

Differential Modulation of the Cardiac L- and T-type Calcium Channel Currents by Isoflurane

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Background: Volatile anesthetics exert their negative chronotropic and inotropic effects, in part by depressing the L- and T-type calcium channels. This study examines and compares the dose-dependent effects of isoflurane on atrial L- and T-type calcium currents ($I_{Ca,L}$ and $I_{Ca,T}$) and ventricular $I_{Ca,L}$.

Methods: Whole cell I_{Ca} was recorded from enzymatically isolated guinea pig cardiomyocytes. Current-voltage relations for atrial and ventricular $I_{Ca,L}$ was obtained from holding potentials of -90 and -50 mV to test a potential of $+60$ mV in 10-mV increments. Atrial $I_{Ca,T}$ was determined by subtraction of currents obtained from holding potentials of -50 and -90 mV. Steady state inactivation was determined using standard two-pulse protocols, and data were fitted with the Boltzmann equation.

Results: Isoflurane depressed I_{Ca} in a dose-dependent manner, with K_d values of 0.23 ± 0.03 , 0.34 ± 0.03 , and 0.71 ± 0.02 mM of anesthetic for atrial $I_{Ca,T}$ and $I_{Ca,L}$ and ventricular $I_{Ca,L}$, respectively, and caused a significant ($P < 0.05$) hyperpolarizing shift in steady state inactivation. At 1.2 and 1.6 mM, isoflurane caused a significant ($P < 0.05$) depolarizing shift in the steady state activation in ventricular $I_{Ca,L}$ but not in atrial $I_{Ca,L}$ or $I_{Ca,T}$. In addition to the depression of $I_{Ca,L}$, isoflurane also induced a hyperpolarizing shift in the reversal potential of I_{Ca} for both atrial and ventricular L-type calcium channels.

Conclusion: The results show that atrial $I_{Ca,T}$ is more sensitive to isoflurane than atrial $I_{Ca,L}$ and ventricular $I_{Ca,L}$ is the least responsive to the anesthetic. These differential sensitivities of the calcium channels in the atrial and ventricular chambers might reflect phenotypic differences in the calcium channels or differences in modulation by the anesthetic.

VOLATILE anesthetics have negative inotropic and chronotropic effects on cardiac function.¹ *In vivo* and *in vitro* studies have shown that these anesthetics depress automaticity of the sinoatrial node and conduction velocity in the atrioventricular node. In so far as the action potentials of these cardiac tissues depend on calcium influx through calcium channels, one of the inhibitory effects of these anesthetics may be by blocking the L- and T-type calcium channels.² Volatile anesthetics are known to depress cardiac contractility, in part by inhibiting L- ($I_{Ca,L}$) and T-type calcium channel currents ($I_{Ca,T}$), necessary for excitation-contraction coupling.^{3,4}

In the adult cardiac muscle, the two types of calcium

channels play different functional roles. $I_{Ca,L}$ underlies the "slow inward" current of the action potential in atrial and ventricular myocytes, and $I_{Ca,T}$ underlies the transient current that may regulate action potential in pacemaker cells and atrial cells. $I_{Ca,T}$ have been reported also in ventricular cells, but its current magnitude is significantly smaller than the ventricular L-type.⁵ Because the main physiologic function of ventricular cells is contraction, L-type calcium 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 calcium channel currents are also important in the generation of and plateau phase of the slow action potential. Unlike the L-type calcium channel, the role of the cardiac T-type calcium channel contributes little to the Ca^{2+} influx responsible for muscle contraction. However, other studies have reported that activation of ventricular T-type calcium channels could lead to Ca^{2+} release from the sarcoplasmic reticulum.⁵ The T-type calcium channel, because of its voltages of activation and inactivation, may be involved in the late phase of diastolic depolarization in pacemaker cells.⁶ The two channels can also be separated by their voltage-dependent activation and inactivation; $I_{Ca,L}$ requires a strong depolarization for activation and decays slowly during maintained depolarization. The T-type calcium current, on the other hand, is activated at more negative potentials than the L-type and is rapidly inactivated during sustained depolarization.⁶ In addition, the two calcium channels can be distinguished pharmacologically; pharmacologic agents such as dihydropyridines, phenylalkylamines, and benzothiazepines inhibit $I_{Ca,L}$, while nickel and mibefradil preferentially block $I_{Ca,T}$.⁷⁻⁹

Although volatile anesthetics depress calcium currents, differences in the sensitivities of the L- and T-type calcium channels to the anesthetics have been reported. McDowell *et al.*¹⁰ showed that low-voltage activated calcium channels of the thyroid C-cell line were more sensitive to isoflurane and enflurane than high-voltage activated calcium channels. In a recent study, McDowell *et al.*¹¹ also demonstrated differential effects of volatile anesthetics on T-type calcium channels from different types of tissue. However, previous studies from this laboratory have shown that volatile anesthetics inhibit Purkinje fiber L- and T-type calcium channels equally.¹² Therefore, to further characterize how volatile anesthetics alter cardiac function, we investigated the effects of isoflurane on atrial L- and T-type calcium channels and compared anesthetic effects on the L-type calcium channels of atrial and ventricular myocytes. The results show

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that isoflurane reversibly suppressed atrial $I_{Ca,T}$ more than atrial $I_{Ca,L}$, and the ventricular $I_{Ca,L}$ was the least sensitive to the anesthetic. The magnitude of the depressions of atrial and ventricular $I_{Ca,L}$ induced by isoflurane was also associated with a reversible hyperpolarizing shift in the reversal potential for calcium.

Materials and Methods

Atrial and Ventricular Cell Isolation

The isolation procedure for cardiac myocytes from guinea pigs was approved by the Medical College of Wisconsin Animal Care and Use Committee. Single atrial and ventricular myocytes were isolated from the hearts of adult guinea pigs (SASCO, Wilmington, MA) weighing 180–250 g. The procedure of the cell isolation is a modification of that of Mitra and Morad¹³ and has been described previously.¹⁴ Briefly, the guinea pigs were injected intraperitoneally with 10,000 USP units of heparin and anesthetized with pentobarbital sodium (250 mg/kg intraperitoneally). Hearts were rapidly extracted after thoracotomy and mounted on a Langendorff apparatus for perfusion *via* the ascending aorta. The hearts were perfused at a rate of 7 ml/min at 37°C with Joklik medium (Gibco minimum essential medium) containing heparin at pH 7.23 (95% O₂-5% CO₂ oxygenation). After 3–4 min, the Joklik solution was removed, and the hearts were perfused with recirculating enzyme solutions containing a heparin-free Joklik medium with 0.6 mg/ml collagenase (GIBCO type II; Life Technologies, Grand Island, NY), 0.1 mg/ml protease Type XIV (Sigma Chemical Co., St. Louis MO), and 1 mg/ml bovine serum albumin solution (Pentex, Bayer Corp., Kankakee IL), pH 7.23 (95% O₂-5% CO₂, oxygenation).

Isolation of Atrial Cells

Atrial cells were obtained during the initial stage of the cell isolation procedure. After 3–4 min of perfusion with enzymes on the Langendorff apparatus, the auricles were cut and minced in 10 ml of Tyrode solution at 37°C. The minced auricles were then transferred into a small flask, placed in a water bath (37°C), and shaken for 3 min. After shaking, the dispersed tissue and cells were filtered through a 210- μ m spectral mesh in a test tube and left to settle before experimentation.

Isolation of Ventricular Cells

From the same heart after the auricles were cut, the ventricles were removed after 14 min of enzyme digestion and minced in the enzyme solution. They were then transferred into small flasks containing enzyme solution and incubated for an additional 3–8 min in a shaker water bath (37°C) to disperse the cells. The dispersed cells were then filtered and centrifuged. The resulting pellet was first washed in the recovery solution (Joklik me-

dium supplemented with 0.1 mM CaCl₂ and 1 mg/ml bovine albumin fraction V; Pentex) and then with Tyrode solution. The isolated ventricular myocytes were stored in Tyrode solution at room temperature for studies.

Solutions and Reagents

Modified Tyrode solution contained 132 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl₂, 10 mM HEPES, 5 mM dextrose, and 1 mM CaCl₂ and was adjusted to pH 7.32 with NaOH. For recording of calcium currents, Ca²⁺ was used as the charge carrier in all experiments. To isolate the calcium current, the following reagents were used for the external solution: 2 mM CaCl₂, 132 mM NMDG (for sodium replacement), 4.8 mM CsCl to block potassium currents, 2 mM MgCl₂, 10 mM HEPES, and 5 mM glucose, adjusted to pH 7.4 with HCl. The pipette solution contained 110 mM CsCl to block potassium currents, 10 mM HEPES, 1 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, and 5 mM potassium-sensitive adenosine triphosphate adjusted to pH 7.3 with CsOH. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Isoflurane (Ohmeda Caribe Inc., Liberty Corner, NJ) was delivered in the superfusate after a vigorous mixture of an aliquot of the anesthetic with a fixed volume of external Ca²⁺ solution. To determine the effect of isoflurane on I_{Ca} , the inflow perfusate was changed to one in which a given concentration of the anesthetic agent had been equilibrated. At the end of each experiment, solution samples of the chamber were taken and analyzed by gas chromatography (GC-8AIF, Shimadzu Corp., Kyoto, Japan) to verify the anesthetic concentration surrounding the cell.

Whole Cell Patch Configuration

A drop of cell suspension (atrial or ventricular myocytes) was placed on a Plexiglas chamber mounted on the stage of an inverted microscope (Olympus IMT2, Tokyo, Japan) and superfused with Tyrode solution. Experiments involving atrial cells were conducted within 6 h after isolation, and the selected cells were spindle shaped and showed distinct borders and striations. For ventricular myocytes, the cells were used within 10 h after isolation, and rod shaped cells with clear borders and striations were selected. Pipettes from borosilicate glass were pulled with a multistage puller (Sachs-Flaming, PC-84; Sutter Instrument Company, Novato, CA) and heat polished (Narishige microfuge, MF-83; Setagaya-KU, Tokyo, Japan). The polished pipettes had tip resistances of 2.5–4.5 m Ω . High-giga-ohm seals and whole cell voltage clamp were attained in Tyrode solution, followed by superfusion of the chamber with the external Ca²⁺ solution.

Whole cell calcium currents using the patch clamp technique were recorded at room temperature. Currents were recorded using a List EPC-7 patch clamp amplifier (Adams and List Assoc., Great Neck, NY), and the output

was filtered at 3 kHz to reduce high frequency noise. Data acquisition and analysis were performed using the pCLAMP software package (Version 6.0; Axon Instruments, Inc., Foster City, CA). Additional analysis was performed using ORIGIN version 4.0 software (Microcal Software Inc., Northampton, MA).

In ventricular and atrial myocytes, $I_{Ca,L}$ was elicited during 50-ms depolarizing test pulses (200 ms duration) to +60 mV (10-mV increments) from a holding potential of -50 mV. To elicit $I_{Ca,T}$ in atrial cells, depolarizing test pulses were initiated from holding potentials of -90 and -50 mV. $I_{Ca,T}$ was obtained by digital subtraction of currents obtained at holding potential of -50 mV (where the L-type is elicited) from currents elicited from a holding potential of -90 mV (where both L- and T-type are elicited) in the same cell.

Data Analysis

After obtaining whole cell configuration, calcium current was initially recorded for 15 min to monitor time-dependent changes in peak calcium current amplitude. Cells that showed a significant change in current amplitude because of rundown (*i.e.*, > 5%) during the stabilization period of 15-20 min, or did not show at least 80% recovery during washout, were not included in analysis of the data. Current-voltage (I-V) relations were obtained during control (anesthetic-free external Ca^{2+} solution), in the presence of anesthetic, and during washout (recovery) for each cell. The effects of anesthetic on peak I_{Ca} are reported as percentage block of the current amplitude in the presence of isoflurane compared with control. Concentration-response curves for block of the peak I_{Ca} by isoflurane were obtained by nonlinear regression to a sigmoidal equation:

$$B/M = 1/[1 + (K_d/[A])^{n_H}] \quad (1)$$

where B is a block by anesthetic, M is the maximum block by the anesthetic, [A] is concentration of the anesthetic, K_d is the anesthetic concentration for half maximal effect, and n_H is the Hill coefficient.

Steady-state activation was obtained by calculating conductance from:

$$g = I_{Ca}/(V - E_{rev}) \quad (2)$$

where I_{Ca} is the current amplitude, V is the test potential, and E_{rev} is the reversal potential for calcium, which was approximately 50-60 mV during control conditions. The conductance was normalized to maximum conductance, g_{max} . To monitor steady-state inactivation of atrial and ventricular $I_{Ca,L}$, cells were subjected to 1-s preconditioning test pulses ranging from -50 mV (holding potential) to +40 mV (in 10-mV increments) before stepping to the test pulse of +20 or +30 mV, the potential at which peak $I_{Ca,L}$ current occurred. Steady state T-type current inactivation was determined by imposing 1-s preconditioning voltage pulses ranging from

-90 (holding potential) to -30 mV before stepping to the test pulse of -30 mV, the potential at which peak $I_{Ca,T}$ occurs. At -30 mV test pulse, $I_{Ca,L}$ was not activated. Peak currents obtained at the test potential were normalized to the maximum current (I_{max}). Both steady-state activation and inactivation were fitted to a Boltzmann equation of the form:

$$g/g_{max} \text{ (or } I/I_{max}) = 1/[1 + \exp[(V - V_{1/2})/k]] \quad (3)$$

where V is the membrane potential (for activation) or preconditioning potential (for inactivation), $V_{1/2}$ is the voltage where activation or inactivation is half maximal, and k is the slope factor.

Statistical Analysis

Data are presented as mean \pm SEM. Dose-related inhibition of I_{Ca} was analyzed using one-way analysis of variance. Multiple-comparison analysis of variance with repeated measures was used to analyze the between-group differences. The Student-Newman-Keuls procedure (least significant range) was used *a posteriori* to determine differences between mean values. All statistical analyses were conducted using commercially available software from SigmaStat (Jandel Scientific, Corte Madera, CA). A test result was considered significant when $P \leq 0.05$.

Results

Figure 1 depicts a representation of the L- and T-type calcium channel currents recorded from atrial cells. Whole cell inward I_{Ca} and corresponding I-V relations were recorded from atrial and ventricular cells bathed in Na^+ -free external solution containing 2 mM Ca^{2+} . Current traces in figures 1A and B show $I_{Ca,L}$ elicited by depolarizing test pulses to -30 mV and +10 mV from holding potentials of -50 and -90 mV, respectively. With the cell held at -50 mV, $I_{Ca,L}$ was present during a test step to +10 mV but not at -30 mV. From a holding potential of -90 mV, both $I_{Ca,T}$ and $I_{Ca,L}$ were elicited by depolarizing step pulses to -30 and at +10 mV, respectively. Subsequent subtraction of traces recorded from a holding potential of -50 mV from those obtained at -90 mV yielded the T-type calcium current (fig. 1C) in atrial myocytes. During our recording conditions, ventricular T-type calcium channel currents were not studied because they were not observed.

Figure 2 depicts representative whole cell ventricular L- and atrial L- and T-type calcium current traces and their corresponding I-V relations recorded in the absence (control) and presence of isoflurane. The average peak current amplitude during control conditions (2 mM external Ca^{2+}) for atrial T- and L-type and ventricular L-type calcium currents were 101 ± 4 (n = 51), 211 ± 6 (n = 56), and 511 ± 13 pA (n = 61), respectively. In the

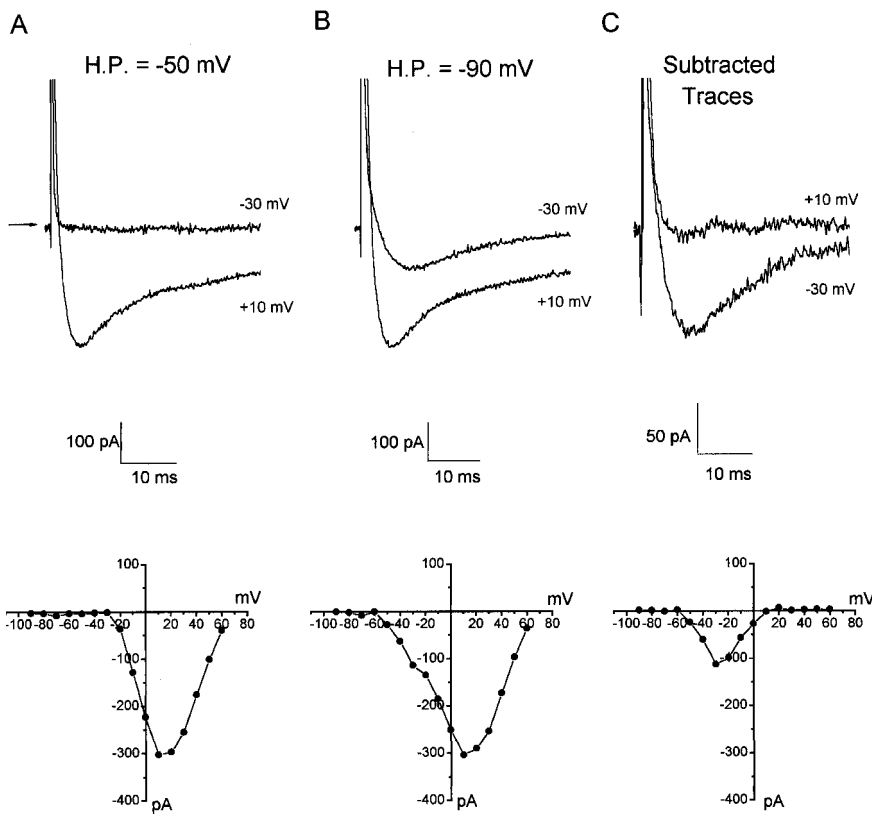


Fig. 1. T- and L-type calcium currents in isolated guinea pig atrial myocyte. L-type Ca^{2+} currents were activated by depolarizing voltage pulses from holding potentials (HP) of -50 (A) and -90 mV (B). (C) Differences obtained by subtracting currents elicited from HP -50 mV from currents elicited from HP -90 mV. (Top) Current traces recorded at test potential of -30 and $+10$ mV. (Bottom) Corresponding current-voltage (I-V) values of peak currents elicited from HP -90 and HP -50 mV and the subtracted I-V representing T-type current. The slow inactivating current, representing the L-type channel, peaked at $+10$ to $+20$ mV, whereas the fast decaying inward current peaked at -30 mV is indicative of T-type calcium current. The arrows indicate zero-current levels.

examples shown in figure 2, isoflurane at 0.9 mM depressed peak I_{Ca} in both L- (atrial and ventricular) and T-type (atrial) calcium channels. The corresponding I-V values show that the depression of I_{Ca} by isoflurane was reversed after washout of the anesthetic.

To compare the relative sensitivity of atrial L- and T-type calcium channels and ventricular L-type calcium channels to isoflurane, concentration-response curves were determined. Isoflurane block was calculated as a percent reduction in the peak current amplitude measured during depolarizing test pulses in the presence of various concentrations of the anesthetic compared with control. The respective K_d values for atrial T- and L-type and ventricular L-type calcium channels were 0.23 ± 0.03 , 0.34 ± 0.03 , and 0.71 ± 0.06 mM (fig. 3). These significant differences in K_d values show that the atrial T-type calcium channel is most sensitive and the ventricular L-type calcium channel is the least sensitive to the blocking action of isoflurane. The Hill coefficients were 1.5 ± 0.3 and 1.7 ± 0.2 for atrial T- and L-type calcium channels, respectively, and 2.8 ± 0.5 for the ventricular L-type calcium channel. The differences were not statistically significant.

Figure 4 demonstrates the effects of isoflurane on steady-state inactivation in atrial T- and L-type and ventricular L-type calcium channels. In the example shown, isoflurane produced a shift in the hyperpolarizing direction for all three cases. During control conditions, the average $V_{1/2}$ and k values for atrial T-type calcium chan-

nel current were -57.4 ± 0.6 and 6.9 ± 0.3 mV, respectively. The average values for $V_{1/2}$ and k for atrial and ventricular L-type calcium channel currents during control conditions were -15.1 ± 0.6 and 5.3 ± 0.3 mV and -14.9 ± 0.1 and 5.2 ± 0.1 mV, respectively. After the administration of isoflurane, there was a dose-dependent hyperpolarizing shift in $V_{1/2}$ for both atrial and ventricular calcium channels. Table 1 summarizes the dose-dependent shifts in $V_{1/2}$ of steady-state inactivation by isoflurane. At 0.25 mM isoflurane, a small but significant shift in $V_{1/2}$ occurred in atrial T-type channel but not in the atrial and ventricular L-type channels. At 0.9 mM isoflurane, the shift in steady state inactivation was significantly greater ($P < 0.05$) in the atrial L-type than the atrial T- and ventricular L-type calcium channels. However, at 1.20 mM isoflurane, the shift in steady state inactivation was significantly ($P < 0.05$) greater in both atrial and ventricular L-type calcium channels than the atrial T-type calcium channels. The k values did not change significantly in the presence of isoflurane.

The effects of isoflurane on steady-state activation for atrial L- and T-type and ventricular L-type calcium channel currents were less pronounced than those observed for steady-state inactivation (fig. 5). During control conditions, the average $V_{1/2}$ for steady-state activation for atrial T- and L-type and ventricular L-type calcium channels were -30.6 ± 0.8 , 7.4 ± 0.8 , and 2.0 ± 0.4 mV, respectively. The k values for activation for the atrial L- and T-type channels were 10.1 ± 0.2 and 9.1 ± 0.4 ,

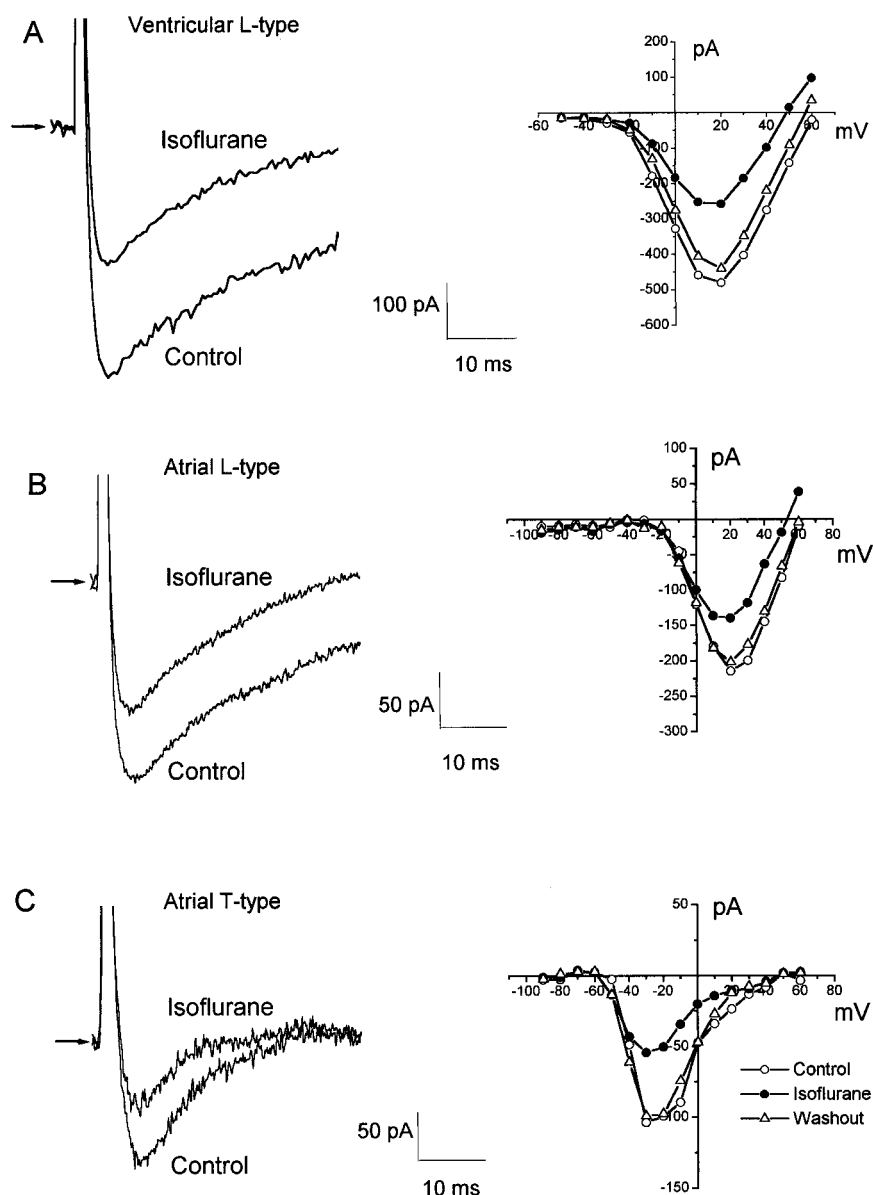


Fig. 2. Effects of isoflurane (0.9 mM) on ventricular L-type and atrial L- and T-type Ca²⁺ channel currents. Peak calcium current traces and corresponding current-voltage curves in the absence (control: open circle; washout: open triangle) and presence of isoflurane (filled circle) are shown for ventricular L-type Ca²⁺ channel (A) and atrial L- (B) and T-type (C) Ca²⁺ channels, respectively. The L- and T-type calcium currents were elicited as described in figure 1. The arrows indicate zero-current levels.

respectively, and 8.3 ± 0.2 mV for the ventricular L-type calcium channel. Table 2 summarizes the effects of isoflurane on steady-state activation in atrial and ventricular L- and T-type calcium channels. For the ventricular L-type calcium channel, only at higher isoflurane concentrations of 1.2 and 1.6 mM was a depolarizing shift in $V_{1/2}$ significant. Isoflurane did not significantly affect the steady state activation of the atrial L- and T-type calcium currents. The slope factor, k , did not change significantly in all cases after the addition of isoflurane.

In addition to the inhibitory effect on I_{Ca} , isoflurane also caused a significant hyperpolarizing shift in the apparent reversal potential (E_{rev}) for the atrial and ventricular L- but not for the T-type calcium channels (fig. 2). E_{rev} was determined graphically from the I-V relation. During control conditions (2 mM extracellular Ca²⁺), the average E_{rev} for the atrial and ventricular L-type calcium channels were 59.7 ± 2.3 and 59.1 ± 0.7 mV, respec-

tively. Isoflurane induced dose-dependent negative changes in E_{rev} in atrial and ventricular L-type calcium channel currents (figure 6A). These hyperpolarizing shifts in E_{rev} were reversed toward control values after washout of the anesthetic. To test whether these hyperpolarizing shifts in E_{rev} were dependent on extracellular Ca²⁺, experiments were conducted using ventricular myocytes to determine the effects of 0.9 mM isoflurane on E_{rev} in the presence of increased extracellular Ca²⁺ concentration (fig. 6B). At higher concentrations of extracellular Ca²⁺, the shifts in E_{rev} induced by isoflurane were diminished.

Discussion

This study compared the effects of isoflurane on atrial L- and T-type and ventricular L-type calcium channel

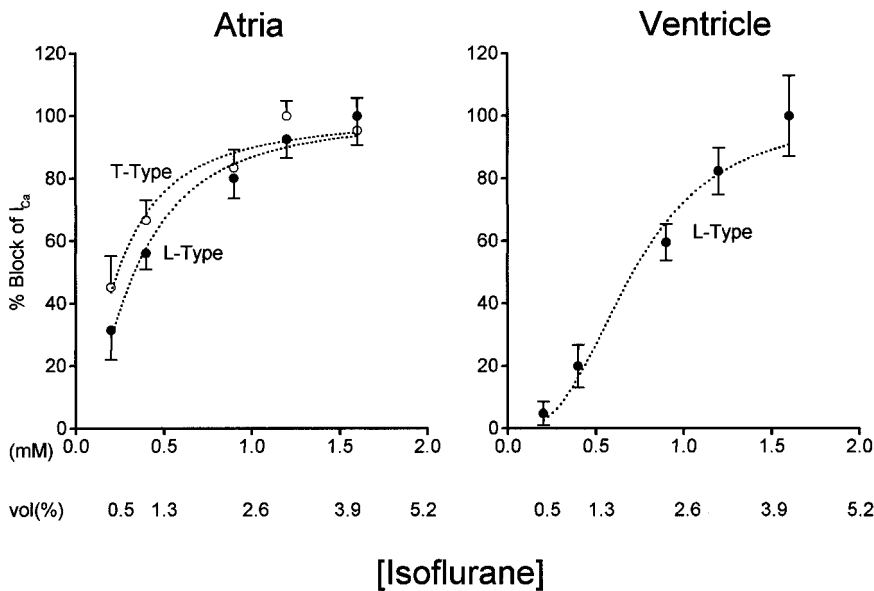


Fig. 3. Concentration-dependent depression of peak L- and T-type Ca^{2+} channel currents by isoflurane. Percent block was determined as percent reduction in peak current amplitude in the presence of isoflurane. (*Left*) Percent block of atrial L- (filled circle) and T-type (open circle) calcium channels; (*right*) percent block of ventricular L-type calcium channel current (filled circle). Data were normalized to maximum block of calcium current, and curves were fitted by nonlinear regression to a sigmoidal equation: $B/M = 1/[1 + (K_d/[A])^{n_H}]$, where B is block by isoflurane, M is the maximum block by the anesthetic, [A] represents the different concentrations of the anesthetic, K_d is the anesthetic concentration for half-maximal effect, and n_H is the Hill coefficient.

currents from the guinea pig heart. At clinically relevant concentrations, isoflurane differentially depressed atrial and ventricular calcium channel currents. Atrial T-type calcium channels showed the greatest sensitivity to isoflurane, followed by atrial L-type and the ventricular L-type calcium channels. This dose-dependent block of I_{Ca} by isoflurane within the clinical range shows saturation at higher concentrations for atrial L- and T-type but not for the ventricular L-type calcium channel. Isoflurane also induced dose-dependent hyperpolarizing shifts in $V_{1/2}$ of steady-state inactivation in atrial L- and T-type and ventricular L-type calcium channel currents. At higher concentrations, isoflurane induced a significant depolarizing shift in steady-state activation in ventricular L-type but not in atrial L- or T-type calcium channel current. Isoflurane also induced a hyperpolarizing shift in E_{rev} for the atrial and ventricular L-type calcium channel currents, and this effect was diminished in the presence of elevated extracellular Ca^{2+} concentrations.

Studies by other investigators reported similar hyperpolarizing shifts in steady-state inactivation by volatile anesthetics in ventricular $I_{\text{Ca,L}}$.^{3,15} The hyperpolarizing shift in $V_{1/2}$ of the inactivation curve suggests that isoflurane depresses I_{Ca} in guinea pig atrial and ventricular myocytes, in part by reducing the availability of calcium channels necessary for the influx of Ca^{2+} ions on depolarization. Furthermore, in ventricular cells, the depolarizing shift in activation curve at 1.2 and 1.6 mM isoflurane indicates that, at higher concentrations (above clinical levels), the depression of peak $I_{\text{Ca,L}}$ may also involve a decrease in ion conductance. The change in steady-state activation may be caused by isoflurane-induced alteration in the rate constants from closed to open states. The magnitude of shifts in steady-state inactivation curve was less in the atrial T-type than the L-type calcium channels (table 1), although the depression of peak I_{Ca} was greater in the T-type channel (fig. 3). These differences in shifts in inactivation curves between the atrial

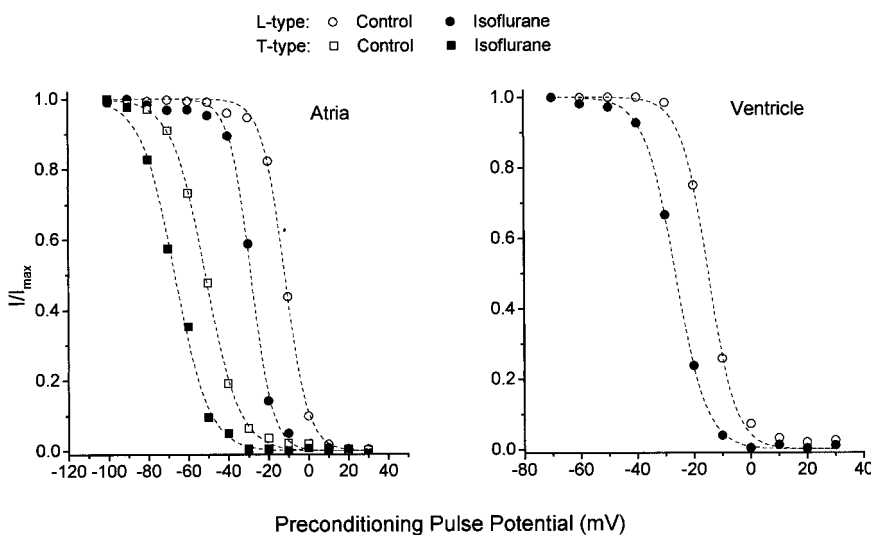


Fig. 4. Effects of isoflurane on steady-state inactivation of cardiac calcium channel currents. Inactivation curves using standard pulse protocol were obtained for L- (circles) and T-type (squares) calcium channel currents before (open symbols) and after isoflurane (0.9 mM; filled symbols). Normalized current amplitude is plotted versus preconditioning pulse potential. The data were fitted with a Boltzmann equation (see Methods). (*Left*) Steady state inactivation curves for atrial L- and T-type calcium channel; (*right*) inactivation curves for ventricular L-type calcium channel.

Table 1. Shifts in Steady-state Inactivation $V_{1/2}$ (Δ mV) of $I_{Ca,L}$ and $I_{Ca,T}$

Isoflurane (mm)	Atrial Cells				Ventricular Cells	
	T-type	n	L-type	n	L-type	n
0.25	$-3.2 \pm 1.8^*$	8	-1.8 ± 1.3	8	-1.3 ± 2.4	7
0.40	$-6.0 \pm 2.4^*$	7	$-8.4 \pm 1.1^*$	8	$-3.8 \pm 1.1^*$	7
0.90	$-6.5 \pm 1.8^*$	9	$-11.9 \pm 1.2^*\dagger$	12	$-8.0 \pm 0.7^*$	10
1.20	$-9.6 \pm 2.1^*$	8	$-14.7 \pm 0.8^*\ddagger$	9	$-12.8 \pm 1.5^*\ddagger$	8
1.60	$-12.5 \pm 5.5^*$	7	$-12.9 \pm 2.2^*$	7	$-13.5 \pm 0.7^*$	9

Summary of the effects of isoflurane on steady-state inactivation of the Ca^{2+} channel currents in atrial and ventricular cells. Data are mean \pm SEM of changes in $V_{1/2}$ from control for the different doses of isoflurane. The changes are denoted as Δ mV. Control $V_{1/2}$ (half maximal voltage) values of -57.4 ± 0.6 , -15.1 ± 0.6 , and -14.9 ± 0.3 mV were obtained for atrial T-, L-, and ventricular L-type Ca channels ($I_{Ca,T}$, $I_{Ca,L}$), respectively. The slope factors k were not significantly affected by isoflurane (see Results).

* Values are significantly different ($P < 0.05$) from control. † Significant difference in the hyperpolarizing shift in steady-state inactivation from atrial T- and ventricular L-type Ca channels. ‡ Significant ($P < 0.05$) differences in the hyperpolarizing shift in steady-state inactivation from atrial T-type Ca channels.

and ventricular L- and T-type calcium channels suggest that the depression of $I_{Ca,T}$ in atrial cells may depend less on the effects of isoflurane on calcium channel availability when compared with the L-type calcium channel. Furthermore, the shift in steady-state activation in the ventricular but not in the atrial L-type calcium channels by high concentrations of isoflurane suggest that the L-type calcium channels in the two chambers may be modulated differently by volatile anesthetics. No significant changes in k by isoflurane were observed, indicating that the channels voltage sensor was not affected.

The pharmacologic results of this study may suggest heterogeneity in the L- and T-type calcium channels in the guinea pig heart. Studies by other investigators have compared the effects of volatile anesthetics on calcium channels between and also within cell types. For example, McDowell *et al.*¹⁰ showed that, in a thyroid C-cell line, isoflurane and enflurane blocked T-type calcium channels more potently than either L- or N-type calcium channels. In a recent preliminary study, Yamakage *et al.*¹⁶ reported that, in porcine bronchial smooth muscle cells, the T-type calcium channel was more responsive than the L-type calcium channel to inhibition by volatile

anesthetics. However, anesthetic depression of the bronchial L-type calcium channel was similar to its depression of the tracheal L-type calcium channel. In smooth muscle cells obtained from guinea pig portal vein, the T-type calcium channel was less sensitive than the L-type calcium channel to isoflurane.¹⁷ Previous studies from this laboratory showed that, in the canine Purkinje fiber, the L- and T-type calcium channels showed similar sensitivities to isoflurane, enflurane, and halothane.¹² However, in the current study using guinea pig hearts, the atrial T-type calcium channels were more sensitive to isoflurane than the atrial and ventricular L-type calcium channels. In addition, the atrial L-type calcium channels were more responsive to isoflurane than the ventricular L-type calcium channel. The discrepancies in these studies may be attributed to tissue and functional differences between atrial and Purkinje fibers. In the canine heart, the ventricular L-type calcium channel current has been shown to have properties that are different from the conducting Purkinje fiber L-type calcium channel. For example, the decay of $I_{Ca,L}$ is slower in ventricular cells than in Purkinje cells.¹⁸ The differences in responses may also be ascribed to species differences.

Fig. 5. Effects of isoflurane on steady-state activation of cardiac calcium channel currents. Activation curves for L- (circle) and T-type (square) Ca^{2+} channel currents in atrial (left) and ventricular myocytes (right) were obtained by dividing the peak current recorded during each test potential by the driving force (see Methods). The normalized peak conductance for each calcium channel is plotted against test potential before (control; open symbols) and after isoflurane (0.9 mm; filled symbols). The dotted curves represent the Boltzmann fits to the data.

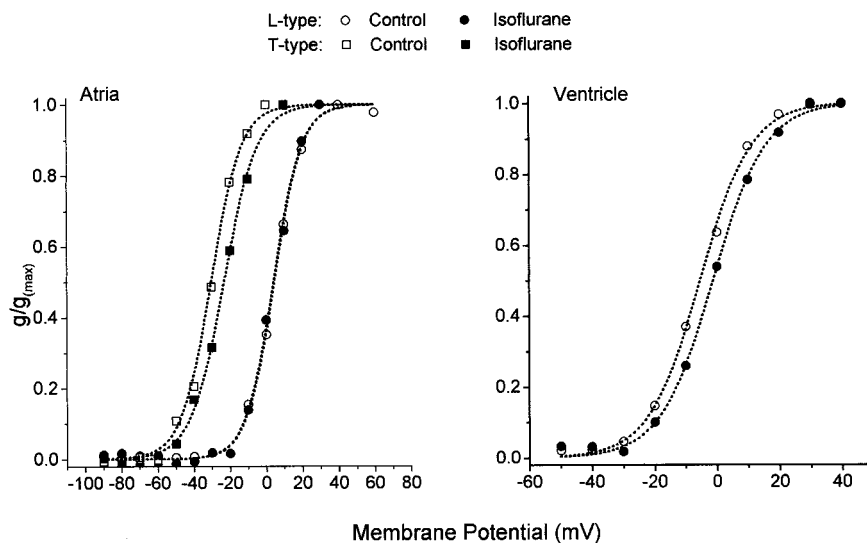


Table 2. Shifts in Steady-state Activation $V_{1/2}$ (Δ mV) of $I_{Ca,L}$ and $I_{Ca,T}$

Isoflurane (mM)	Atrial Cells				Ventricular Cells	
	T-type	n	L-type	n	L-type	n
0.25	-1.2 ± 2.8	8	-0.8 ± 1.5	8	-0.3 ± 2.2	9
0.40	-1.5 ± 4.1	10	$+3.9 \pm 1.6$	13	$+0.3 \pm 1.1$	15
0.90	$+3.1 \pm 2.2$	10	$+2.0 \pm 1.7$	16	$+1.5 \pm 0.7$	16
1.20	-4.5 ± 3.2	9	$+2.7 \pm 1.3$	12	$+2.5 \pm 0.8^*$	12
1.60	-1.5 ± 3.5	7	-1.3 ± 2.2	7	$+4.6 \pm 1.2^*$	10

Summary of the effects of isoflurane on steady-state activation of the Ca^{2+} currents in atrial and ventricular cells. Data are mean \pm SEM of changes in $V_{1/2}$ from control for the different doses of isoflurane. Control $V_{1/2}$ (half maximal voltage) values were -30.6 ± 0.8 , 7.4 ± 0.8 , and 2.0 ± 0.4 mV for atrial T- and L-type and ventricular L-type channels ($I_{Ca,T}$, $I_{Ca,L}$), respectively.

* Values significantly different ($P < 0.05$) from control.

Heterogeneity in the L- and T-type calcium channel functions have been attributed to the expression of distinct pore forming α_1 subunits, the selective association with accessory subunits and other modulatory proteins associated with the channels.¹⁹ The underlying molecular structure of the L-type calcium α_{1C} subunit present in heart, smooth muscle, and neurons²⁰⁻²² is different from the recently cloned human heart α_{1H} subunit of the T-type calcium channel.²³⁻²⁶ The α_{1C} subunit is also modulated by accessory subunits that may be different from the accessory subunits associated with the α_{1H} subunit of the T-type calcium channel.^{19,24,25,27,28} The L- and T-type calcium channels are also modulated differentially by neuromediators and intracellular mechanisms involving protein kinases and phosphorylation-dependent pathway.^{8,18} Takenoshita and Steinbach²⁹ reported that, in rat DRG cells, the greater sensitivity of the low-voltage (T-type) calcium channel to halothane when compared with the high-voltage (L-type) calcium channel might be attributed to the differences in signal transduction pathways that modulate the two channels.²⁹ These differences in α_1 isoforms and differences in intracellular modulators could contribute to the differences in responses of the L- and T-type calcium channels to isoflurane.

The differences in sensitivities to isoflurane by the atrial and ventricular L-type calcium channels may also suggest the existence of variants of the cardiac L-type calcium α_{1C} subunit. Splice variants of the human fibroblast α_{1C} subunit for the L-type calcium channel have been shown to respond differently to the inhibitory action of isradipine.³⁰ In the cardiovascular system, variants of the α_{1C} subunit are responsible for greater sensitivity of smooth muscle L-type calcium channel to nifedipine when compared with cardiac L-type calcium channel.²⁰ These differential responses may be attributed to specific segments of the transmembrane protein and splice variants of the accessory subunits that modulate the α_1 subunit.^{19,21,30} If similar variations in isoforms of the L-type exist in the heart, this may explain why the atrial L- and ventricular L-type calcium channels showed different sensitivities to isoflurane. However, whether isoforms of the L-type calcium channel α_{1C} subunit and variations in their accessory subunits exist for atrial and

ventricular myocytes of the two chambers remains to be tested.

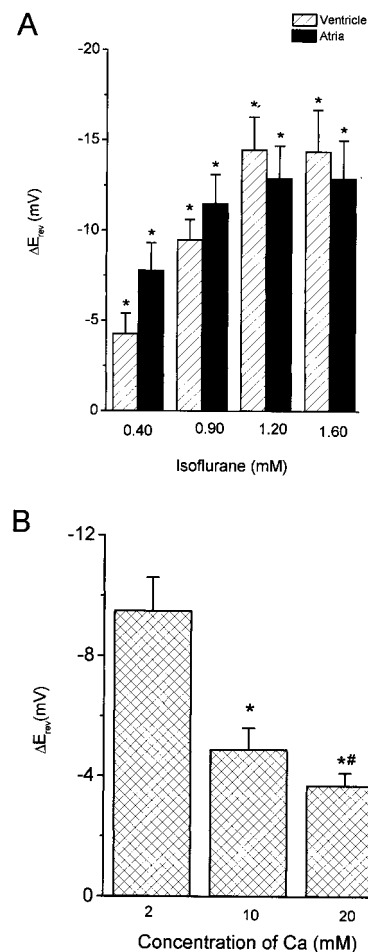


Fig. 6. Isoflurane induced shifts in reversal potential. (A) Dose-dependent effects of isoflurane on the apparent reversal potential (E_{rev}) in atrial (shaded gray) and ventricular (hatch bars) L-type Ca^{2+} channel currents are shown. The average E_{rev} for the atrial and ventricular L-type calcium channels were 59.7 ± 2.3 and 59.1 ± 0.7 mV, respectively; shifts in reversal potential are depicted as ΔE_{rev} from control. *Significantly different ($P < 0.05$) from control. **(B)** Increased extracellular Ca^{2+} concentration attenuates the magnitude of shift in E_{rev} induced by 0.9 mM isoflurane. #Shift in E_{rev} is significantly ($P \leq 0.05$) less when compared with 2 mM extracellular Ca^{2+} . #Significant difference between 10 and 20 mM extracellular Ca^{2+} in reducing isoflurane-induced hyperpolarizing shift in E_{rev} .

Another plausible explanation for the differences in the sensitivity of the atrial and ventricular L-type calcium channels could be diversity in the regulation of the L-type calcium channels in the two chambers. Thus, in the presence of isoflurane, the modulation of the L-type calcium channels in the atria and ventricle could involve other modulatory proteins in the local environment around the channel or in subsequent steps in signal transduction pathways. In support of this notion, we have recently shown that isoflurane might depress atrial and ventricular L-type calcium channel currents by different regulatory pathways. Preliminary results show that, at clinically relevant concentrations, isoflurane might inhibit ventricular but not atrial $I_{Ca,L}$ via a cholinergic-dependent signal transduction cascade.^{31,32} Thus, atrial and ventricular cells may contain receptors or intracellular mediators that may contribute to differential anesthetic sensitivity.

An interesting observation of this study is the reversible dose-dependent hyperpolarizing shift in the apparent E_{rev} of the atrial and ventricular $I_{Ca,L}$ induced by isoflurane. This negative shift in reversal potential by isoflurane was attenuated by increases in extracellular Ca^{2+} concentration (fig. 6). This reduction in the shift in E_{rev} by isoflurane in the presence of elevated external Ca^{2+} suggests that the shift may partly be attributed to selectivity changes of the calcium channel by the anesthetic, although further experiments are needed to confirm this. The notion that the calcium channel may be permeable to other monovalent ions has been suggested.^{33,34} Study³⁵ reported a similar hyperpolarizing shift in the calcium E_{rev} in the presence of isoflurane in hippocampal pyramidal neurons. According to Study, the shift in the reversal potential may be attributed to an increase in outward rectification caused, in part, by movement of Cl^- ,³⁶ which is reversed in the presence of Cd^{2+} .³⁵ Studies by Mitra and Morad³⁷ have also shown that Cs^+ ion fluxes through the inward rectifier potassium channel may contribute to shift in the reversal potential. In the current study, however, it is unlikely that Cs^+ flux through the inward rectifier potassium channel contributed to the shift in the reversal potential. At the two holding potentials used in this study, -50 and -90 mV, the holding current amplitude was zero in control and in the presence of isoflurane. Furthermore, the concentrations of extracellular Cs^+ , 30 – 120 mM, was significantly higher in the study by Mitra and Morad.³⁷ Consequently, the contribution of the inward rectifier potassium channel to changes in the observed reversal potential is likely negligible.

In summary, the current study shows that the calcium channels in the atria and ventricles respond differently to isoflurane. The differences in sensitivity and actions of the anesthetic on the channels may represent phenotypic differences in the calcium channels or differences in the regulation of the channels. Nonetheless, the dif-

ferential sensitivities of the channels to isoflurane, particularly at the lower concentrations, could cause a disparity in the refractory periods in the two chambers, which could cause conduction abnormalities during anesthesia.

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