

Effects of Isoflurane, Sevoflurane, and Halothane on Myofilament Ca^{2+} Sensitivity and Sarcoplasmic Reticulum Ca^{2+} Release in Rat Ventricular Myocytes

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Background: **The aim of this study was to describe and compare the effects of isoflurane, sevoflurane, and halothane at selected concentrations (i.e., concentrations that led to equivalent depression of the electrically evoked Ca^{2+} transient) on myofilament Ca^{2+} sensitivity, sarcoplasmic reticulum (SR) Ca^{2+} content, and the fraction of SR Ca^{2+} released during electrical stimulation (fractional release) in rat ventricular myocytes.**

Methods: **Single rat ventricular myocytes loaded with fura-2 were electrically stimulated at 1 Hz, and the Ca^{2+} transients and contractions were recorded optically. Cells were exposed to each anesthetic for 1 min. Changes in myofilament Ca^{2+} sensitivity were assessed by comparing the changes in the Ca^{2+} transient and contraction during exposure to anesthetic and low Ca^{2+}. SR Ca^{2+} content was assessed by exposure to 20 mM caffeine.**

Results: **Isoflurane and halothane caused a depression of myofilament Ca^{2+} sensitivity, unlike sevoflurane, which had no effect on myofilament Ca^{2+} sensitivity. All three anesthetics decreased the electrically stimulated Ca^{2+} transient. SR Ca^{2+} content was reduced by both isoflurane and halothane but was unchanged by sevoflurane. Fractional release was reduced by both isoflurane and sevoflurane, but was unchanged by halothane.**

Conclusions: **Depressed myofilament Ca^{2+} sensitivity contributes to the negative inotropic effects of isoflurane and halothane but not sevoflurane. The decrease in the Ca^{2+} transient is either responsible for or contributory to the negative inotropic effects of all three anesthetics and is either primarily the result of a decrease in fractional release (isoflurane and sevoflurane) or primarily the result of a decrease in SR Ca^{2+} content (halothane). (Key words: Heart; sarcoplasmic reticulum; volatile anesthetics.)**

THE volatile anesthetics, halothane, isoflurane, and sevoflurane, in addition to inducing unconsciousness, exert a potent negative inotropic effect on the heart.[1–7] The mechanisms underlying the cardiac-depressant effects of these agents are yet to be fully elucidated. A decrease in contraction can only be the result of a decrease in the sensitivity of the myofilaments or a decrease in the cytosolic Ca^{2+} transient. A decrease in the Ca^{2+} transient could result from a decrease in the Ca^{2+} content of the sarcoplasmic reticulum (SR) or a decrease in the fraction of the SR Ca^{2+} content released on electrical stimulation (fractional release).

The influence of halothane and isoflurane on myofilament Ca^{2+} sensitivity has been studied in skinned cardiac muscle, but the results of such experiments have generated conflicting data; some investigators reported little change,[8–10] and others a decrease of myofilament Ca^{2+} sensitivity.[11,12] Herland *et al.*[13] suggested that the conflicting data could result from the techniques used to skin cardiac muscle. Data have been published from intact cardiac muscle tissue (single cells or trabeculae) that are consistent with a depression of myofilament Ca^{2+} sensitivity induced by halothane and isoflurane,[1] although other investigators have reported that any contribution of reduced myofilament Ca^{2+} sensitivity does not play a major role in the negative inotropic effect of halothane.[14–16] Currently, the effects of sevoflurane on myofilament Ca^{2+} sensitivity are undocumented. One aim of the present study was to establish the effect of sevoflurane on myofilament Ca^{2+} sensitivity, confirm that isoflurane decreases myofilament Ca^{2+} sensitivity and compare the effects of sevoflurane and isoflurane on myofilament Ca^{2+} sensitivity to that of halothane.

We have demonstrated previously that halothane decreases the SR Ca^{2+} content in stimulated rat ventricular cells.[17] Jiang and Julian[18] argued that isoflurane causes a substantial decrease of SR Ca^{2+} content in intact stimulated trabeculae. However, these investigators used both rapid cooling and caffeine to measure SR Ca^{2+} content (because, in the presence of isoflurane, rapid cooling alone did not cause release of SR Ca^{2+}), which is not an established technique. In contrast, Wilde *et al.*[19] reported no significant change in SR Ca^{2+} content measured using caffeine in isolated rat ventricular myocytes in the presence of isoflurane. The effect of sevoflurane on SR Ca^{2+} content is unknown. Another aim of the present study was to establish the effect of sevoflurane on SR Ca^{2+} content, reexamine the effect of isoflurane on SR Ca^{2+} content, and compare the effects of isoflurane and sevoflurane on SR Ca^{2+} content to that of halothane.

Bassani *et al.*[20] introduced the concept of fractional release. Their work showed that fractional release in ferret ventricular myocytes is strongly influenced by the magnitude of the L-type Ca^{2+} current, I_{Ca}, as well as the Ca^{2+} content of the SR. Isoflurane, sevoflurane, and

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halothane have been shown to depress I_{Ca}, and halothane, at least, is known to decrease SR Ca^{2+} content; therefore, it is possible that the volatile anesthetics may affect fractional release. Furthermore, volatile anesthetics may alter fractional release by acting directly on the SR Ca^{2+} release channel. However, the effect on fractional release of volatile anesthetics is unknown, and the final aim of the present study was to compare the effects of isoflurane, sevoflurane, and halothane on fractional release to understand further the mechanisms underlying their negative inotropic effect.

Materials and Methods

Cell Isolation

The experiments described were conducted on Wistar rats weighing between 200 and 250 g. Animals were bred in the Biomedical Services Unit, University of Leeds, maintained under a 12-h light–dark cycle, and provided with food and water *ad libitum*. All procedures concerning animals conformed to the UK Home Office Animals (Scientific Procedures) Act, 1986. Animals were killed using a schedule 1 procedure sanctioned by the UK Home Office under project license 60/02087, and the heart was rapidly excised into an "isolation solution" (see below for composition), supplemented with 750 μM $CaCl_2$ and equilibrated with 100% O_2. The heart was flushed of blood by retrograde perfusion *via* the coronary arteries with the above solution and then perfused for 4 min with the isolation solution, to which 100 μM Na_2EGTA was added.[21] The heart was then perfused for 9 min with the isolation solution supplemented with 1 mg/ml collagenase (type 1; Worthington Biochemical Corp., Lakewood, NJ) and 0.1 mg/ml protease (type XIV; Sigma, Poole, Dorset, United Kingdom), after which the ventricles were cut from the heart, finely chopped, and shaken in the collected enzyme solution (to which 1% bovine serum albumin was added) for 5-min intervals. Dissociated cells were collected by filtration at the end of each 5-min digestion, and the remaining tissue was returned for further enzyme treatment. The dissociated cells were centrifuged at 30*g* for 40 s and resuspended in the 750 μM $CaCl_2$ solution and stored at 4°C until needed.

Single ventricular myocytes are an ideal preparation for investigating the mechanisms involved in the inotropic actions of anesthetics because equilibration is rapid throughout the entire preparation and therefore minimizes any problems associated with diffusion delays that could occur in multicellular preparations. Furthermore, single cells appear to respond to inotropic interventions in qualitatively the same way as multicellular preparations.[22,23] These experiments were conducted at 30°C on unloaded rat ventricular myocytes stimulated at 1 Hz. This temperature was chosen to maximize retention of fura-2 during experimental procedures, but it should be noted that the balance between Ca^{2+} entry and efflux from the cell would differ slightly from that at 37°C. Furthermore, as the cells are unloaded, shortening-induced alterations in the Ca^{2+} binding to troponin-C may occur, which would not be observed in preparations during isometric conditions. However, length-dependent changes in Ca^{2+} binding to troponin-C at physiological levels of Ca^{2+} are likely to be minimal.[24]

Solutions

The isolation solution was composed of 130 mM NaCl, 5.4 mM KCl, 1.4 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5 mM HEPES, 10 mM glucose, 20 mM taurine, 10 mM creatine, pH 7.1 (NaOH) at 37°C. After dissociation, cells were perfused with a physiologic salt solution of the following composition: 140 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5 mM HEPES, 10 mM glucose, 1 mM $CaCl_2$, pH 7.4 (NaOH) at 30°C. Concentrations of 0.3 mM halothane, 1 mM isoflurane, and 0.6 mM sevoflurane were delivered from stock solutions made up in dimethyl sulfoxide. After dilution of the stock solutions, the final concentration of dimethyl sulfoxide in the superfusate never exceeded 0.2%, a concentration that had no significant effect on contractions (not shown). Unless stated otherwise, all solution constituents were from Sigma (Poole, Dorset, United Kingdom).

Recording Cell Length

Freshly dissociated cells were transferred to a tissue chamber (volume, 0.1 ml) attached to the stage of an inverted microscope (Nikon Diaphot; Nikon UK, Kingston-Upon-Thames, Surrey, United Kingdom). The cells were allowed to settle for several minutes onto the glass bottom of the chamber before being superfused at a rate of ~ 3 ml/min with the physiological salt solution. Solutions were delivered to the experimental chamber by magnetic drive gear metering pumps (Micropump, Concord, CA), and solution level and temperature (30°C) were maintained by feedback circuits.[25] All experiments were conducted at 30°C.

Cells were stimulated electrically at a frequency of 1 Hz (stimulus duration, 2 ms) *via* two platinum electrodes situated in the sides of the chamber. Cell length was recorded continuously using an optical system based on a photodiode array[26] and displayed on a chart recorder (Gould 2600S; Gould Electronics Ltd., Ilford, Essex, United Kingdom). A sample and hold circuit[27] was used to display active shortening of a cell during each contraction (twitch shortening) on the chart recorder. This circuit has the effect of excluding changes in resting cell length, although the time course of the twitch is recorded faithfully.

Recording Ca^{2+} Transients

Cells were loaded with fura-2 by gentle agitation of a 2-ml aliquot of cell suspension with 6.25 μl of 1 mM

fura-2 acetoxy methyl ester in dimethyl sulfoxide for 12 min. After centrifugation as before, the supernatant was removed by suction, and the pellet of cells was resuspended in 750 μM Ca^{2+} solution. The fura-2–loaded cells were left for at least 30 min before use to allow de-esterification of the dye to take place. These cells were then transferred to the tissue chamber and stimulated as above. To record Ca^{2+} transients, the fura-2–loaded cells were excited alternately with light at two wavelengths (340 and 380 nm), and fluorescence was detected at 510 nm using a spectrophotometer (Cairn Research Ltd., Faversham, Kent, United Kingdom). The ratio of fluorescence at 510 nm in response to excitation at 340 and 380 nm was used as a measure of the intracellular Ca^{2+} concentration.

Cell length, Ca^{2+} transients, and twitch shortening were recorded using a pulse code modulator (Neuro-Corder DR-890; Neuro Data Instruments Corp., New York, New York) coupled to a standard VHS video recorder. Averaged cell contraction and Ca^{2+} transients were digitized at 0.2 kHz and displayed using an Axoscope (Axon Instruments, Foster City, CA).

Statistical Analysis

Data are presented as mean values ± SEM, and statistical comparisons were conducted with the Student paired *t* test or Wilcoxon signed rank test as appropriate, using SigmaStat 2.0 (Jandel Scientific, Erkrath, Germany). All graphs were prepared using SigmaPlot 4.0 (Jandel Scientific).

Results

Effects on Ca^{2+} Transient and Contraction

This study focuses on the mechanisms contributing to the inhibitory action of isoflurane, sevoflurane, and halothane on myocyte contractility. The approach taken here was to use concentrations of each anesthetic that led to a similar level of inhibition of the electrically evoked Ca^{2+} transient to compare the involvement of various mechanisms that could contribute to the negative inotropic effects of these agents. The concentrations chosen were 1 mM isoflurane, 0.6 mM sevoflurane, and 0.3 mM halothane, which reduced the Ca^{2+} transient to approximately 70–80% of control. However, as these concentrations differ in their anesthetic potency, other experiments were also conducted with 0.6 mM of each anesthetic, a concentration that approximates to twice the minimum alveolar concentration [MAC_{50}] for each anesthetic.

Figure 1 shows paired recordings of Ca^{2+} transients and contractions before, during, and after a 1-min exposure to anesthetic. Figure 1A shows that on application of 1 mM isoflurane, both the Ca^{2+} transients and contractions decreased. During the 1-min exposure there was a small degree of recovery of both Ca^{2+} transients and contractions toward control. On wash-off of isoflurane, contractions increased to a level slightly greater than that observed during control conditions before returning to control levels. Figure 1B shows that the Ca^{2+} transients and contractions were rapidly reduced in the presence of 0.6 mM sevoflurane before returning toward control levels during the exposure. On wash-off of sevoflurane, both the Ca^{2+} transients and contractions increased to levels in excess of those observed during control conditions before returning to control levels. Figure 1C shows that on application of 0.3 mM halothane, there was an initial increase in contraction, followed by a decrease that was sustained until wash-off. On removal of halothane, there was a small decrease of both Ca^{2+} transients and contractions before they returned to control levels.

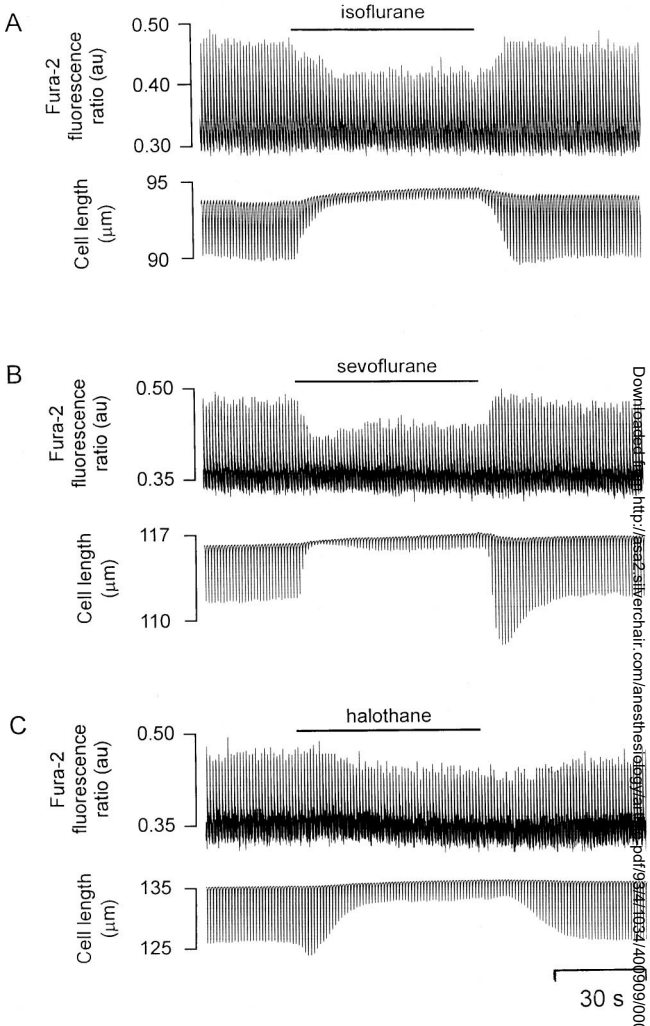

Fig. 1. Effects of isoflurane, sevoflurane, and halothane on the Ca^{2+} transient and contraction. Representative paired chart records of Ca^{2+} transients (*top*) and cell length (*bottom*) from three cells, on a slow time base, before, during, and after a 1-min exposure to 1 mM isoflurane (*A*), 0.6 mM sevoflurane (*B*) and 0.3 mM halothane (*C*). Exposure to anesthetic is indicated by the solid bars. Stimulation rate, 1 Hz. Similar results have been observed in numerous cells.

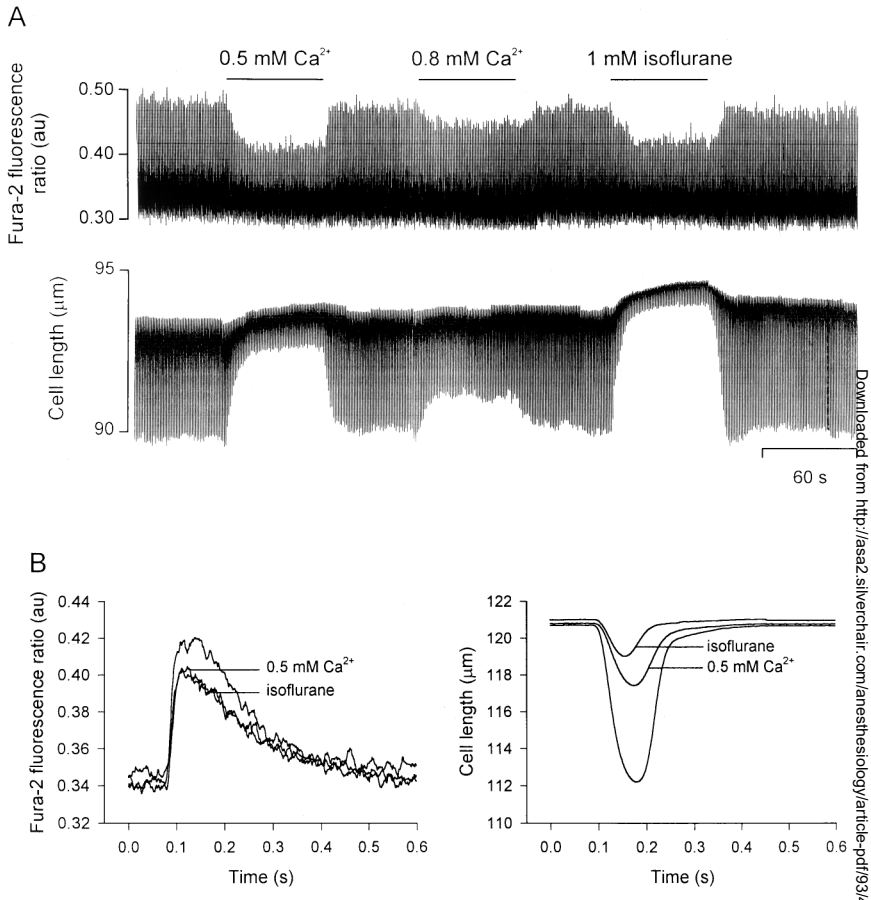

Fig. 2. Isoflurane depresses myofilament Ca^{2+} sensitivity. (*A*) Representative paired chart records of Ca^{2+} transients (*top*) and cell length (*bottom*) on a slow time base. The cell was exposed to low Ca^{2+} solutions (0.5 and 0.8 mM Ca^{2+}) and 1 mM isoflurane as shown by the bars. (*B*) Averaged Ca^{2+} transients (*left*) and contractions (*right*) of a different cell on a fast time base during control conditions (unlabelled) and in 0.5 mM Ca^{2+} and 1 mM isoflurane. A total of 15 consecutive traces were used to create the average records. Similar results have been obtained from seven myocytes.

These results illustrate that the negative inotropic effects of volatile anesthetics are caused, at least in part, by a reduction in the intracellular Ca^{2+} transient. Further experimental work was conducted to assess whether the effects of the agents were also the result of a decrease in myofilament Ca^{2+} sensitivity.

Effects on Myofilament Ca^{2+} Sensitivity

Figure 2A shows Ca^{2+} transients and contractions from a cell during control conditions (1 mM Ca^{2+}), in the presence of reduced Ca^{2+} (0.5 and 0.8 mM Ca^{2+}) and in the presence of 1 mM isoflurane. As expected, reducing extracellular Ca^{2+} decreased the Ca^{2+} transient and contraction. Isoflurane decreased the Ca^{2+} transient to an equivalent extent to 0.5 mM Ca^{2+}, but the contraction was depressed to a greater degree. Figure 2B shows representative fast time base Ca^{2+} transients and contractions during control conditions (1 mM Ca^{2+}) and in the presence of 0.5 mM Ca^{2+} and 1 mM isoflurane. In this example, 0.5 mM Ca^{2+} and 1 mM isoflurane depressed the peak of the Ca^{2+} transient to a similar extent, but the contraction was depressed to a greater degree by isoflurane. These data illustrate that isoflurane caused a greater depression of contraction than can be explained by the depression of the Ca^{2+} transient alone, suggesting that myofilament Ca^{2+} sensitivity was reduced by isoflurane.

Figure 3 shows similarly acquired data for 0.6 mM sevoflurane and 0.3 mM halothane. In the example shown in figure 3A, 0.6 mM sevoflurane and 0.5 mM Ca^{2+} depressed the Ca^{2+} transient to a similar degree; however, the contraction was slightly larger in the presence of sevoflurane than in the presence of 0.5 mM Ca^{2+}. This might suggest that in this example, myofilament Ca^{2+} sensitivity was increased by sevoflurane; however, this result was not typical, and on balance, the result from a group of cells were consistent with little or no effect of sevoflurane on myofilament Ca^{2+} sensitivity (see below). In the example shown in figure 3B, halothane and 0.5 mM Ca^{2+} reduced the peak of the Ca^{2+} transient to similar levels, whereas halothane depressed the contraction to a much greater extent. This result supports previous data that halothane causes a depression of myofilament Ca^{2+} sensitivity.[17] It should be noted that with both isoflurane and halothane, but not sevoflurane, diastolic cell length increased during application of anesthetic with no change in diastolic Ca^{2+}; these observations also suggest that myofilament Ca^{2+} sensitivity is reduced by isoflurane and halothane but not by sevoflurane.

Figure 4 shows mean data summarizing the results from the experiments shown in figures 2 and 3. Each panel illustrates the amplitude of contraction (expressed as a percentage of control) plotted against the amplitude of the Ca^{2+} transient (expressed as a percentage of control). The solid lines represent the observed changes

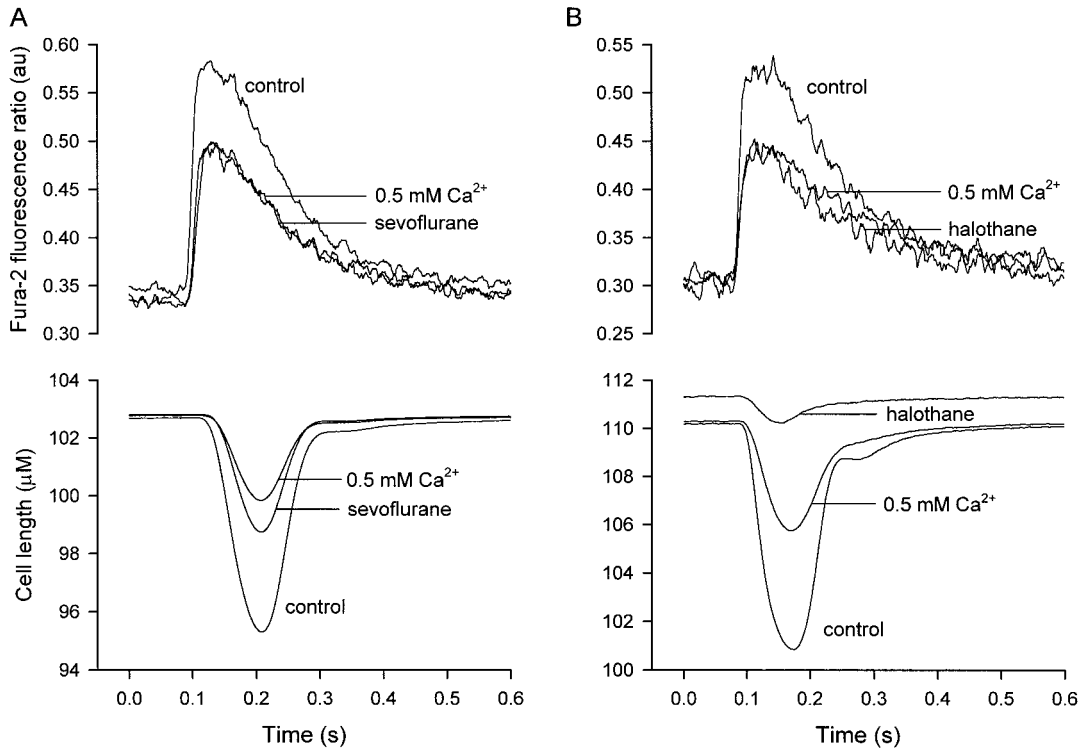

Fig. 3. Sevoflurane does not, whereas halothane does, depress myofilament Ca^{2+} sensitivity. Averaged Ca^{2+} transients (*top*) and contractions (*bottom*) during control conditions and in 0.5 mM Ca^{2+} and 0.6 mM sevoflurane (*A*) or 0.3 mM halothane (*B*) on a fast time base. A total of 15 consecutive traces were used to create the averaged records. Similar results were obtained from 9 and 11 myocytes for sevoflurane and halothane, respectively.

of the Ca^{2+} transient and contraction when the extracellular Ca^{2+} concentration was altered from 1 mM to 0.8 or 0.5 mM. The same data are shown in each panel and represent mean responses from 27 cells. The dashed lines represent the relation between the magnitude of the Ca^{2+} transient and contraction in the presence of the chosen concentration of anesthetic. If myofilament Ca^{2+} sensitivity was unchanged by the anesthetics, then the relation between the magnitude of the Ca^{2+} transient and contraction would also be expected to be unchanged, *i.e.*, the data for each anesthetic should lie on the same trajectory as that obtained by varying extracellular Ca^{2+} concentration. The vertical dotted lines in figures 4A and 4C show that in the presence of isoflurane and halothane, there was deviation from the relation between the contraction and the Ca^{2+} transient when extracellular Ca^{2+} was varied. The deviation is consistent with a depression of myofilament Ca^{2+} sensitivity induced by these concentrations of anesthetic. In figure 4B, the data for sevoflurane lie on the same trajectory as the data obtained in 1, 0.8, and 0.5 mM Ca^{2+}, suggesting that sevoflurane has little or no effect on myofilament Ca^{2+} sensitivity.

Figure 5 shows the relation between cell length and intracellular Ca^{2+} during a single contraction during control conditions and in the presence of each anesthetic. Spurgeon *et al.*[28] argued that during the relaxation phase of a contraction, the myofilaments come into quasi-equilibrium with cytosolic Ca^{2+}. Therefore, by plotting cell length against the fura-2 fluorescence ratio during the relaxation phase of a contraction, it is possible to obtain an index of the Ca^{2+} sensitivity of the myofilaments. For a given contraction, the data proceed in an anticlockwise direction. Figure 5 illustrates such plots from contractions recorded in the absence and presence of isoflurane, sevoflurane, and halothane. The inset in each panel shows data throughout the Ca^{2+} transient and contraction. The main plot of each panel shows these data during the final phase of relaxation. The solid lines are the result of linear regression of these data. In figures 5A and 5C, the relation between the fura-2 fluorescence ratio and cell length in the presence of both isoflurane and halothane is less steep (n = 7 and 11 cells; $P = 0.008$ and $P = 0.031$, respectively) and is shifted down. The decrease in the gradient of the regression lines and the downward shift is suggestive of a depression in myofilament Ca^{2+} sensitivity. Figure 5B shows that in the presence of sevoflurane there was no significant change in the gradient of the regression line (n = 9 cells; $P = 0.22$) and no substantial downward shift, suggesting that there was little or no change in the myofilament Ca^{2+} sensitivity.

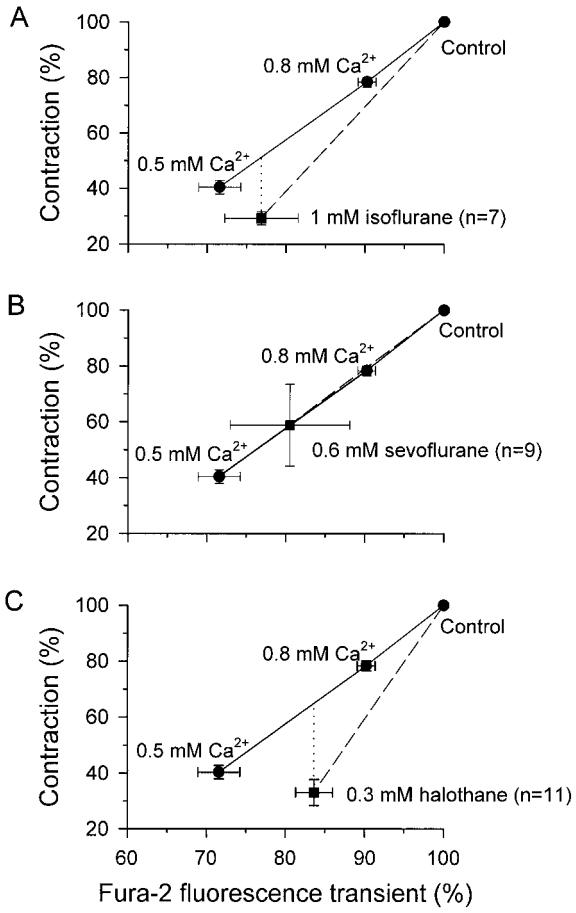

Fig. 4. Effects of isoflurane, sevoflurane, and halothane on myofilament Ca^{2+} sensitivity, mean data. Amplitude of contraction as a percentage of control is plotted against the amplitude of the Ca^{2+} transient (as a percentage of control) for low Ca^{2+} solutions (0.5 and 0.8 mM Ca^{2+}) and 1 mM isoflurane (*A*), 0.6 mM sevoflurane (*B*), or 0.3 mM halothane (*C*). Control and low Ca^{2+} data are represented by circles, and anesthetic data are represented by squares. Mean values and SEMs are shown. Numbers of cells are shown in each panel. See text for further details.

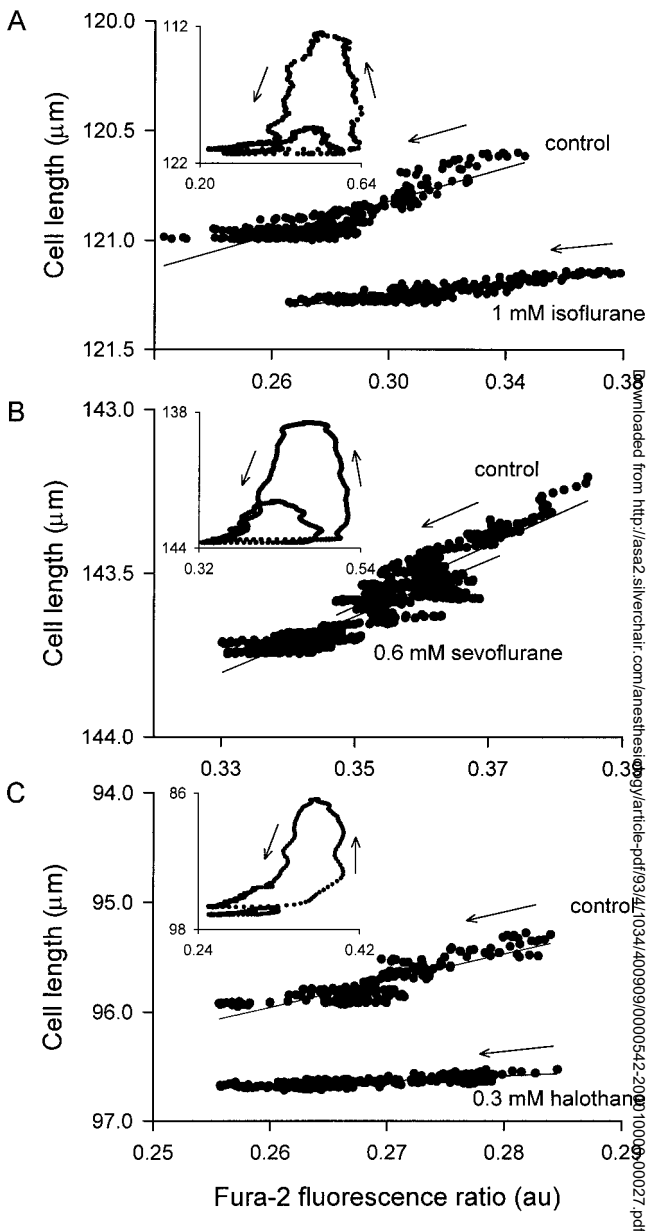

Fig. 5. Effects of isoflurane, sevoflurane, and halothane on myofilament Ca^{2+} sensitivity measured during a single contraction. Relation between cell length and intracellular Ca^{2+} (as measured by the fura-2 fluorescence ratio) during a contraction with control conditions and in the presence of 1 mM isoflurane (*A*), 0.6 mM sevoflurane (*B*), or 0.3 mM halothane (*C*). The insets show the relation throughout the contraction, and the main plots show the relation just during the final phase of relaxation (the arrows show the sequence of data). The traces shown are averages from 15 consecutive records.

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

The aim of these experiments was to determine the extent to which anesthetic-induced changes in SR Ca^{2+} content and fractional release contributed to the ~ 20–30% decrease in the electrically evoked Ca^{2+} transient that was observed during application of the chosen concentrations of anesthetic. The left panels of figure 6 show records of electrically stimulated Ca^{2+} transients during control conditions, whereas the right panels illustrate the corresponding data in the presence of isoflurane (fig. 6A), sevoflurane (fig. 6B), and halothane (fig. 6C). Both during control conditions and in the presence of anesthetic, stimulation was stopped, and 20 mM caffeine was applied rapidly to release Ca^{2+} from the SR. The peak of the caffeine-evoked Ca^{2+} transient is a measure of the Ca^{2+} content of the SR.[29] Once the caffeine-evoked Ca^{2+} transient had decayed, the caffeine was washed off with anesthetic-free control solution, and stimulation was recommenced. In the left panel of figure 6A, the dashed line represents the mean peak of the electrically stimulated Ca^{2+} transient, and the arrows show how fractional release was measured: the peak of the electrically stimulated Ca^{2+} transient was expressed as a percentage of the peak of the caffeine-evoked Ca^{2+} transient; in this example, fractional release was 61%. The horizontal lines in the right panels in figure 6 represent the peaks of the electrically and caffeine-evoked Ca^{2+} transients during

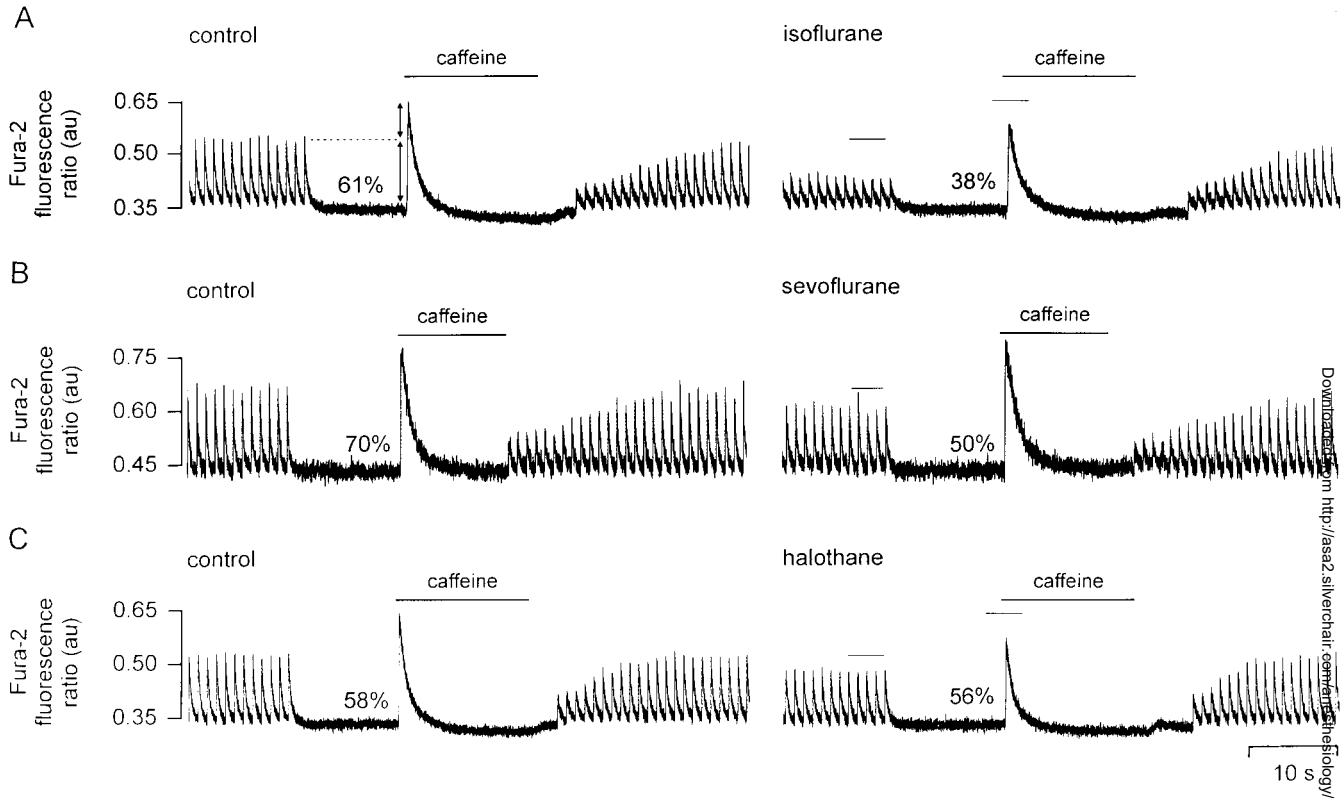

Fig. 6. Effects of isoflurane, sevoflurane, and halothane on sarcoplasmic reticulum (SR) Ca^{2+} content and fractional release. Representative chart records of Ca^{2+} transients during control conditions (*left*) and in the presence of anesthetics (*right*). (*A*) Data for 1 mM isoflurane; (*B*) data for 0.6 mM sevoflurane; (*C*) data for 0.3 mM halothane, from three different cells. Initially the cells were electrically stimulated at 1 Hz. Then the stimulation was stopped and after 10 s, 20 mM caffeine was applied (as shown by the bars) to evoke the release of Ca^{2+} from the SR. When the caffeine-evoked Ca^{2+} transient had decayed, stimulation was recommenced (*Right*) After the application of caffeine, caffeine-free and anesthetic-free solution was applied. In each panel, the percentage value is the fractional release (see text for details). The short horizontal bars show the amplitude of the electrically stimulated and caffeine-evoked Ca^{2+} transients during control conditions.

control conditions. Figure 7 represents mean data from these experiments. The first three bars of each panel in figure 7 show mean data (n = 7) in the presence of isoflurane, sevoflurane, and halothane. Figure 7A shows the sustained effects of the anesthetics on the electrically stimulated Ca^{2+} transient, figure 7B shows the effects on SR Ca^{2+} content, and figure 7C shows the effects on fractional release.

Figures 6 and 7A confirm that the three anesthetics decreased the Ca^{2+} transient significantly by 20–30%. Figures 6 and 7B show that the effects on SR Ca^{2+} content varied between the anesthetics—isoflurane caused a modest but significant decrease of SR Ca^{2+} content to 90 ± 3.9% of control ($P = 0.045$), sevoflurane caused no change (SR Ca^{2+} content, 99 ± 1.1% of control), and halothane caused a substantial and significant decrease to 72 ± 1.3% of control ($P < 0.001$).

Other experiments were performed to assess the effects of 0.6 mM isoflurane and halothane on the Ca^{2+} transient, SR Ca^{2+} content, and fractional release for comparison with data for 0.6 mM sevoflurane; 0.6 mM is approximately equivalent to 2 × MAC_{50} for all three anesthetics. As might be expected, the measured parameters changed in a dose-dependent manner: with 0.6 mM isoflurane, the Ca^{2+} transient, SR Ca^{2+} content, and fractional release were reduced to a lesser extent (to 85 ± 3%, 95 ± 2%, and 90 ± 4%, respectively; n = 9) than with 1 mM isoflurane. In 16 cells with 0.6 mM halothane, the Ca^{2+} transient and SR Ca^{2+} content were reduced to 68 ± 2% and 72 ± 3%, respectively, but fractional release was maintained at 97 ± 3%. Data at equi-anesthetic concentrations as well as at concentrations that induce a similar depression of the electrically evoked Ca^{2+} transient (figs. 6 and 7C) show that fractional release was reduced significantly in the presence of isoflurane and sevoflurane but was maintained by halothane despite a large reduction in the SR Ca^{2+} content.

To assess the possible mechanisms underlying the action of these anesthetics, experiments such as those in figure 6 were conducted using 0.5 μM nifedipine and 0.25 mM caffeine. The data obtained are summarized in figure 7. Nifedipine, a dihydropyridine antagonist, is known to selectively block I_{Ca}. Volatile anesthetics are also known to reduce I_{Ca} (see Discussion). To assess the effects of partial blockade of I_{Ca} alone on SR Ca^{2+} content and fractional release, 0.5 μM nifedipine, a concentration that depressed the electrically stimulated Ca^{2+} transient to a similar degree as the chosen concentration

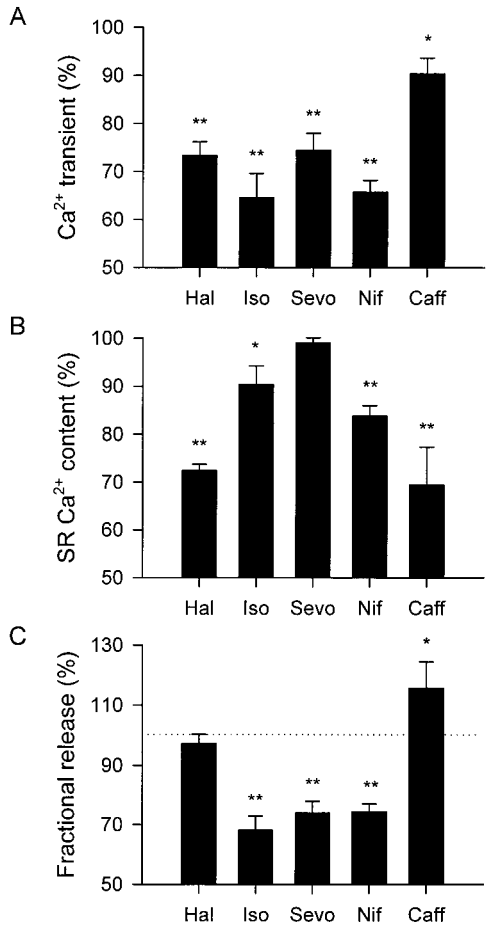

Fig. 7. Effects of isoflurane, sevoflurane, and halothane on sarcoplasmic reticulum (SR) Ca^{2+} content and fractional release, mean data. (*A*) Amplitude of electrically stimulated Ca^{2+} transients (as a percentage of that during control conditions). (*B*) SR Ca^{2+} content assessed using 20 mM caffeine (as a percentage of that during control conditions). (*C*) Fractional release (as a percentage of that observed during control conditions). In (*C*), the dotted line represents 100%. Data (mean ± SEM) are shown in the presence of 1 mM isoflurane (Iso; n = 7), 0.6 mM sevoflurane (Sevo; n = 7), 0.3 mM halothane (Hal; n = 7), 0.5 μM nifedipine (Nif; n = 8), or 0.25 mM caffeine (Caff; n = 7).

of each anesthetic, was used. These data showed that 0.5 μM nifedipine caused significant reductions in the Ca^{2+} transient ($P < 0.001$), SR Ca^{2+} content ($P = 0.008$), and fractional release ($P < 0.001$; fig. 7).

Caffeine is known to cause a sensitization of Ca^{2+}-induced Ca^{2+} release (CICR) from the SR.[30] Volatile anesthetics may also affect the sensitivity of CICR[6]; therefore, to assess the effects of a change (increase) in the sensitivity of CICR alone on SR Ca^{2+} content and fractional release, 0.25 mM caffeine, a concentration known to sensitize CICR[30] but not to deplete the SR of Ca^{2+} fully, was applied in experiments such as that shown in figure 6. These data showed that 0.25 mM caffeine caused a small but significant reduction in the Ca^{2+} transient ($P = 0.036$), a significant reduction in SR Ca^{2+} content ($P = 0.009$) but a significant increase in fractional release ($P < 0.001$; fig. 7). The significance of these findings is considered in the Discussion.

Discussion

The majority of experiments conducted in the present study used concentrations of anesthetic that induced a 20–30% reduction in the magnitude of the electrically evoked Ca^{2+} transient to further investigate the mechanisms underlying their negative inotropic effect. A reduction in the Ca^{2+} transient would reduce contractility; however, at anesthetic concentrations that led to a similar depression of the Ca^{2+} transient, isoflurane and halothane, but not sevoflurane, also reduced myofilament Ca^{2+} sensitivity, which would contribute to the negative inotropic effect of isoflurane and halothane.

In the present study, 0.6 and 1 mM isoflurane caused a modest decrease in SR Ca^{2+} content, which conflicts with previous data from Jiang and Julian,[18] whereas sevoflurane had little or no effect on SR Ca^{2+} content despite decreasing the electrically stimulated Ca^{2+} transient. On the other hand, 0.3 mM halothane reduced the SR Ca^{2+} content to a greater extent than isoflurane. Both isoflurane and sevoflurane caused a decrease in fractional release, whereas halothane did not. The effects of sevoflurane on myofilament Ca^{2+} sensitivity and SR Ca^{2+} content are novel findings, as are the effects of all three anesthetics on fractional release.

Myofilament Ca^{2+} Sensitivity

Previous reports concerning the effects of halothane and isoflurane on myofilament Ca^{2+} sensitivity are contradictory (see Introduction). The findings of Herland *et al.*[13] suggest that the presence of the cell membrane is needed for the effect of halothane on myofilament Ca^{2+} sensitivity to be observed. It follows from this that the effect of halothane is mediated by the cell membrane, although how this occurs is not known. However, in intact cardiac muscle tissue, some investigators report data that are consistent with a depression of myofilament Ca^{2+} sensitivity by both halothane and isoflurane,[8,10,12,17,31–33] whereas others suggest that a reduction in myofilament Ca^{2+} sensitivity plays little or no role in the negative inotropic effects of these agents.[14–16] In the present study, 1 mM isoflurane and 0.3 mM halothane depressed myofilament Ca^{2+} sensitivity, whereas sevoflurane did not affect myofilament Ca^{2+} sensitivity.

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

The reported effects of isoflurane on SR Ca^{2+} content are inconsistent. Wilde *et al.*[19] reported a modest but not significant decrease in SR Ca^{2+} content to 88% of control by 1.5% isoflurane. Although Jiang and Julian[18] recorded a significant decrease to 71% of control by 0.62 mM

isoflurane, the method used to measure SR Ca^{2+} content was not standard (see Introduction). In the present study, we observed a modest but significant decrease in SR Ca^{2+} content to 90 ± 3.9% in the presence of 1 mM isoflurane, no effect of sevoflurane on SR Ca^{2+} content, whereas halothane reduced the SR Ca^{2+} content to a greater extent, as has been observed previously.[34] Finally, isoflurane and sevoflurane, but not halothane, reduced fractional release. Data from experiments using equivalent concentrations of each anesthetic (0.6 mM) gave a broadly similar picture; the decrease in the Ca^{2+} transient with isoflurane and sevoflurane appeared to result from a decrease in fractional release, whereas with halothane, was primarily a result of a reduction in SR Ca^{2+} content as fractional release was maintained.

The effects of the anesthetics on SR Ca^{2+} content and fractional release could be the result of a decrease in I_{Ca} or a direct effect on the SR Ca^{2+} release channel. Isoflurane, sevoflurane, and halothane at concentrations greater than 1.65%,[35] 1%,[36] and 1.2%,[37] respectively, decrease I_{Ca}. There is evidence that halothane affects the SR Ca^{2+} release channel: halothane has been shown to increase both the open probability and the open time, but not the conductance of Ca^{2+} release channels (whereas isoflurane does not show this behavior).[34] In quiescent preparations, halothane, but not isoflurane, has been shown to increase intracellular free Ca^{2+},[31,38] whereas in electrically stimulated myocytes, halothane, but not isoflurane or sevoflurane, results in a brief increase in contractions and Ca^{2+} transients[6,17,32,39,40]; these effects of halothane have been suggested to be the result of an enhancement of CICR from the SR by halothane.[6,34,41,42]

To assess the effect of a reduction of I_{Ca} alone on the SR Ca^{2+} content and fractional release, the effects of the anesthetics were compared with those of 0.5 μM nifedipine, a known Ca^{2+} channel antagonist. Figure 7 shows that nifedipine decreased both the SR Ca^{2+} content and fractional release. The decrease in the SR Ca^{2+} content after block of I_{Ca} is an expected consequence of a decrease in Ca^{2+} influx into the cell.[43] Both a decrease in I_{Ca} (the trigger for SR Ca^{2+} release) and a decrease in SR Ca^{2+} content would be expected to reduce fractional release,[20] and either or both of these factors could be the cause of the decrease in fractional release in the presence of nifedipine. In figure 7, the mean data for isoflurane and nifedipine are essentially very similar. Both depressed the Ca^{2+} transient, SR Ca^{2+} content, and fractional release to a similar degree. This is consistent with the possibility that the effects of isoflurane on the Ca^{2+} transient, SR Ca^{2+} content, and fractional release are the result of a decrease in I_{Ca} alone.

The profile of changes with sevoflurane is unlike that with nifedipine (fig. 7): although sevoflurane resulted in a decrease in fractional release, the SR Ca^{2+} content remained unchanged. An alternative possibility is that sevoflurane inhibits CICR from the SR, as does tetracaine.[44] However, although this would explain the decrease in fractional release, such an effect would be expected to result in an increase in SR Ca^{2+} content similar to that with tetracaine.[44] Such an increase in SR Ca^{2+} content was not observed in the present study. We propose that the effects of sevoflurane are the result of inhibition of I_{Ca} and an inhibition of CICR from the SR (or suppression of Ca^{2+} extrusion *via* Na^{+}–Ca^{2+} exchange). This could explain why there is a decrease in fractional release and no change in SR Ca^{2+} content with sevoflurane.

The profile of changes with halothane is also unlike that with nifedipine (fig. 7); although halothane resulted in a decrease in SR Ca^{2+} content, fractional release remained unchanged. As discussed previously, there is evidence that halothane enhances CICR from the SR. To assess the effect of an enhancement of CICR alone, the effect of 0.25 mM caffeine, which is known to enhance CICR,[30] was studied. As expected, caffeine increased fractional release and decreased SR Ca^{2+} content (fig. 7). Although halothane decreased SR Ca^{2+} content like caffeine, it did not increase fractional release. We propose

Table 1. Mechanisms Underlying the Negative Inotropic Effect of Isoflurane, Sevoflurane, and Halothane

	Decrease in Contraction Because of:	
Anesthetic	Reduced Myofilament Ca^{2+} Sensitivity (%)*	Reduced Electrically Stimulated Ca^{2+} Transient (%)*
1 mM isoflurane	31	69
0.6 mM sevoflurane	0	100
0.3 mM halothane	48	52
	Decrease in Ca^{2+} Transient Because of:	
	Reduced SR Ca^{2+} Content (%)	Reduced Fractional Release (%)
1 mM isoflurane	10	32
0.6 mM sevoflurane	1	26
0.3 mM halothane	28	3

* Estimated from figure 4.

SR = sarcoplasmic reticulum.

that the effects of halothane (at this concentration) are the result of inhibition of I_{Ca} and an enhancement of CICR from the SR. This could explain why there is a decrease in SR Ca^{2+} content and no net change in fractional release with halothane.

Working Hypothesis

The data from the present study are summarized in table 1. It should be noted that these data refer to concentrations of anesthetic that induce a similar depression of the electrically evoked Ca^{2+} transient and are not equi-anesthetic concentrations. These data show that approximately one third of the negative inotropic effect of 1 mM isoflurane is the result of a decrease in myofilament Ca^{2+} sensitivity and approximately two thirds is the result of a decrease in the Ca^{2+} transient. The decrease in the Ca^{2+} transient is the result of decreases in both the SR Ca^{2+} content and fractional release (table 1); we have argued that these effects are the result of an inhibition of I_{Ca}. The data summarized in table 1 show that the negative inotropic effect of 0.6 mM sevoflurane is the result of a decrease in the Ca^{2+} transient, and the decrease in the Ca^{2+} transient results from a reduction in fractional release. We have argued that the decrease in fractional release could be the result of an inhibition of I_{Ca}. Finally, the data summarized in table 1 suggest that half of the negative inotropic effect of 0.3 mM halothane results from a decrease in myofilament Ca^{2+} sensitivity, and half results from a decrease in the Ca^{2+} transient, which in turn reflects the decrease in SR Ca^{2+} content. We have argued that the decrease in the SR Ca^{2+} content is the result of both an inhibition of I_{Ca} and an enhancement of the CICR from the SR.

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