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Effects of Volatile Anesthetics on Sarcolemmal Calcium Transport and Sarcoplasmic Reticulum Calcium Content in Isolated Myocytes

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Background: **The surface membrane Ca^{2+}–adenosine triphosphatase and Na^{+}–Ca^{2+} exchanger transport Ca^{2+} out of the ventricular myocyte, competing for cytosolic Ca^{2+} with the Ca^{2+}-adenosine triphosphatase located in the sarcoplasmic reticulum. In this study the authors examined the effects of halothane, isoflurane, and sevoflurane on Ca^{2+} extrusion from the cell and sarcoplasmic reticulum Ca^{2+} content.**

Methods: **Single myocytes from the right ventricular free wall of adult male ferret hearts were isolated, loaded with the acetoxymethyl ester of the fluorescent Ca^{2+} indicator fluo-3, and electrically stimulated at 0.25 Hz to reach a steady state level of intracellular Ca^{2+} stores. The effects of halothane, isoflurane, and sevoflurane (1 minimum alveolar concentration) on the peak and rate of decline of the Ca^{2+} transient induced by 10 mM caffeine were examined. The peak was used as an index of sarcoplasmic reticulum Ca^{2+} content, and the rate of decline was used to monitor Ca^{2+} extrusion from the cell.**

Results: **During control conditions, halothane reduced the Ca^{2+} content of the sarcoplasmic reticulum, isoflurane maintained it, and sevoflurane caused it to increase. Halothane did not affect Ca^{2+} extrusion from the cell, but both isoflurane and sevoflurane inhibited it. When Na^{+}–Ca^{2+} exchange was inhibited by ionic substitution, isoflurane and sevoflurane still reduced the rate of Ca^{2+} efflux from the cell. However, when the sarcolemmal Ca^{2+}–adenosine triphosphatase was inhibited by carboxyeosin, isoflurane and sevoflurane had no effect on Ca^{2+} efflux.**

Conclusions: **These results suggest that isoflurane and sevoflurane inhibit Ca^{2+} transport from the cell *via* the sarcolemmal Ca^{2+}–adenosine triphosphatase. This effect seems to counteract the decrease in Ca^{2+} influx through sarcolemmal L-type Ca^{2+} channels and maintains sarcoplasmic reticulum Ca^{2+} stores.**

PREVIOUS studies have shown that volatile anesthetics have direct effects on the heart and depress myocardial contractility *in vitro* in a dose-dependent, reversible manner.[1–7] In general, the effects of the volatile anesthetics on myocardial contractility can be attributed to their effects on mechanisms involved in intracellular Ca^{2+} homeostasis,[3,5,8–11] but changes in Ca^{2+} sensitivity[7,11,12] and cross-bridge cycling have been reported.[13–16]

At least four processes are involved in regulating intracellular Ca^{2+} in the myocyte, and individual volatile anesthetics could theoretically affect each one differently. Three of these processes are important in the beat-to-beat regulation of intracellular Ca^{2+}. The sarcolemmal Ca^{2+}–adenosine triphosphatase (PMCA) and Na^{+}–Ca^{2+} exchanger (NCX) transport Ca^{2+} out of the ventricular myocyte, competing for cytosolic Ca^{2+} with the Ca^{2+}–adenosine triphosphatase located in the sarcoplasmic reticulum (SERCA). During steady state contractions in the heart, Ca^{2+} entry across the surface membrane through L-type Ca^{2+} channels (I_{Ca}) is balanced by an equal efflux of Ca^{2+} from the cell *via* the NCX[17,18] and the PMCA.[19,20] In this way, intracellular Ca^{2+} and sarcoplasmic reticulum (SR) Ca^{2+} content are regulated and maintained.

Because of the multiple sites involved in maintaining the intricate balance necessary for normal Ca^{2+} homeostasis, it is not surprising that individual volatile anesthetics seem to differ in their effects on SR Ca^{2+} storage. For example, halothane and enflurane have been reported to decrease steady state SR Ca^{2+} content,[7,21–26] whereas isoflurane seems to maintain it.[24,27] Theoretically, the volatile anesthetics could alter the amount of Ca^{2+} stored in the SR by increasing or decreasing Ca^{2+} extrusion from the cell, for example, by stimulating or inhibiting NCX or PMCA. The purpose of this study was to examine the effects of volatile anesthetics (halothane, isoflurane, and sevoflurane) on SR Ca^{2+} content and Ca^{2+} flux out of the ventricular myocyte.

Methods

Tissue Preparation

All experimental procedures were reviewed and approved by the Animal Care and Use Committee of the Mayo Foundation (Rochester, MN). Protocols were completed in accordance with National Institutes of Health guidelines and in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council). Adult male ferrets (mean weight, 1.2 kg; Marshall Farms, North Rose, NY) were anesthetized with 3% halothane in 100% oxygen, and their hearts were rapidly excised. The aorta was then cannulated and the heart perfused with physiologic salt solution (PSS) bubbled with 100% oxygen. The composition of this solution was 117.5 mM NaCl, 5 mM KCl, 1.0 mM MgCl, 1.0 mM $CaCl_2$, 5 mM MOPS, 20 mM Na-acetate, and 10 mM glucose (pH 7.40).

Ferret ventricle was chosen because the balance between SERCA, NCX, and PMCA is similar to human

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ventricle.[20,28] Functionally, this results in both rest potentiation of SR Ca^{2+} release and rest decay of the SR Ca^{2+} content.

To isolate single myocytes, the right ventricular free wall was removed *en bloc* and rinsed for 5 min in PSS containing no added Ca^{2+}. The tissue was then minced into small pieces and gently stirred in a Teflon beaker containing a dispersion solution[29] composed of 75 mM NaCl, 2.4 mM KCl, 1.0 mM MgCl, 10 mM HEPES, 58 mM sucrose, 10 mM dextrose, 5 mM $NaHCO_3$, and 2.5 mM L-glutamic acid (pH 6.96) with 160 μg/ml type II collagenase and 0.6 mg/ml bovine serum albumin. The solution was decanted and replaced four times at 15-min intervals. Myocytes were harvested by low-speed centrifugation and then resuspended in culture medium (Medium 199).

Detection of the Intracellular Ca^{2+} Transient

Single isolated myocytes were loaded for 2–3 min with the acetoxymethyl ester of the fluorescent Ca^{2+} indicator fluo-3 (Molecular Probes, Eugene, OR) at an extracellular concentration of 1 μM. Fluo-3 was chosen for the intracellular Ca^{2+} indicator because it undergoes an approximately 100-fold increase in fluorescence on Ca^{2+} binding. This property makes it particularly well suited for measuring the kinetics of Ca^{2+} transients because it improves the signal-to-noise ratio. The rate and extent of shortening of fluo-3–loaded cells was not different from unloaded controls. A comparison of fura-2 and fluo-3 in pilot studies indicated that the two indicators yielded similar results and that the fluo-3 signals were less noisy.

Cells were subsequently rinsed with Medium 199 and rested for 30 min to allow complete deesterification of the fluo-3. For individual experiments, an aliquot of cells was transferred to a small plastic chamber (90-μl volume) mounted on the stage of the inverted microscope and allowed to adhere to a laminin-coated coverslip. The adhering myocytes were then superfused with PSS delivered to the chamber by gravity at a rate of 5 ml/min. Cells were electrically stimulated by a pair of platinum electrodes to reach a steady state level of intracellular Ca^{2+}. The electrodes carried square-wave pulses of 5-ms duration, at a voltage 10% greater than threshold and at a rate of 0.25 Hz. All experiments were conducted at 23°C. At this temperature, the extrusion of fluo-3 from ferret ventricular myocytes is minimal compared with that which occurs at 37°C (unpublished observation, September 4, 1998).

Fluo-3 fluorescence was measured by a fluorometer (C&L Instruments, Hummelstown, PA) mounted on an inverted microscope (TE300; Nikon, Melville, NY). The fluo-3 was excited with 485 nm light from a xenon bulb, and Ca^{2+}-dependent fluorescence emission was detected at 535 nm. Dye photobleaching was minimized by restricting the power level of the light source and by preventing exposure to light when data were not being acquired.

Administration of Anesthetic

In all experiments an in-line calibrated anesthetic vaporizer was used to add the appropriate concentration of isoflurane to the solution that bathed the preparation. Concentrations of volatile anesthetics in the gas over the bathing solution were monitored by Raman spectroscopy (Ohmeda Rascal II, Madison, WI) and were also verified in the chamber solution by gas chromatography (5880A; Hewlett-Packard, Palo Alto, CA).[30] The 1 minimum alveolar concentration (MAC) value for the three anesthetics at 23°C (isoflurane = 0.87%, halothane = 0.48%, sevoflurane = 1.53%) was calculated from published values measured at 37°C in the ferret (halothane and isoflurane)[31] and rat (sevoflurane).[32] The MAC values at different temperatures are essentially constant when expressed as aqueous phase concentrations.[32]

Experimental Protocols

In high concentration (10 mM), caffeine is known to release all SR Ca^{2+} content and prevent functional reuptake of Ca^{2+}.[33] When a myocyte bathed in PSS is exposed to caffeine, the intracellular [Ca^{2+}] rapidly increases to a peak and then decreases exponentially because of the action of the NCX (approximately 86%) and PMCA (approximately 14%).[20] Uptake of Ca^{2+} into the mitochondria contributes relatively little to the rate of decline.[19]

In some experiments, the NCX was inhibited by bathing the cells in 0 Na^+–0 Ca^{2+} solution, which contained no added Na^+ (LiCl substituted for NaCl) and 1 mM EGTA.[34] When caffeine is applied to the myocytes bathed in this solution, PMCA is the predominant mechanism responsible for the decline of the Ca^{2+} transient. In another set of experiments, we inhibited the PMCA by exposing cells to 5-(and-6) carboxyeosin diacetate (succinimidyl ester; Molecular Probes). Carboxyeosin has been shown to block cardiac PMCA more effectively than ionic substitution (0 Na^+, 10 mM Ca^{2+}) in ferret and rabbit ventricular myocytes.[19,35,36] A 10-mM stock solution in dimethyl sulfoxide was added to the control PSS to achieve a final concentration of 20 μM. Cells were loaded with carboxyeosin by exposing them to this solution for 15 min. They were then incubated in normal PSS without stimulation for 15 min to allow for deesterification of carboxyeosin. We performed only a single contracture in each of these cells (no time control) because the resting fluorescence did not return to baseline after caffeine application in the presence of carboxyeosin.

Signals were digitized and recorded at a sampling rate of 200 Hz. The least squares method was used to fit a single exponential curve ($Y = Y_0 + ae^{-kt}$) to the declining phase of the data.

Statistical Analysis

Individual measurements obtained in the presence of an anesthetic agent were normalized to the value ob-

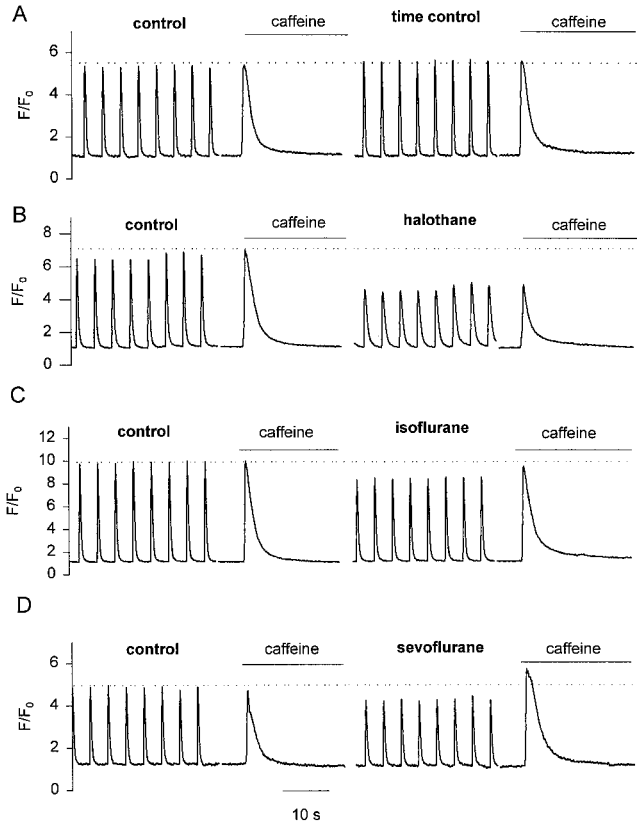

Fig. 1. Effects of halothane, isoflurane, and sevoflurane on electrically stimulated and caffeine-induced Ca^{2+} transients in isolated myocytes. The protocol consisted of electrical stimulation at 0.25 Hz for 5 min to reach steady state intracellular Ca^{2+} concentrations, at which point stimulation was stopped for 30 s (data not shown) and 10 mM caffeine was administered. Representative records obtained in four cells. The left half of the figure was recorded in the absence of anesthetic. The right half of the figure shows the effect of repeating the protocol in the same cell either in the absence (time control) or presence of anesthetic. Data are shown for (*A*) for time control, (*B*) 1 MAC halothane, (*C*) 1 MAC isoflurane, and (*D*) 1 MAC sevoflurane.

tained in the same cell in the absence of the anesthetic. For time control cells, the same protocol was followed except that they were not exposed to anesthetic. For statistical analysis, measurements in anesthetic-treated cells were compared with time controls, except in cells treated with carboxyeosin, where this was not possible. All measurements are reported as mean ± SD. Statistical significance ($P < 0.05$) was determined using the one-way analysis of variance with Dunnett test for multiple comparisons *versus* control.

Results

Effects on Sarcoplasmic Reticulum Ca^{2+} Release, Ca^{2+} Content, and Fractional Release

Figure 1 shows representative records from four cells used in these experiments. The left half of the figure shows normal Ca^{2+} transients recorded during electrical stimulation followed by a prolonged transient obtained during the application of caffeine. We used the peak of the caffeine-induced transient to monitor the Ca^{2+} content of the SR, and the rate of decline as an index of the rate of Ca^{2+} extrusion from the cell.[33,34] Caffeine apparently opens the Ca^{2+} release channel in the SR, resulting in the prolonged transients. In this situation, the intracellular [Ca^{2+}] slowly declines because of the continued function of the NCX and PMCA mechanisms that move Ca^{2+} out of the cell.

The right half of the figure repeats this sequence in the absence of anesthetic (time control, fig. 1A) and in the presence of halothane (fig. 1B), isoflurane (fig. 1C), and sevoflurane (fig. 1D), each at 1 MAC. Control experiments showed that repeated exposures to caffeine were well tolerated. However, we felt the use of time controls was necessary to account for the possibility of cell rundown and that the level of fluo-3 might decrease over the course of the experiment because of photobleaching or loss from the cell.

Figure 2 shows the mean data for the relative amplitude of the electrically stimulated (fig. 2A) and caffeine-induced (fig. 2B) Ca^{2+} transients. Figure 2A shows that each anesthetic decreased the peak intracellular Ca^{2+} during electrical stimulation. The peak of the electrically stimulated Ca^{2+} transient (time control = 100 ± 19%) in 1 MAC anesthetic decreased for halothane (64 ± 12%), isoflurane (82 ± 14%), and sevoflurane (86 ± 17%).

In contrast, the results are different for the caffeine-induced Ca^{2+} transient. Figure 2B shows that total SR Ca^{2+} content was unchanged in time controls (94 ± 14%) and decreased in the presence of halothane (60 ± 7%). Of note, it was unchanged in the presence of isoflurane (97 ± 14%) and actually increased in the presence of sevoflurane (110 ± 21%).

We also compared the effect of anesthetics on the fractional release of Ca^{2+} from the SR. Fractional release was calculated by dividing the peak of the electrically stimulated transient (amount released) by the peak of the subsequent caffeine-induced transient (amount available).[7,28,37] In the absence of anesthetic, the fractional release is high (very close to 100%; fig. 2C) during the conditions of the experiment. The slow rate of electrical stimulation (0.25 Hz) allows more SR Ca^{2+} release channels to recover between beats (increases the electrically stimulated transient), and a certain amount of Ca^{2+} leaks from the SR during the 30-s rest period before the application of caffeine (decreases the caffeine-induced transient).

During these circumstances, the fractional Ca^{2+} release (time control = 102 ± 22%) remained unchanged in the presence of halothane (104 ± 24%). In contrast, both isoflurane (84 ± 27%) and sevoflurane (79 ± 21%) decreased the relative amount of available Ca^{2+} that was released by electrical stimulation.

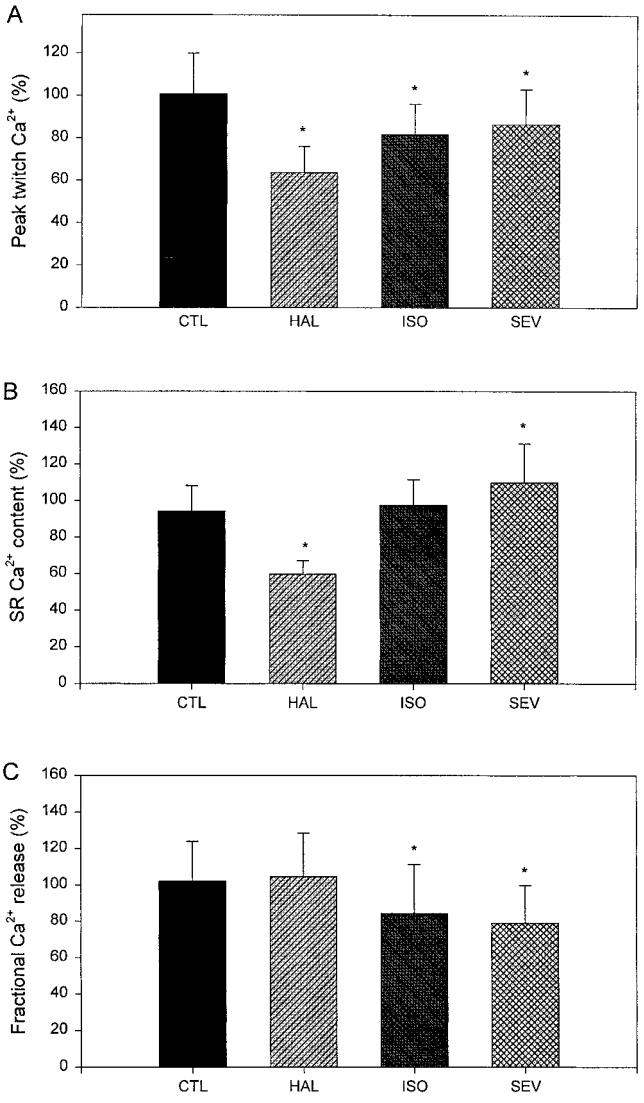

Fig. 2. Effects of halothane, isoflurane, and sevoflurane on the peak of the electrically stimulated Ca^{2+} transients, sarcoplasmic reticulum (SR) Ca^{2+} content, and fractional release of Ca^{2+} from the SR (see text) as a percentage of that obtained at baseline in the same cell. (*A*) Amplitude of the electrically stimulated Ca^{2+} transients; (*B*) SR Ca^{2+} content determined by application of 10 mM caffeine; (*C*) Fractional release of Ca^{2+} from the SR. Data (mean ± SD) are shown in the presence of halothane (HAL, n = 12), isoflurane (ISO, n = 14), and sevoflurane (SEV, n = 16) each at 1 MAC, and in time controls in the absence of anesthetic (CTL, n = 13). *$P < 0.05$.

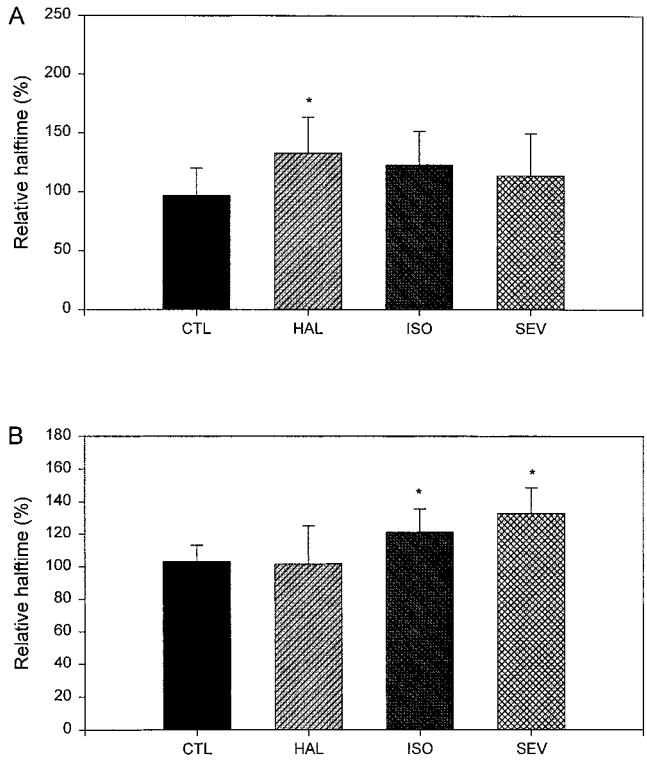

Fig. 3. Effects of halothane, isoflurane, and sevoflurane on the rate of decline of electrically stimulated and caffeine-induced Ca^{2+} transients when cells were bathed in physiologic salt solution. Results expressed as a percentage of that obtained at baseline in the same cell. (*A*) Half-time of decline of the electrically stimulated Ca^{2+} transients; (*B*) half-time of decline of the caffeine-induced transient. Data (mean ± SD) are shown in the presence of halothane (HAL, n = 12), isoflurane (ISO, n = 14), and sevoflurane (SEV, n = 16) each at 1 MAC, and in time controls in the absence of anesthetic (CTL, n = 13). *$P < 0.05$.

Effects on Ca^{2+} Uptake by the Sarcoplasmic Reticulum

Only halothane prolonged the duration of the electrically stimulated intracellular Ca^{2+} transient compared with time controls, indicating that it decreases the reuptake of Ca^{2+} from the myoplasm by the SR. Figure 3A shows the relative effects of each anesthetic on the half-time of the electrically stimulated Ca^{2+} transient. The greatest effect in percentage terms was seen in the presence of halothane (133 ± 31%). Isoflurane (123 ± 29%) and sevoflurane (114 ± 36%) did not produce a statistically significant prolongation compared with the time controls (97 ± 23%). The half-time of relaxation of the Ca^{2+} transient was 160 ± 55 ms during time control conditions and increased to 252 ± 107 ms in the presence of halothane ($P < 0.05$). Again, there was not a statistically significant prolongation of the electrically stimulated transient by isoflurane (170 ± 74 ms) or sevoflurane (155 ± 68 ms).

Effects on Ca^{2+} Extrusion from the Cell

When a myocyte is exposed to caffeine in the presence of normal PSS, the function of the SR is inhibited by caffeine.[33] However, the intracellular [Ca^{2+}] declines because both NCX and PMCA mechanisms are active in extruding Ca^{2+} from the cell. Table 1 shows the effects of inhibition of NCX (0 [Na^+] and 0 [Ca^{2+}]) and PMCA (carboxyeosin) compared with control conditions (PSS) on the caffeine-induced Ca^{2+} transients. We did not attempt to inhibit mitochondrial uptake of Ca^{2+} because it does not appear to make a substantial contribution to the rate of decline of intracellular Ca^{2+} during twitches or caffeine contractures.[19,33]

When the myocyte is bathed in normal PSS, the rate of decline decreased in the presence of isoflurane and

Table 1. Half-time of Decrease of Electrically Stimulated and Caffeine-induced Ca^{2+} Transients under Various Conditions

	PSS	0 Na^+, 0 Ca^{2+}	Carboxyeosin
Time control			
TWI (s^{-1})	0.16 ± 0.06	—	0.13 ± 0.03
%	97 ± 23	—	—
CAF (s^{-1})	1.21 ± 0.21	2.26 ± 0.61	2.12 ± 0.38
%	103 ± 10	89 ± 14	—
n	13	20	8
Sevoflurane			
TWI (s^{-1})	0.15 ± 0.07	—	0.14 ± 0.04
%	114 ± 36	—	—
CAF (s^{-1})	1.49 ± 0.23	3.07 ± 1.07	2.10 ± 0.41
%	133 ± 16*	126 ± 22*	—
n	16	11	8
Isoflurane			
TWI (s^{-1})	0.17 ± 0.07	—	0.15 ± 0.02
%	123 ± 29	—	—
CAF (s^{-1})	1.31 ± 0.34	3.28 ± 1.24	2.05 ± 0.30
%	121 ± 14*	120 ± 24*	—
n	14	8	8
Halothane			
TWI (s^{-1})	0.25 ± 0.11	—	0.17 ± 0.04
%	133 ± 31*	—	—
CAF (s^{-1})	1.24 ± 0.28	1.94 ± .32	1.57 ± 0.44*
%	102 ± 23	75 ± 9	—
n	12	9	8

Mean ± SD.

* $P < 0.05$ compared with time control in the same solution.

PSS = physiologic saline solution; 0 Na^+, 0 Ca^{2+} = lithium chloride substituted for sodium chloride, 1 mM EGTA; TWI = electrically stimulated twitch; CAF = caffeine-induced contracture.

sevoflurane, but not halothane. Figure 3B shows that, during these circumstances, the duration of the caffeine-induced Ca^{2+} transient (time control = 103 ± 10%) is unchanged in the presence of halothane (102 ± 23%) but increases in the presence of isoflurane (121 ± 14%) and sevoflurane (133 ± 16%). This suggests that isoflurane and sevoflurane inhibit Ca^{2+} efflux from the cell. Figure 4 shows representative traces illustrating the effect of no anesthetic (simply repeating the contracture; fig. 4A), halothane (fig. 4B), isoflurane (fig. 4C), and sevoflurane (fig. 4D) on the rate of decline of the caffeine-induced Ca^{2+} transient. During these circumstances, only isoflurane and sevoflurane significantly inhibited Ca^{2+} extrusion.

To further clarify this issue, we examined the effect of isoflurane and sevoflurane on the duration of the caffeine-induced transient when NCX was inhibited in a solution lacking Na^+ and Ca^{2+} (0 Na^+ and 0 Ca^{2+}). Figure 5A shows the mean data from these experiments. Although halothane had no effect, both isoflurane and sevoflurane slowed the rate of decline. The relative half-time of decline was 120 ± 53% in the presence of isoflurane and 126 ± 22% in the presence of sevoflurane, compared with 89 ± 14% for time controls. These results suggest that both isoflurane and sevoflurane inhibit PMCA.

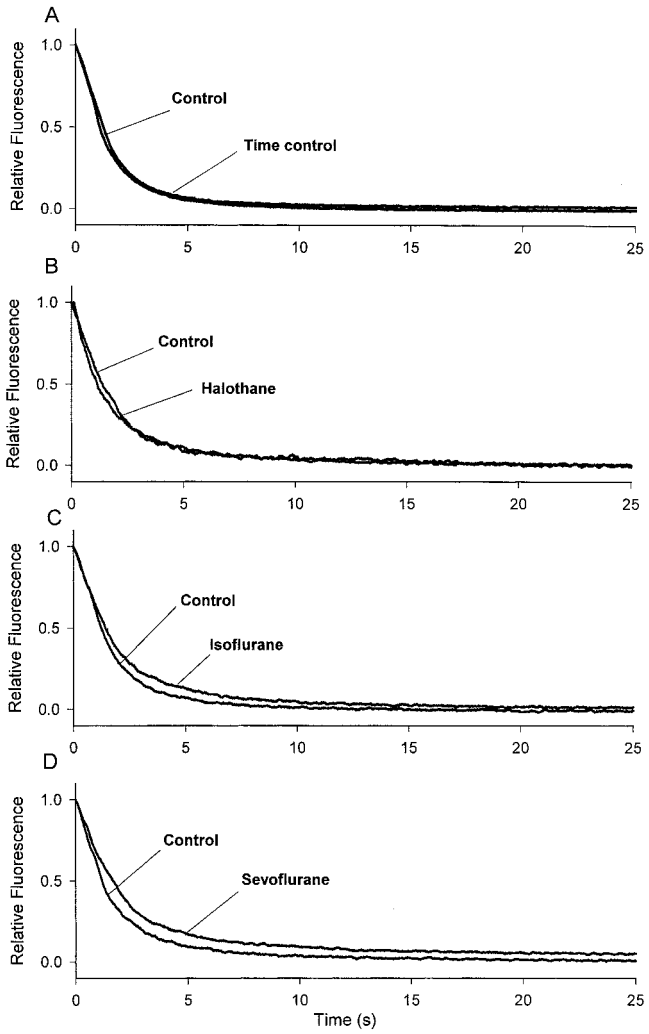

Fig. 4. Representative traces of declining phase of the Ca^{2+} transient obtained during caffeine application in four cells. The effect of repeating the caffeine exposure in the same cell either in the absence (time control) or presence of anesthetic is shown for (*A*) time control, (*B*) 1 MAC halothane, (*C*) 1 MAC isoflurane, and (*D*) 1 MAC sevoflurane.

In another set of experiments, we examined the effects of isoflurane and sevoflurane when only NCX was active in extruding Ca^{2+} from the cell. In this case, we inhibited the surface membrane Ca^{2+} pump with carboxyeosin.[19,35] Carboxyeosin has been shown to more effectively block the PMCA than ionic substitution in ferret ventricular myocytes.[19] Figure 5B summarizes the results of these experiments. Although halothane decreased the half-time of decline of the caffeine-induced transient (half-time = 1.57 ± 0.44 s^{-1}), it does not appear to actually increase the rate of extrusion because the resting level of Ca^{2+} increased during these circumstances. On the other hand, isoflurane (half-time = 2.0 ± 0.30 s^{-1}) and sevoflurane (half-time = 2.1 ± 0.41 s^{-1}) had no effect on the duration of the caffeine-induced

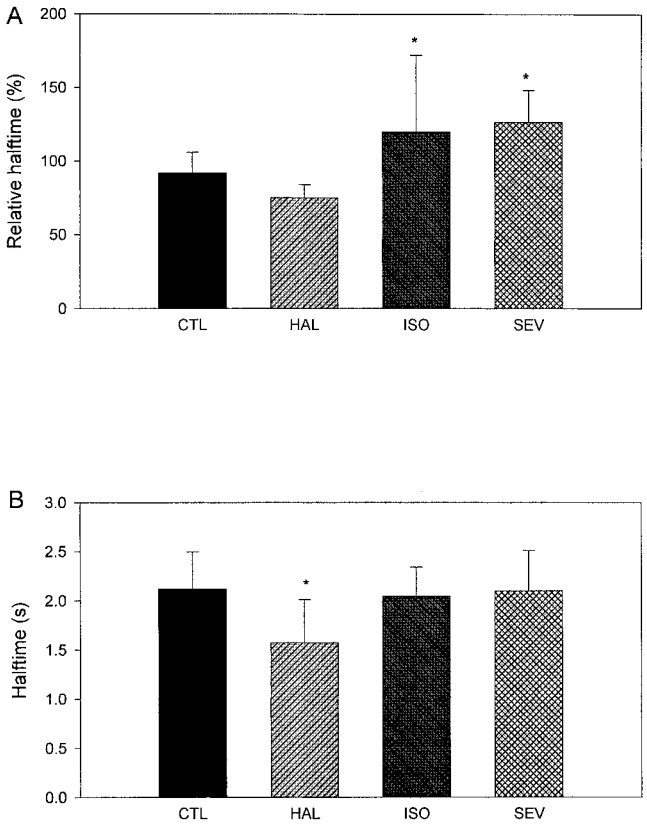

Fig. 5. Effects of isoflurane and sevoflurane on the rate of decline of the caffeine-induced Ca^{2+} transient when the function of either Na^+–Ca^{2+} exchanger (NCX) or sarcolemmal Ca^{2+}–adenosine triphosphatase (PMCA) was inhibited. (*A*) Effects of halothane, isoflurane, and sevoflurane in cells bathed in 0 Na^+, 0 Ca^{2+} (NCX inhibited). Results expressed as a percentage of that obtained at baseline in the same cell. (*B*) Effects of isoflurane and sevoflurane in cells exposed to carboxyeosin (PMCA inhibited). Data are mean ± SD. *$P < 0.05$. CTL = control; HAL = halothane; ISO = isoflurane; SEV = sevoflurane.

contracture when compared with controls (half-time = 2.1 ± 0.38 s^{-1}) and had no apparent effect on resting Ca^{2+}. This result is consistent with the interpretation that isoflurane and sevoflurane prevent Ca^{2+} extrusion by inhibiting the PMCA. Assuming that carboxyeosin causes 100% inhibition of the PMCA, we can use the rate constants of the decline of the caffeine-induced calcium transient to estimate the approximate inhibition caused by 1 MAC anesthetic. In this case, sevoflurane caused 88% inhibition and isoflurane 56%.

Discussion

The interpretation of our experimental data must take into account the ability of individual volatile anesthetics to affect multiple sites within the myocardial cell in different ways. The interplay between the capacity of each system involved in Ca^{2+} homeostasis and the degree to which it is affected by a particular volatile anesthetic determines the amount of Ca^{2+} stored in and released from the SR. In this study we found that halothane, isoflurane, and sevoflurane decrease the electrically stimulated intracellular Ca^{2+} transient, but that only isoflurane and sevoflurane tend to preserve the Ca^{2+} content of the SR. The latter observation is somewhat surprising, because all three anesthetics have been reported to inhibit I_{Ca}[9,38] and therefore might be expected to decrease SR Ca^{2+} content in the absence of other antagonistic effects. However, our data also indicate that isoflurane and sevoflurane inhibit Ca^{2+} extrusion from the cell by the PMCA. This effect is important because it apparently allows the SR to accumulate relatively more Ca^{2+} than it would otherwise.

Effects on Electrically Stimulated Transients and Fractional Release

The ability of volatile anesthetics to inhibit I_{Ca} is important in explaining our results for two reasons. First, the relatively small amount of Ca^{2+} that enters the cell in this way triggers the release of a much greater amount of Ca^{2+} from the SR in a process known as Ca^{2+}-induced Ca^{2+} release (CICR), and fractional Ca^{2+} release can be affected by interventions that alter this process. Second, the relatively small amount of Ca^{2+} that makes up I_{Ca} can accumulate in the SR during some circumstances and contribute to the larger pool of Ca^{2+} released from the SR in subsequent beats. For example, this process probably contributes substantially to the positive force–frequency relation seen in some species (rabbit, ferret, and human, but not rat or mouse).[20]

Therefore, the volatile anesthetics could potentially affect fractional Ca^{2+} release or SR Ca^{2+} content by affecting I_{Ca}. In the presence of isoflurane and sevoflurane, the most likely explantation for the observed decrease in the electrically stimulated Ca^{2+} transient despite normal or increased SR Ca^{2+} content is that CICR is impaired when Ca^{2+} current decreases.[37] This effect on fractional release is what would be expected solely because of inhibition of surface membrane Ca^{2+} current, and has been documented for nifedipine in rat ventricular myocytes.[7]

Effects on Sarcoplasmic Reticulum Ca^{2+} Content and Transarcolemmal Ca^{2+} Transport

Our observation that the SR Ca^{2+} content can be preserved (isoflurane) or increased (sevoflurane) suggests that other mechanisms must be involved, because an agent that only decreased I_{Ca} would be expected to reduce SR Ca^{2+} content. Among the three major mechanisms responsible for controlling the free intracellular [Ca^{2+}] and balancing systolic Ca^{2+} influx, SERCA sequesters Ca^{2+} in the SR for later release during systole, whereas NCX and PMCA decrease the amount of Ca^{2+} available for release by transporting Ca^{2+} out of the cell. During a normal twitch, SERCA is responsible for transporting most (65–92%) of the cytosolic Ca^{2+}, NCX re-

moves less (7–30%), and the PMCA still less (1–3%).[33,34] Clearly, volatile anesthetic interactions with any or all of these processes could affect the SR Ca^{2+} content.

At equilibrium, by definition the amount of Ca^{2+} entering the cell each beat equals the amount leaving. Interventions that inhibit I_{Ca}, for example, the volatile anesthetics, could possibly decrease the Ca^{2+} transient by two mechanisms. First, less Ca^{2+} could be available for release (I_{ca} is the main determinant of SR Ca^{2+} content), and second, the trigger mechanism could be less effective (decreased fractional release, inhibition of CICR). Indeed, it has been consistently observed that the amount of Ca^{2+} released from the SR during electrical stimulation decreases in the presence of volatile anesthetics (halothane, enflurane, isoflurane, and sevoflurane) and the peak free intracellular Ca^{2+} concentration declines.[3,7,11,39]

However, halothane clearly affects Ca^{2+} homeostasis differently from isoflurane and sevoflurane, and the most likely explanation is that, of the anesthetics tested in this study, only halothane opens the Ca^{2+} release channel.[40–42] Halothane apparently causes Ca^{2+} to leak into the cytoplasm and depletes SR Ca^{2+} content.[22–24] On the other hand, halothane does not appear to alter fractional Ca^{2+} release even though it decreases I_{Ca}, indicating that it may enhance CICR. This facilitation of SR Ca^{2+} release (CICR) after abrupt exposure to halothane (and enflurane) appears to be responsible for the ability of these anesthetics to cause a short-lived increase in intracellular Ca^{2+}.[26] It has been suggested that, in the steady state situation, halothane may have offsetting effects on Ca^{2+} current and CICR.[7]

Our results are consistent with those of Davies *et al.*,[7] who reported the effects of halothane, isoflurane, and sevoflurane on fractional Ca^{2+} release and SR Ca^{2+} content in isolated rat ventricular myocytes. They also found that halothane reduced SR Ca^{2+} content but not fractional release, and that isoflurane and sevoflurane decrease fractional Ca^{2+} release. In contrast, they found that isoflurane decreased and sevoflurane maintained SR Ca^{2+} content. They proposed that the effects of sevoflurane were caused by the combination of inhibition of Ca^{2+} current and inhibition of CICR or suppression of Ca^{2+} extrusion from the cell. Our results indicate that suppression of Ca^{2+} efflux is the more likely of the latter two mechanisms.

Effects on Transsarcolemmal Ca^{2+} Transport

Our experiments indicate that both isoflurane and sevoflurane inhibit Ca^{2+} extrusion from the cell by affecting the PMCA. We found that when both NCX and PMCA are functioning, the rate of decline of the Ca^{2+} transient is slower in the presence of isoflurane or sevoflurane, but not halothane. When only NCX was inhibited, both isoflurane and sevoflurane still slowed the rate of decline, indicating an effect on PMCA. Consistent with this interpretation, when only PMCA was inhibited, the rate of decline was unchanged by these anesthetics.

We should point out that when the function of the SR is inhibited by rapid application of caffeine, PMCA apparently assumes a greater role in Ca^{2+} transport, especially in ferret myocytes.[19,20] It is also noteworthy that the relative contribution of PMCA to this process in ferret heart is similar to that in human heart (and much higher than in rat heart).[19]

Volatile anesthetics have been shown to decrease PMCA activity in neural cells,[43] and it has been postulated that this effect may be at least partly responsible for alteration of the sensitivity to volatile anesthetics in disease states and with aging. Mammalian PMCAs are encoded by four separate genes, and additional isoforms are generated by alternative splicing of RNA.[44,45] The expression of PMCAs is determined by the stage of development, tissue, and cell type. PMCAs 1 and 4 are expressed in most tissues, but PMCAs 2 and 3 are found mainly in the brain and striated muscle.[44,45]

Inhibition of PMCA activity by isoflurane and sevoflurane would be expected to maintain or increase SR Ca^{2+} content if SR function (SERCA and Ca^{2+} release channel) was not affected to any appreciable extent. Evidence from other studies supports the hypothesis that isoflurane and sevoflurane exert relatively little influence on the SR. For example, isoflurane has been reported to have no effect[24,40,41] or to make the SR less leaky,[27] and sevoflurane has also been reported to leave SR Ca^{2+} content intact.[7]

Because both isoflurane and sevoflurane are known to decrease L-type Ca^{2+} current, our results suggest that inhibition of PMCA activity counteracts this and any affect on SR function to leave SR content intact (isoflurane) or actually increased (sevoflurane) during the conditions of these experiments. These results are significant because they help to explain the decreased depression of myocardial contractility seen with isoflurane and sevoflurane when compared with halothane.

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