co-workers found that halothane attenuates

endothelium-induced vasodilation in dog coronary arteries.¹⁶

The halothane-induced vasorelaxation, as examined in intact rat aortic rings, is likely induced by a combination of events including decreased Ca^{2+} accumulation by the sarcoplasmic reticulum, decreased sensitivity of contractile proteins to Ca^{2+} ,¹⁰ and/or decreased Ca^{2+} entry from extracellular space, as shown in the present study. In addition to these events, there is evidence that anesthetic agents directly alter other ionic fluxes at the vascular muscle membrane.¹⁷ Halothane is more potent than isoflurane in reducing the amount of Ca^{2+} rapidly released from the sarcoplasmic reticulum in rat ventricular myocardium.²⁹ In cerebral arterial muscle, halothane caused depolarization despite simultaneous vascular relaxation, suggesting electromechanical uncoupling of membrane potential and vascular contraction.¹⁷ This unusual action of halothane remains unexplained, but the authors suggested that an intracellular action of halothane or alteration of membrane ionic fluxes might be involved.

The purpose of the present study was to identify the actions of halothane and isoflurane on macroscopic I_K and I_{Ca} currents in the coronary arterial cell membrane. Our findings show that these volatile anesthetics reduce the amplitude of I_K and I_{Ca} in the vascular muscle membrane. However, these two channel types were not equally sensitive to block by halothane and isoflurane: I_{Ca} was blocked more effectively than I_K . These findings concur with previous voltage-clamp experiments performed in cardiac myocytes,^{18,19,30} where halothane and isoflurane produced similar depression of L-type and transient (T-type) I_{Ca} ³⁰ but less depression of delayed rectifier I_K .¹⁹ Halothane was a more potent depressor of I_K and I_{Ca} than was isoflurane. These depressant effects of halothane and isoflurane on I_{Ca} persisted after terminating the anesthetics. This may explain the irreversible depressant effect of halothane on vascular contraction in isolated rat aortic rings, both *in vivo* and *in vitro*.³¹ Because volatile anesthetics have widespread effects on Ca^{2+} translocation,³ the mechanism for this effect is not readily apparent.

The macroscopic I_K measured in our study was 4-AP sensitive, and slowly inactivating. A 4-AP-sensitive, delayed-rectifier K^+ channel also has been described in neuronal,³² cardiac,³³ skeletal,³⁴ and vascular smooth muscle cells³⁵ and recently in coronary arterial cells.³⁶ In our study, I_K showed a whole-cell conductance slope of 18 nS. This represents a relatively high K^+ conductance, compared with estimated slope conductance of 6 nS in cerebral arterial cells³⁷ and 7 nS in rabbit portal vein.³⁸ This efflux of K^+ through these channels functions as an endogenous dilating mechanism and may play an important role in regulating coronary arterial tone.³⁶ Activation of membrane I_K causes cell hyperpolarization, rendering Ca^{2+} channels inactive and leading to vascular relaxation.³⁹ It

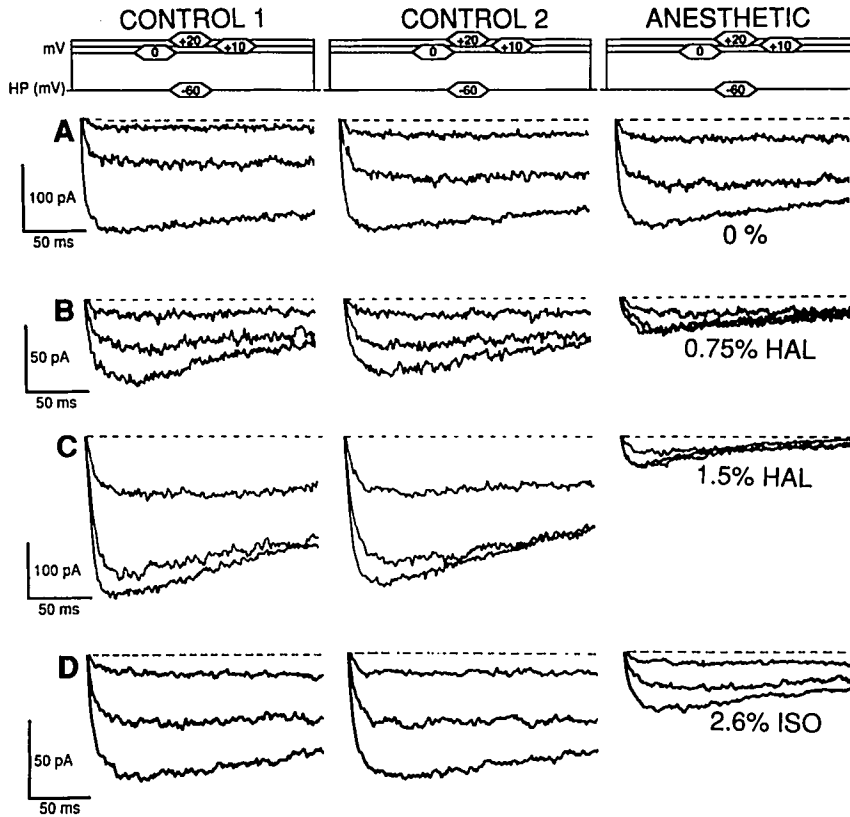


FIG. 6. Recordings of Ca^{2+} current (I_{Ca}) generated by progressive depolarizing pulses from -60 mV to $+40$ mV (10 -mV increments). Only selected episodes are shown: -60 mV (dashed line) and 0 , $+10$, and $+20$ mV. A: Three measurements (control 1, control 2, and control 3) were taken 3 min apart without exposure to volatile anesthetics in order to determine time-dependent changes (decay) in I_{Ca} . B: In other cells, recordings of I_{Ca} were obtained in control solution (control 1, control 2) and then in the presence of 0.75% halothane, 1.5% halothane (C), or 2.6% isoflurane (D). Control measurements were taken 3 min apart, and measurements after anesthetic application were taken 3 min after control 2 in the same cell.

is estimated that the opening of only a few K^+ channels can significantly hyperpolarize a vascular muscle cell. Moreover, opening of a single K^+ channel could, by some calculations, hyperpolarize arterial cells from -55 to -56.2 mV.⁴⁰

Two distinct Ca^{2+} channel types have been identified in vascular smooth muscle.²⁴ The T-type current activates at more negative membrane potentials and decays rapidly, whereas L-type current activates at more positive membrane potentials and decays slowly. The inward current

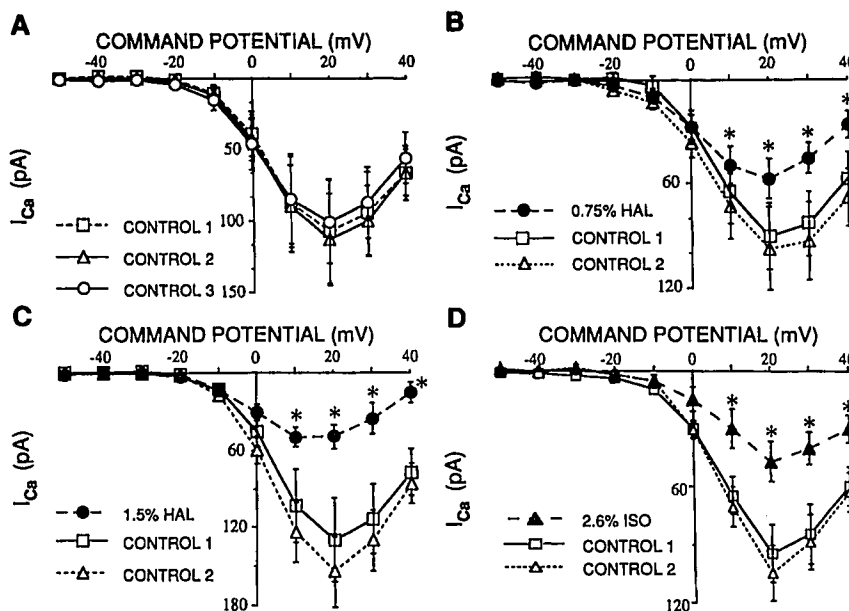


FIG. 7. The current-voltage relationships for Ca^{2+} current (I_{Ca}) activation. A: Stable current-voltage relationship for I_{Ca} activation in three consecutive trials was confirmed in eight cells. B: The current-voltage relationship for I_{Ca} activation in control solution and in the presence of 0.75% halothane ($n = 8$) or (C) 1.5% halothane ($n = 7$). D: The effect of 2.6% isoflurane ($n = 6$) on I_{Ca} at all voltages. Both halothane and isoflurane produced significant depression of I_{Ca} . * $P \leq 0.05$ versus control 1 and control 2.

in this preparation resembled the L-type I_{Ca} . The high threshold for I_{Ca} activation, enhancement of I_{Ca} amplitude by equimolar substitution of Ba^{2+} for Ca^{2+} , and block by nifedipine are consistent with the voltage-dependent and pharmacologic properties of the L-type Ca^{2+} channel in vascular muscle.^{24,41} The predominance of L-type channels in the coronary circulation has been suggested recently by a study in rabbit coronary arterial cells.⁴²

The halothane and isoflurane-induced block of I_{Ca} may help to explain the direct dilatory effect of these anesthetics on vascular smooth muscle. The halothane-induced vasorelaxation is likely induced by a combination of events, including reduction of sensitivity of contractile proteins for Ca^{2+10} and reduction of Ca^{2+} influx. The depression of I_{Ca} by halothane and isoflurane may be particularly important in the coronary circulation, which relies predominantly on influx of external Ca^{2+} for maintenance of contraction.⁴³ Concomitantly to this depression of I_{Ca} , the volatile anesthetic-induced depression of I_K would favor membrane depolarization, because voltage-dependent I_K is crucial for membrane repolarization and control of arterial tone. While this membrane depolarization would favor increased Ca^{2+} influx, the simultaneous and direct depressant action of anesthetics on Ca^{2+} channels may reduce net voltage-dependent Ca^{2+} entry.

From our voltage-clamp data it is not possible to speculate on the molecular basis of the more potent block by halothane and isoflurane of Ca^{2+} as compared to I_K . The depressant effect of volatile anesthetics on whole-cell I_K and I_{Ca} were voltage-independent in this study, suggesting that halothane and isoflurane also likely reduce ionic currents at resting membrane potential in vascular muscle. Measurement of these events by the single-channel, patch-clamp method may provide more detailed information on the exact mechanism by which volatile anesthetics alter channel activity at more physiologic voltages, where net ionic flux is low. However, to judge from our data on macroscopic ionic currents, it is possible that the preferential depression of I_{Ca} by halothane and isoflurane may be one mechanism to reduce cytosolic Ca^{2+} levels in arterial cells during general anesthesia. In the intact coronary circulation, this membrane alteration may interact with the other actions of anesthetic agents to determine coronary arterial tone and modulate blood flow to the heart.

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