Anesthesiology 2006; 105:58–64

Antagonistic Actions of Halothane and Sevoflurane on Spontaneous Ca^{2+} Release in Rat Ventricular Myocytes

Mark D. Graham, B.Sc., Philip M. Hopkins, M.D.,† Simon M. Harrison, Ph.D.‡*

Background: **Halothane has been reported to sensitize Ca^{2+} release from the sarcoplasmic reticulum (SR), which is thought to contribute to its initial positive inotropic effect. However, little is known about whether isoflurane or sevoflurane affect the SR Ca^{2+} release process, which may contribute to the inotropic profile of these anesthetics.**

Methods: **Mild Ca^{2+} overload was induced in isolated rat ventricular myocytes by increase of extracellular Ca^{2+} to 2 mM. The resultant Ca^{2+} transients due to spontaneous Ca^{2+} release from the SR were detected optically (fura-2). Cells were exposed to 0.6 mM anesthetic for a period of 4 min, and the frequency and amplitude of spontaneous Ca^{2+} transients were measured.**

Results: **Halothane caused a temporary threefold increase in frequency and decreased the amplitude (to 54% of control) of spontaneous Ca^{2+} transients. Removal of halothane inhibited spontaneous Ca^{2+} release before it returned to control. In contrast, sevoflurane initially inhibited frequency of Ca^{2+} release (to 10% of control), whereas its removal induced a burst of spontaneous Ca^{2+} release. Isoflurane had no significant effect on either frequency or amplitude of spontaneous Ca^{2+} release on application or removal. Sevoflurane was able to ameliorate the effects of halothane on the frequency and amplitude of spontaneous Ca^{2+} release both on application and wash-off.**

Conclusions: **Application of halothane and removal of sevoflurane sensitize the SR Ca^{2+} release process (and *vice versa* on removal). Sevoflurane reversed the effects of halothane, suggesting they may act at the same subcellular target on the SR.**

HALOTHANE and sevoflurane have been reported to induce both positive and negative inotropic effects in isolated ventricular myocytes when these agents are either applied or removed, the mechanisms of which are not fully elucidated. For example, application of halothane induces an initial positive inotropic effect[1–6] thought to result from sensitization of the sarcoplasmic reticulum (SR) Ca^{2+} release process,[3,4,7,8] followed by a sustained negative inotropic effect due primarily to reductions in SR Ca^{2+} content,[3,4,9,10] the L-type Ca^{2+} current (I_{Ca}),[1,11–15] and myofilament Ca^{2+} sensitivity.[4,6,16,17] In contrast, sevoflurane initially depresses contractility, which then recovers toward control during the exposure,[5,6,18] and on removal, a short-lived positive inotropic effect is induced[5,6,18] before contractions return to control values. In cells equilibrated with ryanodine to inhibit SR function, the initial positive inotropic effect of halothane is blocked, the extent of initial inhibition of contraction on exposure to sevoflurane is reduced, and the magnitude of the positive inotropic effect on removal is greatly diminished, suggesting a pivotal role of SR Ca^{2+} regulation in these inotropic effects.[5] The aim of this study was to investigate whether halothane, isoflurane, and sevoflurane affected the sensitivity of the SR Ca^{2+} release process. To assess this function, we used unstimulated single ventricular myocytes in which mild Ca^{2+} overload was induced by increase of extracellular Ca^{2+}.[19,20] This leads to the induction of spontaneous Ca^{2+} transients, which are reproducible in their frequency and amplitude. It has been shown previously that these spontaneous Ca^{2+} transients reflect Ca^{2+} release from the SR as they are abolished by ryanodine.[4] Using this model, changes in the frequency and/or amplitude of spontaneous Ca^{2+} transients in the presence of the anesthetics will provide evidence for the contribution of the sensitivity of the SR Ca^{2+} release process to the inotropic profiles of these anesthetics.

Materials and Methods

These studies conformed to the *Guide for the Care and Use of Laboratory Animals*,[21] and all animal procedures conformed to schedule 1 regulations described in the Animals (Scientific Procedures) Act, 1986, of the United Kingdom government Home Office. Rat ventricular myocytes were prepared as described previously.[6]

Recording Cytosolic Ca^{2+}

Freshly dissociated cells were loaded with fura-2 by gentle agitation of a 2-ml aliquot of cell suspension with 6.25 μl fura-2 AM, 1 mM, in dimethyl sulfoxide for 12 min followed by centrifugation and resuspension in the physiologic salt solution (see Solutions, first sentence). Cells were transferred to a tissue chamber (volume, 0.1 ml) attached to the stage of an inverted microscope (Nikon Diaphot; Nikon, Surrey, United Kingdom) and allowed to settle for several minutes onto the glass bottom of the chamber before being superfused at a rate of approximately 3 ml/min with the physiologic salt solution. Solutions were delivered to the experimental chamber by magnetic drive gear metering pumps (Micropump, Concord, CA), and solution level and temperature were maintained by feedback circuits.[22] All experiments were

* Research Student, ‡ Senior Lecturer, Institute of Membrane and Systems Biology, Faculty of Biological Sciences, † Professor of Anaesthesia, Academic Unit of Anaesthesia, University of Leeds.

Received from the Institute of Membrane and Systems Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom. Submitted for publication November 15, 2005. Accepted for publication February 22, 2006. Supported by the British Heart Foundation, London, United Kingdom, and the Medical Research Council, United Kingdom.

Address correspondence to Dr. Harrison: Institute of Membrane and Systems Biology, Faculty of Biological Sciences, Worsley Building, University of Leeds, LS2 9JT, United Kingdom. s.m.harrison@leeds.ac.uk.

conducted at 30°C to maximize the retention of cytosolic fura-2. To record Ca^{2+} transients, the fura-2–loaded cells were excited alternately with light at 340 and 380 nm using a monochromator based system (Cairn; Faversham, Kent, United Kingdom) and fluorescence detected at 510 nm. 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. Ca^{2+} transients were digitized at 1 kHz and analyzed using Ionoptix software (Milton, MA).

Solutions

During experimentation, cells were perfused with the following physiologic salt solution: 140 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5 mM HEPES, 10 mM glucose, and 1 or 2 mM $CaCl_2$, pH 7.4 (NaOH) at 30°C. Halothane, isoflurane, and sevoflurane were delivered from stock solutions made up in dimethyl sulfoxide; the final concentration of dimethyl sulfoxide never exceeded 0.2%, which had no significant effect on Ca^{2+} transients (not shown). The maximum final bath concentration of anesthetic used in the experiments, 0.6 mM, approximates to twice the minimum alveolar concentration of halothane, isoflurane, and sevoflurane, and therefore, these concentrations are both clinically relevant and broadly equianesthetic in rat ventricle.[23] Concentrations of anesthetics in the superfusate were verified with gas–liquid chromatography[4] and found to be stable over the course of an experiment.

Experimental Protocol

When cells were perfused with solution containing increased (2 mM) Ca^{2+}, spontaneous Ca^{2+} transients were evoked, which propagated down the cells as Ca^{2+} waves. The frequency and amplitude of these transients were recorded in the absence of anesthetic to establish baseline conditions. If frequency or amplitude was not regular over a period of at least 2 min, the cell was discarded. When steady baseline conditions were achieved, the cells were exposed to 0.6 mM of halothane, isoflurane, or sevoflurane (or a mixture of anesthetics with a total anesthetic concentration of 0.6 mM) for a period of 4 min before control perfusate was restored. The frequency and amplitude of spontaneous Ca^{2+} transients were recorded during periods of 30 s. For purposes of analysis of Ca^{2+} transient amplitude, if a cell did not display any spontaneous transients during a 30-s period (*e.g.*, see fig. 1A), that cell was not included in the calculation of mean amplitude for that time period because this would artificially reduce amplitude of transients in cells where they did occur. However, to give a more complete picture of total SR Ca^{2+} release, data are also presented as the product of frequency and amplitude. In these experiments, each cell acted as its own control and was only exposed to one anesthetic (or one anesthetic mixture) except for the protocols in electrically stimulated cells (see Discussion). In all groups of cells, there were no significant differences in either the frequency or amplitude of spontaneous Ca^{2+} release under baseline conditions (analysis of variance).

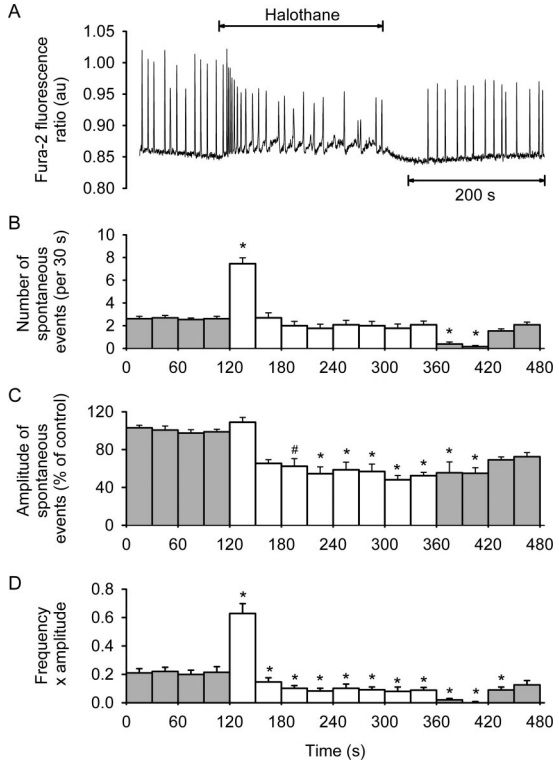

Fig. 1. (*A*) Spontaneous Ca^{2+} transients before, during, and after a 4-min exposure to 0.6 mM halothane. Mean data (± SEM, n = 13) describing the number (*B*), amplitude (*C*), and product of amplitude and frequency (*D*) of spontaneous events per 30-s periods. Control perfusate is shown as *gray bars*, and anesthetic exposure is shown as *white bars*. * $P < 0.05$ to three or more of the four 30-s precontrol periods. # $P < 0.05$ to two of the four precontrol periods and at least one postcontrol period.

Statistical Analysis

Data are presented as mean ± SEM, and statistical comparisons were conducted with SigmaStat, (SPSS, Chicago, IL) using one-way repeated-measures analysis of variance followed by Tukey tests for multiple comparisons or Friedman repeated-measures analysis of variance on ranks followed by Tukey tests for multiple comparison if data failed normality or equal variance tests. Independent sample *t* tests were used for comparison of the effects of halothane alone *versus* halothane–sevoflurane mixtures on the frequency of spontaneous Ca^{2+} release. All figures were prepared using SigmaPlot (SPSS).

Results

Effects of Halothane, Sevoflurane, and Isoflurane on Spontaneous Ca^{2+} Release

Figure 1A illustrates spontaneous Ca^{2+} transients before, during, and after a 4-min exposure to 0.6 mM halo-

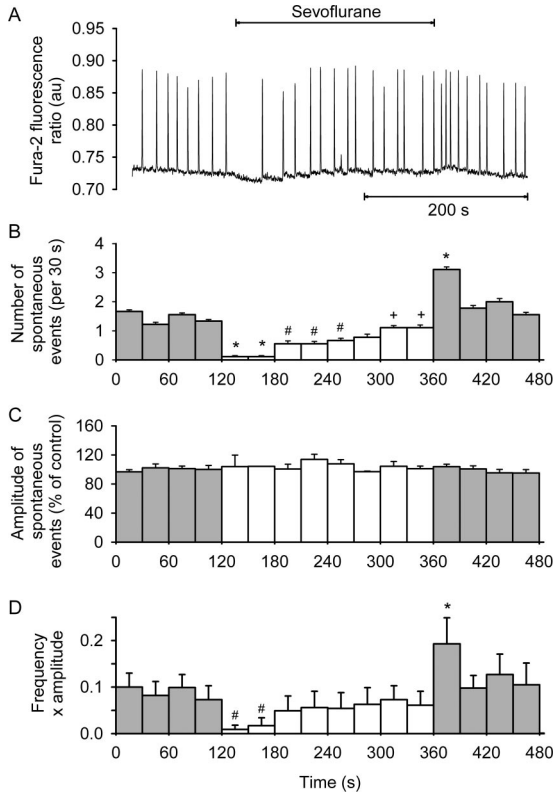

Fig. 2. (*A*) Spontaneous Ca^{2+} transients before, during, and after a 4-min exposure to 0.6 mM sevoflurane. Mean data (± SEM, n = 10) describing the number (*B*), amplitude (*C*), and product of amplitude and frequency (*D*) of spontaneous events per 30-s periods. Control perfusate is shown as *gray bars*, and anesthetic exposure is shown as *white bars*. * $P < 0.05$ to three or more of the four 30-s precontrol periods. # $P < 0.05$ to two of the four precontrol periods and at least one postcontrol period. + $P < 0.05$ *versus* initial exposure (*columns 5* and *6*).

thane. The frequency (fig. 1B) and amplitude (fig. 1C) of spontaneous Ca^{2+} transients assessed over consecutive 30-s periods were stable over the initial control period (0–120 s). Addition of 0.6 mM halothane led to an immediate increase in frequency (from 2.6 ± 0.21 to 7.5 ± 0.5 transients per 30 s; $P < 0.05$ *vs.* control), which then declined to a level not significantly different from control (fig. 1B). At the end of the 4-min exposure, Ca^{2+} transient amplitude was significantly reduced to 54 ± 10% of control ($P < 0.05$). On removal of halothane, the frequency of spontaneous Ca^{2+} release declined ($P < 0.05$) for a period of 60 s before returning to control. The amplitudes of events (when they occurred, see Materials and Methods) remained below control but after 2 min of control perfusate were restored. The product of frequency and amplitude (fig. 1D), which gives a measure of total Ca^{2+} release, reflected changes in both variables.

In contrast, sevoflurane initially reduced the frequency of spontaneous Ca^{2+} release ($P < 0.05$) over a period of 60 s (figs. 2A and B), which returned toward control levels during the 4-min exposure, with no significant change in the amplitude of spontaneous Ca^{2+} transients (fig. 2C). On removal of sevoflurane, frequency was enhanced transiently ($P < 0.05$) before returning to control, but again, this was not accompanied by any change in Ca^{2+} transient amplitude. Figure 2D illustrates that total Ca^{2+} release reflected the effect of sevoflurane on frequency, not amplitude, of release.

Isoflurane (0.6 mM) had no significant effect on the frequency or amplitude of spontaneous Ca^{2+} release when first applied, during the exposure, or on removal (not shown), suggesting that isoflurane had no net influence on the sensitivity of SR Ca^{2+} release.[7]

SR Ca^{2+} Content at the Point of Spontaneous Release

Because spontaneous release of Ca^{2+} from the SR is strongly dependent on SR Ca^{2+} content,[24] this was assessed by rapid application of 20 mM caffeine at the time point when the next spontaneous Ca^{2+} release was expected. Figure 3A illustrates this protocol; under control conditions, 20 mM caffeine was applied to the cell under study to discharge SR Ca^{2+} content. After removal of caffeine, spontaneous Ca^{2+} transients were restored. Anesthetic, 0.6 mM, was then applied, and the caffeine protocol was repeated at the beginning and at the end of the 4-min anesthetic exposure. Figures 3B and C illustrate that sevoflurane significantly increased the magnitude of caffeine-induced release of Ca^{2+} from the SR (a measure of SR Ca^{2+} content) during the exposure (to 110 ± 12%, n = 10; $P < 0.05$), as would be expected if SR Ca^{2+} release were inhibited, whereas halothane reduced content to 77 ± 24% (n = 9; $P < 0.05$), consistent with a halothane-induced enhancement of SR Ca^{2+} release (*i.e.*, spontaneous Ca^{2+} release evoked at a lower SR Ca^{2+} content).

Amelioration of Halothane-induced Effects by Sevoflurane

These results illustrate that halothane and sevoflurane have opposing effects on the SR Ca^{2+} release process, in which case the effects of halothane might be ameliorated or reversed by coapplication of sevoflurane. The experiments in figure 4 illustrate the results of experiments in which mixtures of halothane and sevoflurane were applied to spontaneously active cells; application of a mixture of 0.3 mM halothane and 0.3 mM sevoflurane (fig. 4A) significantly increased frequency of release ($P < 0.05$) from an average value of 1.8 (over the four 30-s control periods) to 3.1 over the first 30-s period of exposure, before returning to control (fig. 4B). This compares to 0.6 mM halothane alone (fig. 1B), where frequency increased from an average value of 2.6 (over four 30-s periods) to 7.5 (over the first 30-s period). This could result from the lower actual concentration of halothane, but this was investigated in a separate series of experiments (see next paragraph, second sentence). The mixture of 0.1 mM halothane and 0.5 mM sevoflurane (fig. 4C) effectively abolished the initial transient increase in

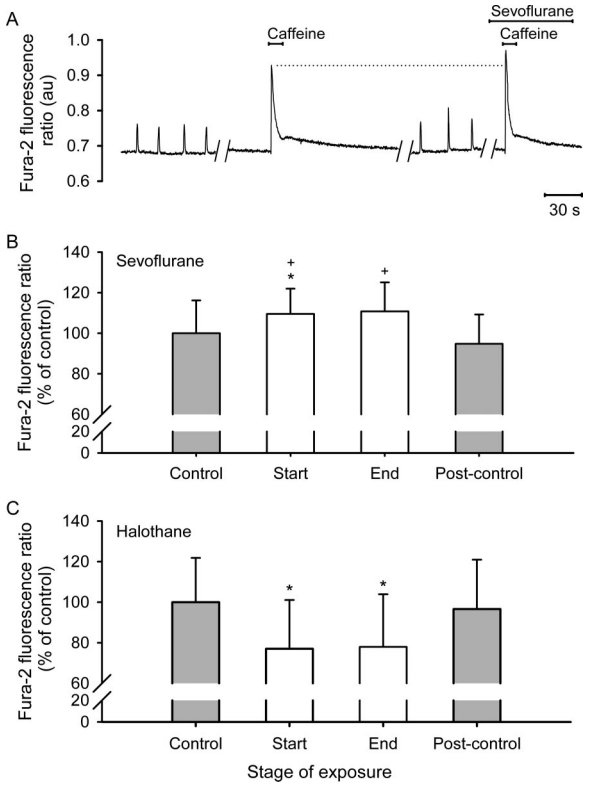

Fig. 3. (*A*) Original trace of protocol for measurement of sarcoplasmic reticulum Ca^{2+} content. Caffeine, 20 mM, was rapidly applied under control conditions at the time when the next spontaneous Ca^{2+} release was expected. Caffeine was then removed, and control conditions were restored. Anesthetic was added, and the caffeine pulse was applied again at the start and end (not shown) of the anesthetic exposure and after removal of anesthetic (postcontrol, not shown). (*B* and *C*) Mean data (± SEM) describing the peak caffeine-induced fura-2 fluorescence under control conditions and at the Start and End of a 4-min exposure to sevoflurane (*B*) or halothane (*C*). For Start data with sevoflurane (n = 10), caffeine was applied at the time when the first spontaneous transient was expected after the addition of the anesthetic, and for halothane (n = 9), caffeine was applied after the initial burst of Ca^{2+} release. For End data, caffeine was applied at the end of the anesthetic exposure. * *P* < 0.05 to precontrol. + *P* < 0.05 to postcontrol.

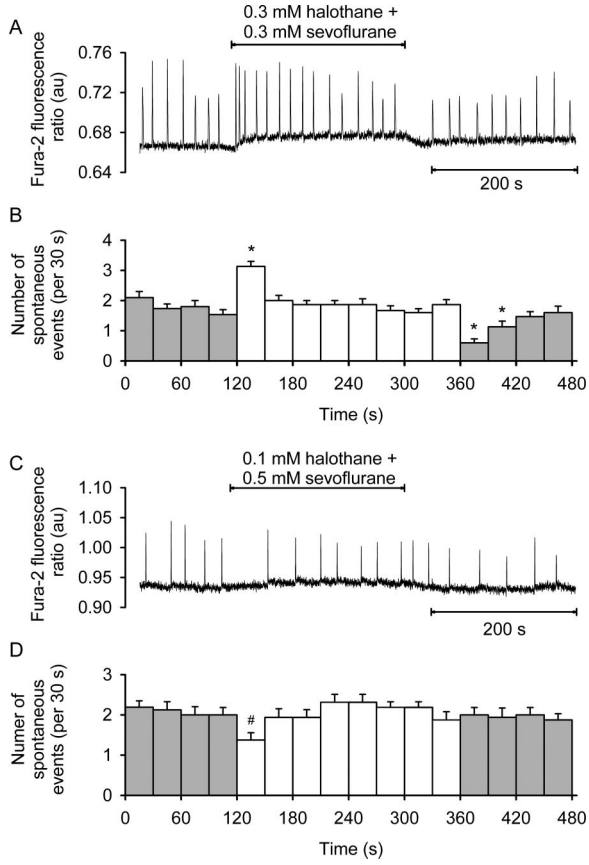

Fig. 4. Spontaneous Ca^{2+} transients before, during, and after a 4-min exposure to 0.3 mM halothane plus 0.3 sevoflurane (*A*) and 0.1 mM halothane plus 0.5 mM sevoflurane (*C*). Mean data (± SEM) describing the number of spontaneous events per 30-s periods for 0.3 mM halothane plus 0.3 sevoflurane (*B*, n = 15) and 0.1 mM halothane plus 0.5 mM sevoflurane (*D*, n = 16). Control perfusate is shown as *gray bars*, and anesthetic exposure is shown as *white bars*. * *P* < 0.05 to three or more of the four 30-s precontrol periods. # *P* < 0.05 to two of the four precontrol periods and at least one postcontrol period.

frequency that we observed with 0.1 mM halothane alone and figure 4D shows that during the first 30-s period, frequency of release was actually reduced. After removal of this anesthetic mixture, there was no effect on the frequency of release.

Figure 5A illustrates that as the proportion of sevoflurane was increased in the mixture, the frequency of spontaneous events on application declined with the line crossing baseline conditions (dotted line) at approximately 0.1 mM halothane–0.5 mM sevoflurane. The effect of 0.1 and 0.3 mM halothane alone (filled symbols) on the initial increase in frequency was significantly ($P < 0.05$) greater than that induced by the anesthetic mixtures containing the same level of halothane. Data for frequency of events on removal (fig. 5B) also illustrates that sevoflurane ameliorates the effect of halothane on suppression of SR Ca^{2+} release, and the line again intercepts baseline conditions at approximately 0.1 mM halothane–0.5 mM sevoflurane.

Changes in Diastolic Ca^{2+}

Halothane (0.6 mM) significantly ($P < 0.05$) increased diastolic Ca^{2+} throughout the exposure (figs. 1A and 6A), which returned to control levels on removal of halothane. In contrast, 0.6 mM sevoflurane significantly reduced diastolic Ca^{2+} for a period of 120 s, after which diastolic Ca^{2+} returned to control levels (figs. 2A and 6B). In cells pretreated with 1 μM ryanodine to inhibit SR function, no significant change in diastolic Ca^{2+} occurred during exposure to halothane (n = 14) or sevoflurane (n = 12). Figure 6C shows that 0.1 mM halothane also led to a sustained increase in diastolic Ca^{2+} similar to that observed with 0.6 mM halothane. Application of a mixture of 0.1 mM halothane and 0.5 mM sevoflurane also produced a sustained increase in diastolic Ca^{2+}; however, there was a 90-s delay before the increase in diastolic Ca^{2+} reached significance.

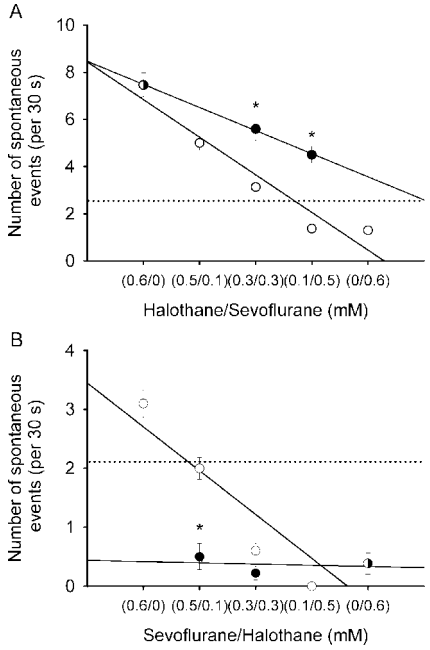

Fig. 5. Composite curves for mixtures of halothane and sevoflurane (total anesthetic concentration of 0.6 mM) on the number of spontaneous Ca^{2+} transients induced on initial exposure (*A*) and upon removal of anesthetic(s) (*B*). *Open symbols* identify data describing the effects of halothane–sevoflurane mixtures, and *filled symbols* identify concentrations of halothane alone. Data are mean ± SEM (n = 15 or 16). * $P < 0.05$ between halothane alone and halothane–sevoflurane mixtures. *Solid lines* are the result of linear regression of these data. *Dotted lines* represent the mean frequency of spontaneous release under baseline conditions.

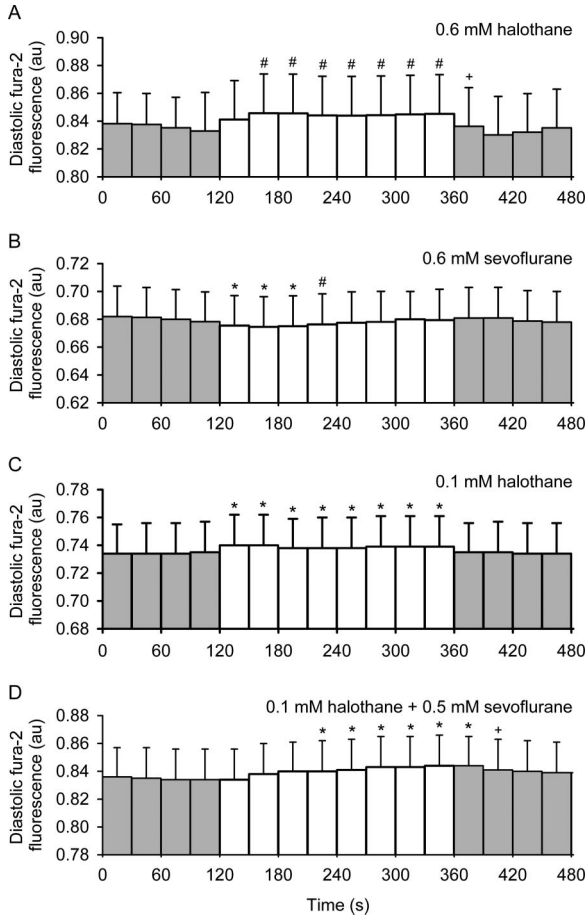

Fig. 6. Mean data (± SEM) describing diastolic fura-2 fluorescence ratio before, during, and after a 4-min exposure to 0.6 mM halothane (*A*, n = 13), 0.6 mM sevoflurane (*B*, n = 9), 0.1 mM halothane (*C*, n = 6), and 0.1 mM halothane plus 0.5 mM sevoflurane (*D*, n = 16). Control perfusate is shown as *gray bars*, and anesthetic exposure is shown as *white bars*. * $P < 0.05$ to three or more of the four 30-s precontrol periods. # $P < 0.05$ to two of the four precontrol periods and at least one postcontrol period. + $P < 0.05$ *versus* steady state (*column 12*).

Discussion

Halothane and Sevoflurane on Spontaneous Ca^{2+} Release

These data describe the effects of halothane, isoflurane, and sevoflurane on the properties of spontaneous Ca^{2+} release in isolated ventricular myocytes and the associated changes in diastolic Ca^{2+} that accompany the application and removal of anesthetic, which help to further our understanding of the inotropic actions of these agents in ventricular muscle. One intriguing aspect of these data is that the effects of halothane on SR Ca^{2+} regulation can be reversed by coapplication of sevoflurane.

During normal excitation–contraction coupling, Ca^{2+} entry *via* I_{Ca} induces the release of Ca^{2+} from the SR *via* the process of calcium-induced calcium release.[25,26] However, in certain pathologic conditions (*e.g.*, glycoside toxicity), cellular Ca^{2+} overload occurs, which can result in arrhythmogenic activity. This results from spontaneous release of Ca^{2+} from the SR, which induces delayed after depolarizations (*via* inward Na^+–Ca^{2+} exchange current[27] as Ca^{2+} is extruded from the cell), potentially leading to the generation of spontaneous action potentials. The extent of spontaneous release of Ca^{2+} from the SR is thought to be heavily influenced by the level of Ca^{2+} within the lumen of the SR,[28] and it has been proposed that luminal Ca^{2+} modulates the sensitivity of the ryanodine receptor (RyR) to cytoplasmic Ca^{2+}. In these studies, we have induced mild Ca^{2+} overload to assess the impact of anesthetics on the sensitivity of the SR Ca^{2+} release process.

Our data show that halothane induced an immediate release of SR Ca^{2+}, which was ryanodine sensitive and consistent with sensitization of the Ca^{2+} release process.[3,4,7] In contrast, application of sevoflurane (although less potent than halothane in molar terms) initially inhibited SR Ca^{2+} release. This result is similar to the decreased efficacy of SR Ca^{2+} release in response to rapid cooling induced by sevoflurane.[29] Coapplication of halothane and sevoflurane significantly ameliorated the sensitizing effect of halothane on the frequency of spontaneous SR Ca^{2+} release, with the mixture of 0.1 mM halothane and 0.5 mM sevoflurane having little impact on the frequency or amplitude of SR Ca^{2+} release, and effectively mimicked the results produced by 0.6 mM isoflurane.

On wash-off of halothane, spontaneous SR Ca^{2+} release was inhibited, whereas removal of sevoflurane induced a burst of Ca^{2+} release. As above, these effects of halothane were ameliorated by sevoflurane (figs. 4 and 5). Although these data do not permit a specific subcellular site to be identified, a plausible explanation of these results is that halothane and sevoflurane bind to the RyR, inducing either an increase[7] or a decrease in its open probability, respectively. Their binding seems antagonistic because 0.5 mM sevoflurane blocked the effects of 0.1 mM halothane almost completely, but again, this may not reflect antagonism at the same site of action as halothane. These data also suggest that isoflurane seems to have no net effect on the RyR under these conditions.

The transient effects of halothane on the frequency of spontaneous Ca^{2+} release are similar to those induced by a low dose of caffeine,[24,30] which are thought to involve luminal feedback of RyR sensitivity. To explain data with halothane (and also caffeine),[24,30] the initial increase in RyR opening would decrease SR Ca^{2+} content (fig. 3). The reduction in SR luminal Ca^{2+} would reduce RyR activation and therefore Ca^{2+} efflux from the SR and a new steady state would be reached when Ca^{2+} efflux was balanced by SR Ca^{2+} uptake. On wash-off of halothane, the decrease in Ca^{2+} release would result from a combination of lower SR Ca^{2+} content and reduced RyR activity.[24]

The effects of sevoflurane on the frequency of spontaneous Ca^{2+} release are similar to those of tetracaine,[24,31] which has been reported to decrease RyR open probability.[31–33] To explain data with sevoflurane (and tetracaine),[23,31] inhibition of SR Ca^{2+} release would increase SR Ca^{2+} content (fig. 3). The consequent increase of luminal Ca^{2+} would overcome the sevoflurane-induced decrease in open probability of the RyR, and Ca^{2+} efflux would increase. This would reach a new steady state when the enhanced efflux at the higher SR Ca^{2+} was balanced by SR Ca^{2+} uptake. On removal of sevoflurane, the burst of spontaneous Ca^{2+} release would result from the restoration of RyR sensitivity coupled with the increased SR Ca^{2+} content.

Changes in Diastolic Ca^{2+}

Although the above-proposed mechanism is capable of explaining the effects of halothane and sevoflurane on the frequency of SR Ca^{2+} release and SR Ca^{2+} content, it can not explain fully the effects on diastolic Ca^{2+}, because a mechanism centered around changes in the sensitivity of the RyR would be expected to induce only transient changes in diastolic Ca^{2+}, as were observed with sevoflurane (and caffeine[30]). Halothane induced a ryanodine-sensitive maintained increase in diastolic Ca^{2+} at doses between 0.1 and 0.6 mM, which suggests that additional mechanisms associated with the SR are affected. This could result from inhibition of SR Ca^{2+} uptake by halothane,[34] because other agents that inhibit SR Ca^{2+} uptake[35,36] also increase diastolic Ca^{2+}. Figure 6D illustrates that sevoflurane was able to delay the increase in diastolic Ca^{2+}, suggesting that the effects of sevoflurane and halothane on diastolic Ca^{2+} were additive rather than antagonistic.

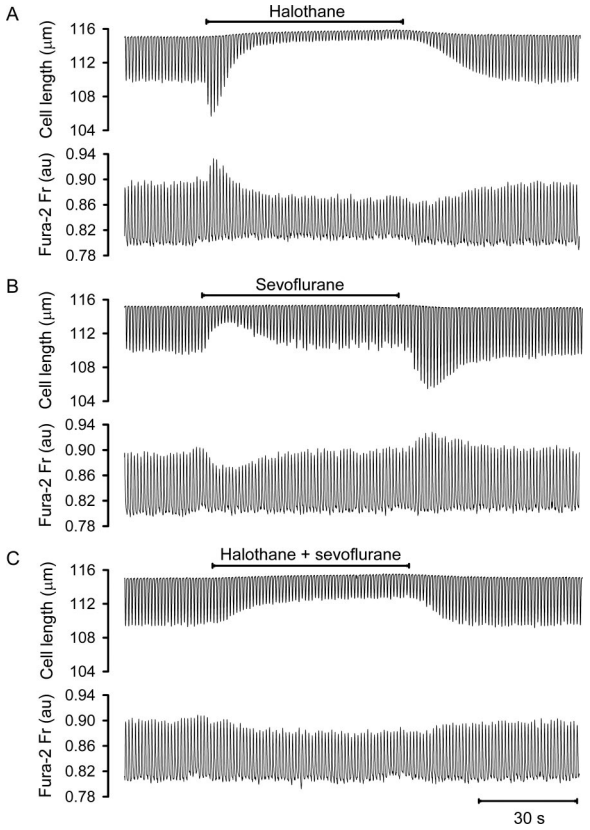

Fig. 7. Recordings of cell length and cytosolic fura-2 fluorescence ratio from a representative myocyte, stimulated electrically at 1 Hz (extracellular [Ca^{2+}] of 1 mM), during sequential 1-min exposures to 0.6 mM halothane (*A*), 0.6 mM sevoflurane (*B*), and a mixture of 0.3 mM halothane and 0.3 mM sevoflurane (*C*). Qualitatively similar results were seen in a total of 12 cells.

Limitations and Summary

One limitation is that these data might overstate the importance of SR-dependent mechanisms in the inotropic profiles of the anesthetics studied as excitation-contraction coupling is heavily SR dependent in the rat. Second, the experiments were conducted at 30°C rather than 37°C—a strategy used to maximize fura-2 retention by cells to allow accurate and consistent measurement of fluorescence signals during long protocols, and the balance of Ca^{2+} fluxes along the various pathways may differ slightly at 30°C compared with 37°C.

To summarize, these data suggest that halothane sensitizes, sevoflurane desensitizes, and isoflurane has no net effect on the SR Ca^{2+} release process. Figure 7 illustrates representative traces of cell shortening and the cytosolic fura-2 fluorescence ratio in an electrically stimulated cell exposed sequentially to halothane, then

sevoflurane, and finally to a mixture of halothane and sevoflurane. Figures 7A and B show the characteristic inotropic profiles induced by halothane and sevoflurane as described in the introduction. Figure 7C illustrates that the initial positive inotropic effect associated with halothane and the positive inotropic effect on removal of sevoflurane are both abolished when halothane and sevoflurane are applied simultaneously, corroborating the results of experiments conducted in spontaneously contracting cells. This illustrates the important contribution of anesthetic-induced changes in the sensitivity of the SR Ca^{2+} release process to the inotropic profiles of these agents and further suggests that halothane and sevoflurane affect SR Ca^{2+} release in an antagonistic manner such that the effects of halothane are ameliorated by sevoflurane.

References

1. Lynch C III, Vogel S, Sperelakis N: Halothane depression of myocardial slow action potentials. ANESTHESIOLOGY 1981; 55:360–8
2. Luk HN, Lin CI, Chang CL, Lee AR: Differential inotropic effects of halothane and isoflurane in dog ventricular tissues. Eur J Pharm 1987; 136:409–13
3. Wheeler DM, Rice RT, Lakatta EG: The action of halothane on spontaneous contractile waves and stimulated contractions on isolated rat and dog heart cells. ANESTHESIOLOGY 1990; 72:911–20
4. Harrison SM, Robinson M, Davies LA, Hopkins PM, Boyett MR: Mechanisms underlying the inotropic action of halothane on intact rat ventricular myocytes. Br J Anaesth 1999; 82:609–21
5. Davies LA, Hamilton DL, Hopkins PM, Boyett MR, Harrison SM: Concentration-dependent inotropic effects of halothane, isoflurane and sevoflurane on rat ventricular myocytes. Br J Anaesth 1999; 82:723–30
6. Davies LA, Gibson CN, Boyett MR, Hopkins PM, Harrison SM: Effects of isoflurane, sevoflurane, and halothane on myofilament Ca^{2+} sensitivity and sarcoplasmic reticulum Ca^{2+} release in rat ventricular myocytes. ANESTHESIOLOGY 2000; 93:1034–44
7. Connelly TJ, Coronado R: Activation of the Ca^{2+} release channel of cardiac sarcoplasmic reticulum by volatile anesthetics. ANESTHESIOLOGY 1994; 81:459–69
8. Lynch C III, Frazer MJ: Anesthetic alteration of ryanodine binding by cardiac calcium release channels. Biochim Biophys Acta 1994; 1194:109–17
9. Herland JS, Julian FJ, Stephenson DG: Halothane increases Ca^{2+} efflux *via* Ca^{2+} channels of sarcoplasmic reticulum in chemically skinned rat myocardium. J Physiol 1990; 426:1–18
10. Hannon JD, Cody MJ: Effects of volatile anesthetics on sarcolemmal calcium transport and sarcoplasmic reticulum calcium content in isolated myocytes. ANESTHESIOLOGY 2002; 96:1457–64
11. Ikemoto Y, Yatani A, Arimura H, Yoshitake J: Reduction of the slow inward current of isolated rat ventricular cells by thiamylal and halothane. Acta Anaesthesiol Scand 1985; 29:583–6
12. Bosnjak ZJ, Supan FD, Rusch NJ: The effects of halothane, enflurane, and isoflurane on calcium current in isolated canine ventricular cells. ANESTHESIOLOGY 1991; 74:340–5
13. Eskinder H, Rusch NJ, Supan FD, Kampine JP, Bosnjak ZJ: The effects of volatile anesthetics on L- and T-type calcium channel currents in canine Purkinje cells. ANESTHESIOLOGY 1991; 74:919–26
14. Pancrazio JJ: Halothane and isoflurane preferentially depress a slow inactivating component of Ca^{2+} channel current in guinea-pig myocytes. J Physiol 1996; 494:94–103
15. Rithalia A, Hopkins PM, Harrison SM: The effects of halothane, isoflurane, and sevoflurane on Ca^{2+} current and transient outward K^+ current in subendocardial and subepicardial myocytes from the rat left ventricle. Anesth Analg 2004; 99:1615–22
16. Hanley PJ, Loiselle DS: Mechanisms of force inhibition by halothane and isoflurane in intact rat cardiac muscle. J Physiol 1998; 506:231–44
17. Jiang YD, Julian FJ: Effects of halothane on $[Ca^{2+}]_i$ transient, SR Ca^{2+} content, and force in intact rat heart trabeculae. Am J Physiol 1998; 43:H106–14
18. Graham MD, Bru-Mercier G, Hopkins PM, Harrison SM: Transient and sustained changes in myofilament sensitivity to Ca^{2+} contribute to the inotropic effects of sevoflurane in rat ventricle. Br J Anaesth 2005; 94:279–86
19. Capogrossi MC, Stern MD, Spurgeon HA, Lakatta EG: Spontaneous Ca^{2+} release from the sarcoplasmic reticulum limits Ca^{2+}-dependent twitch potentiation in individual cardiac myocytes: A mechanism for maximum inotropy in the myocardium. J Gen Physiol 1988; 91:133–55
20. Diaz ME, Trafford AW, O'Neill SC, Eisner DA: Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca^{2+} release. J Physiol 1997; 501:3–16
21. Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council: Guide for the Care and Use of Laboratory Animals. Washington, DC, National Academy Press, 1996
22. Cannell MB, Lederer WJ: A novel experimental chamber for single-cell voltage-clamp and patch-clamp applications with low electrical noise and excellent temperature and flow control. Pflugers Arch Eur J Physiol 1986; 406:536–9
23. Franks NP, Lieb WR: Temperature dependence of the potency of volatile general anesthetics. ANESTHESIOLOGY 1996; 84:716–20
24. Lukyanenko V, Viatchenko-Karpinski S, Smirnov A, Wiesner TF, Gyorke S: Dynamic regulation of sarcoplasmic reticulum Ca^{2+} content and release by luminal Ca^{2+}-sensitive leak in rat ventricular myocytes. Biophys J 2001; 81: 785–98
25. Fabiato A: Time and calcium dependence of activation and inactivation of calcium-induced calcium release from the sarcoplasmic reticulum of a skinned cardiac Purkinje cell. J Gen Physiol 1985; 85:247–89
26. Bers DM: Cardiac excitation-contraction coupling. Nature 2002; 415:198–205
27. Berlin JR, Cannell MB, Lederer WJ: Cellular origins of the transient inward current in cardiac myocytes: Role of fluctuations and waves of elevated intracellular calcium. Circ Res 1989; 65:115–26
28. Lukyanenko V, Subramanian S, Gyorke I, Wiesner TF, Gyorke S: The role of luminal Ca^{2+} in the generation of Ca^{2+} waves in rat ventricular myocytes. J Physiol 1999; 518:173–86
29. Park WK, Pancrazio JJ, Suh CK, Lynch C III: Myocardial depressant effects of sevoflurane: Mechanical and electrophysiologic actions *in vitro*. ANESTHESIOLOGY 1996; 84:1166–76
30. O'Neill SC, Eisner DA: A mechanism for the effects of caffeine on Ca^{2+} release during diastole and systole in isolated rat ventricular myocytes. J Physiol 1990; 430:519–36
31. Overend CL, Eisner DA, O'Neill SC: The effect of tetracaine on spontaneous Ca^{2+} release and sarcoplasmic reticulum calcium content in rat ventricular myocytes. J Physiol 1997; 502:471–9
32. Xu L, Jones R, Meissner G: Effects of local anesthetics on single channel behavior of skeletal muscle calcium release channel. J Gen Physiol 1993; 101: 207–33
33. Overend CL, O'Neill SC, Eisner DA: The effect of tetracaine on stimulated contractions, sarcoplasmic reticulum Ca^{2+} content and membrane current in isolated rat ventricular myocytes. J Physiol 1998; 507:759–69
34. Karon BS, Autry JM, Shi Y, Garnett CE, Inesi G, Jones LR, Kutchai H, Thomas DD: Different anesthetic sensitivities of skeletal and cardiac isoforms of the Ca-ATPase. Biochemistry 1999; 38:9301–7
35. Kawai M, Hussain M, Orchard CH: Cs^+ inhibits spontaneous Ca^{2+} release from sarcoplasmic reticulum of skinned cardiac myocytes. Am J Physiol 1998; 44:H422–30
36. O'Neill SC, Miller L, Hinch R, Eisner DA: Interplay between SERCA and sarcolemmal Ca^{2+} efflux pathways controls spontaneous release of Ca^{2+} from the sarcoplasmic reticulum in rat ventricular myocytes. J Physiol 2004; 559: 121–8