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# Initial Contractile Response of Isolated Rat Heart Cells to Halothane, Enflurane, and Isoflurane

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***Background:*** **In several beating cardiac muscle preparations, a short-lived increase in twitch tension or amplitude has been observed when they were exposed abruptly to solutions containing halothane or enflurane. As exposure to the anesthetics was continued, the expected negative inotropic effect became evident after the short-lived increase in twitch. No such increase in twitch has been reported during exposure to isoflurane. It has been hypothesized that this short-lived increase in twitch is caused by an enhancement of calcium release from the sarcoplasmic reticulum, but other mechanisms have not been excluded.**

***Methods:*** **Freshly isolated, single rat ventricular cells were stimulated to beat at room temperature and abruptly exposed to solutions containing halothane (0.25–0.64 mM), enflurane (0.69–1 mM), or isoflurane (0.31–0.54 mM). During these exposures, twitch amplitude was measured and intracellular calcium concentration was followed using the calcium-sensitive dye indo-1. In some experiments, the whole-cell patch-clamp technique was used to measure membrane current. In addition, in several cells the sarcoplasmic reticulum calcium content was assessed through the response to brief pulses of caffeine.**

***Results:*** **Both the twitch amplitude and the intracellular calcium transient were increased temporarily in cells abruptly exposed to halothane or enflurane. No such behavior was found with isoflurane. After continued exposure to all three agents, both the twitch amplitude and the calcium transient were less than control. During the beats exhibiting an increase in twitch, no alteration in the relation between cell length (twitch amplitude) and the intracellular calcium transient was found compared with control conditions. In addition, the temporary increase in twitch amplitude occurred in cells contracting under voltage-clamp control when halothane was introduced, and it was not associated with any increase in the calcium current. The sarcoplasmic reticulum calcium content at the time of the halothane-induced increase in twitch also was not increased.**

***Conclusions:*** **The short-lived increase in twitch after abrupt exposure to halothane or enflurane is related to increased intracellular calcium during the beat and not to any changes in myofilament sensitivity to calcium. Because these results eliminate most alternative explanations for this phenomenon, the authors conclude that halothane, and probably also enflurane, increases the fraction of calcium released from the sarcoplasmic reticulum with each heart beat. Isoflurane appears to lack this action. (Key words: Anesthetics, volatile: enflurane, halothane, isoflurane. Heart: calcium current, contractility, sarcoplasmic reticulum. Ions: calcium.)**

HALOTHANE has been observed to produce a short-lived increase in twitch tension or twitch amplitude when abruptly introduced in certain beating cardiac muscle preparations from at least three different species.[1-4] Reports of such a finding when enflurane was introduced have also appeared.[4,5] In all cardiac preparations studied, the steady-state action of halothane or enflurane (that is, their effect when exposure to them is continued until no further change occurs) is a decrease in twitch tension or twitch amplitude compared with the control condition. We are aware of no reports that suggest that isoflurane causes a short-lived increase in twitch and several that do indicate that it does not.[2,5] Of course, isoflurane shares with halothane and enflurane a negative inotropic effect at steady-state conditions.

The short-lived increase in twitch has not been obtained in all cardiac muscle preparations exposed to halothane and enflurane; for instance, we are not aware of any such reports in live animals. This lack of uniformity may be due to differences in the rate of change of anesthetic concentration at the cardiac cell membrane in various experimental preparations. A rapid or abrupt exposure of cells to halothane or enflurane may

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be a necessary condition for the short-lived increase in twitch to occur. Thus the transient increase in twitch would be more likely to be found in preparations of small mass (such as single cells) and in those experiments in which solution composition is changed rapidly. In the live animal, of course, there would be difficulty in effecting a sufficiently abrupt anesthetic partial pressure change in the blood. Furthermore, if the anesthetic is delivered *via* the airway, a confounding positive inotropic effect related to airway irritation may occur.

An enhancement of the amount of calcium released from the sarcoplasmic reticulum (SR) with each beat has been hypothesized as the mechanism of the short-lived increase in twitch amplitude or tension with abrupt introduction of halothane.[2,3] Specifically, it has been proposed that when cardiac muscle is suddenly exposed to halothane, the amount of calcium released from the SR during each beat increases because the calcium-release channel is enhanced. With continued halothane exposure, the SR becomes depleted of calcium so that even enhanced release results in a net reduction of the amount of calcium released during each beat. Enflurane is less thoroughly studied, but the available evidence indicates that a parallel argument could be applied to it.

Halothane, enflurane, and isoflurane decrease the intracellular calcium transient that occurs with each beat, once a steady state in each anesthetic is achieved.[6-8] However, calcium transients at the time of anesthetic introduction have not been reported before. The purpose of this study was to analyze intracellular calcium transients and SR calcium content during the introduction of anesthetic so that the hypothesis regarding the mechanism of the short-lived increase in twitch could be tested. The broader significance of this work was to define the mechanisms of the negative inotropic effect of the anesthetics and to determine differences among them. All three agents are known to cause a reduction of the calcium current,[9-12] and halothane, enflurane, and possibly isoflurane reduce SR calcium content.[13-19] Although this reduction of SR calcium content could be secondary to the decrease in sarcolemmal calcium influx (*via* calcium channels), evidence suggests that direct SR actions are contributory, and that an increase in the calcium permeability of the SR may be involved. Thus several components of the anesthetics' action may combine to reduce the intracellular calcium transient found at steady state, and the specific components may be discernible only during dynamic events such as the abrupt introduction of the agents.

## Methods

The animal protocol used in this study was approved by the institutional Animal Care and Use Committee. Killing the rats without first anesthetizing them was deemed acceptable given the speed and reliability of the method of killing and the potential alteration of myocardial calcium metabolism by any anesthetic.

Isolated rat heart cells were obtained by methods previously described.[20] Briefly, male Wistar rats, ages 2 to 6 months (typical weight range, 300 to 500 g), were decapitated by guillotine and the hearts were rapidly excised. The aortic root of each rat was cannulated, and coronary perfusion at constant pressure was begun with a nominally calcium-free Earle's salt solution (142 mM Na, 5.4 mM K, 116 mM Cl, 26.2 mM $HCO_3$, 1 mM $H_2PO_4$, 0.8 mM $MgSO_4$, and 5.6 mM glucose). Once cleared of blood, the heart was perfused for 14 min with 1 g/l collagenase (Worthington Biochemical Corp., Freehold, NJ) and 0.04 g/l protease (Sigma Chemical Co., St. Louis, MO) in an Earle's salt solution with 50 $\mu$M calcium added. The enzyme-containing solution was cleared from the heart using a 5-min perfusion with Earle's salt solution containing 100 $\mu$M calcium. The perfusion was then discontinued, and a suspension of cells was obtained in the final perfusion solution by mincing and straining the ventricles. Sequential resuspensions were performed using Earle's salt solutions containing 250 $\mu$M calcium and 1 mM calcium. The cells were stored for as long as 6 h in a 5% carbon dioxide incubator at 37°C. All solutions were bubbled with a 5% carbon dioxide-95% oxygen mixture.

Aliquots of the heart cell suspension were loaded with the calcium-sensitive, fluorescent dye indo-1 using the following procedure. Cells were exposed for 8 min at room temperature (22-25°C) to a 25-$\mu$M concentration of the acetoxymethyl ester of indo-1 (indo-1 AM, Molecular Probes, Eugene, OR), together with 4.5% fetal calf serum and 0.1% Pluronic-127 (BASF, Wyandotte, MI). The indo-1-loaded cells were resuspended in a HEPES-buffered salt solution of the following composition: 137 mM Na, 5 mM K, 144 mM Cl, 1 mM Ca, 1.2 mM $MgSO_4$, and 20 mM HEPES, with the *p*H adjusted to 7.4. The cells were stored in the dark at room temperature for at least 45 min to allow for intracellular hydrolysis of the indo-1 AM. An aliquot of indo-1-loaded cells was

placed in a glass-bottomed chamber. After 5 min of no flow to permit cell attachment to the glass, perfusion of the chamber was begun with the HEPES-buffered salt solution, as previously described. A single cell of rod-shaped character and without blebs or granulations was selected for observation using an inverted-stage Zeiss microscope. The length of the cell was measured continuously from its bright-field image projected onto a linear photodiode array. A second light source (strobe-driven xenon arc lamp with interference filter) provided light at 350 nm for fluorescence excitation. Fluorescent light emitted by the cell at both 410 and 490 nm was collected and the ratio of the signals obtained. This ratio was used as an index of the free intracellular calcium concentration ($Ca_i$). The apparatus and methods described above have been previously documented in detail.[21] All experiments were performed at room temperature (22–25°C).

Certain cells were exposed to brief pulses of caffeine using the following techniques. Thick-walled glass micropipettes were filled with solution containing 15 mM caffeine. The pipette tip was positioned directly above the cell. Caffeine was delivered on command by applying a 30-mmHg pressure pulse lasting 150 ms to the pipette.

In some cells, membrane currents were measured by standard patch-clamp techniques using the whole-cell voltage clamp mode. Recordings were made using a high-impedance amplifier (Axopatch-1A; Axon Instruments, Burlingame, CA). The patch micropippetes were filled, in most experiments, with a solution containing 120 mM CsCl, 10 mM NaCl, 2 mM MgATP, 20 mM HEPES, and 0.5 μM EGTA, adjusted to a *p*H of 7.1 with CsOH. In the extracellular solution, cesium was also substituted for potassium. The cesium blocked potassium-dependent currents such as the transient outward current (an outward current in the rat that is carried by potassium and temporally overlaps the calcium current)[22] and the inwardly rectifying current. Sodium current was eliminated by including tetrodotoxin (5–20 μM) in the perfusion solution. Several cells were studied with a different voltage-clamp protocol: Sodium current was inactivated using a holding potential of −40 mV, transient outward current was blocked with 4-aminopyridine (2 mM), but cesium was not used in the pipette or in the external solution. Therefore, the inwardly rectifying current of the rat ($I_{K1}$) is present in those records, but because it is time-independent, it will not alter the measurement of calcium current.[23] The magnitude of the calcium current was calculated as the difference between the peak inward current during depolarization and the current present immediately before repolarization. Some of the cells used in the electrical experiments were not loaded with indo-1.

During experimental protocols in which membrane currents were not measured, cells were stimulated to beat using current pulses applied to platinum wire electrodes at opposite ends of the chamber. The beating rates were 0.5 or 0.25 Hz. In all experiments, after baseline measurements were obtained from a cell in control solution and during continuous beating of the cell, the superfusion solution was changed to one containing anesthetic. After a steady state in the presence of the anesthetic was obtained, the superfusion solution was changed back to control. Much of the data analysis focused on the time immediately after the anesthetic-containing solution reached the cell. For halothane and enflurane, this time was readily identified by the occurrence of a short-lived increase in twitch. When no increase in twitch occurred (*i.e.,* in essentially all of the isoflurane runs), the "transient" response to the anesthetic was taken from the same time after the solution change when that cell demonstrated a positive twitch response in the other anesthetics.

The data were normalized based on the results in control solution just before the transition to anesthetic. Differences from control were tested using one-sample, two-tailed *t* tests. The results in anesthetic-containing solution were compared with one another using one-way analysis of variance, with the Student-Newman-Keuls test to determine the significance of individual *post hoc* comparisons. In the analysis in which the comparison involved individual cells exposed to two different anesthetics, the paired *t* test was used to test for significant differences between pairs of anesthetics. For the experiment involving the response to pulses of caffeine, repeated-measures analysis of variance was performed for measurements at the following times: in control solution, during the short-lived increase in twitch, at steady state in halothane, and after return to control. For this analysis of variance, a missing value for the return to control was estimated according to the method described by Li.[24]

Anesthetic concentration in the cell chamber was determined by sampling the aqueous solution there during perfusion of anesthetic-containing solution and performing gas chromatography after heptane extraction. Conversion to vol% units was performed using a Bunsen partition coefficient (buffer:gas) of 1.25 for halothane, 1.28 for enflurane, and 0.98 for isoflurane (at 25°C).

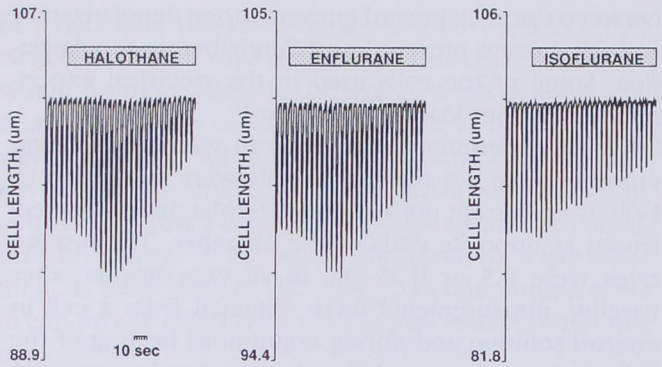


**Fig. 1. Cell length of individual rat heart cells exposed to volatile anesthetics. The cells were field stimulated to beat at a rate of 0.5 Hz. Each vertical deflection represents the extent of shortening (or twitch amplitude) of each beat: The deeper downward deflections represent greater twitch amplitude. Each record shows a different cell during the early moments of its exposure to anesthetic-containing solution. Anesthetic concentrations were 0.40 mM halothane (0.8 vol%), 1 mM enflurane (1.9 vol%), and 0.54 mM isoflurane (1.4 vol%).**

These coefficients were derived by taking published values,[25-27] converting to the Bunsen definition, averaging those values obtained at 37°C, and extrapolating to 25°C based on the temperature trends found in the report by Smith and coworkers.[25] Temperature was assumed to have the same effect on the enflurane coefficient as it did on isoflurane.

## Results

When the solution perfusing the cell chamber was abruptly changed to one containing halothane (in concentrations ranging from 0.25 to 0.64 mM or 0.5 to 1.3 vol%), a short-lived increase in the twitch amplitude of indo-1-loaded cells typically occurred. After this initial increase, twitch amplitude decreased in the continued presence of halothane-containing solution to a value less than that in control solution. Washout of halothane resulted in a return of twitch amplitude to control values. These results are the same as those obtained in rat heart cells at 37°C that were not loaded with any indicator.[3] In cells abruptly exposed to enflurane (0.69 to 1.0 mM or 1.3 to 1.9 vol%), a small transient increase in twitch amplitude also was often observed before the expected decrease in twitch amplitude as enflurane exposure was continued. However, with isoflurane (0.31 to 0.54 mM or 0.8 to 1.4 vol%), there was no evidence of any increase in twitch amplitude (fig. 1). When the twitch amplitude immediately following anesthetic introduction was compared among the three anesthetics, each pairwise comparison yielded a statistically significant difference. Again, these results in indo-1-loaded cells at room temperature mirror those obtained using rat heart cells at 37°C with no indicator.[3,5] Thus the effects of the anesthetics on twitch amplitude cannot be attributed to the presence of the indicator or to the temperature of the experiment.

Coincident with this short-lived increase in twitch amplitude on abrupt exposure to halothane or enflurane was a small and similarly temporary increase in the intracellular calcium transient associated with each beat (fig. 2). As exposure to the anesthetics was continued and twitch amplitude declined, the calcium transient was also reduced, an effect previously demonstrated in detail by others.[6-8] Washout of anesthetic resulted in a return to near-control levels. Thus, for each anesthetic, changes in the intracellular calcium transient paralleled changes in twitch amplitude (fig. 3). When the magnitude of the intracellular calcium transient just after anesthetic introduction was compared among the three agents, there were statistically significant differences among the anesthetics for each pairwise comparison.

To confirm the differences in the initial effect of abrupt exposure among the three agents, cells were exposed to two agents in sequence, with an intervening control superfusion. The anesthetic concentrations used in these experiments were 0.25 mM halothane, 1 mM enflurane, and 0.54 mM isoflurane. An example of

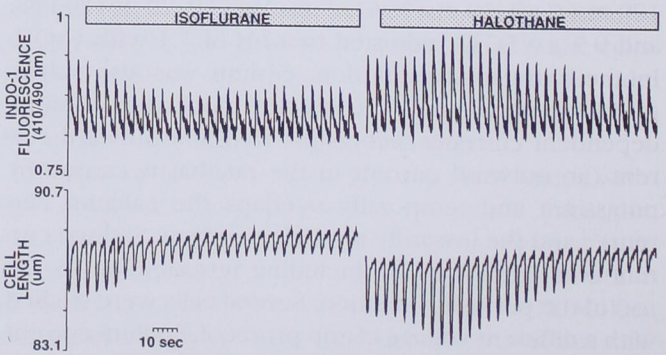


**Fig. 2. The upper traces represent the intracellular calcium concentration measured as the indo-1 fluorescence ratio, and the lower traces represent the simultaneously measured cell length. The height of the peaks in the upper traces thus reflects the magnitude of the intracellular calcium transient, and the depth of the deflections in the lower traces represents the twitch amplitude. These records are taken from a single cell exposed first to 0.31 mM isoflurane (0.8 vol%) and, after a return to control solution (not shown), then to 0.25 mM halothane (0.5 vol%).**

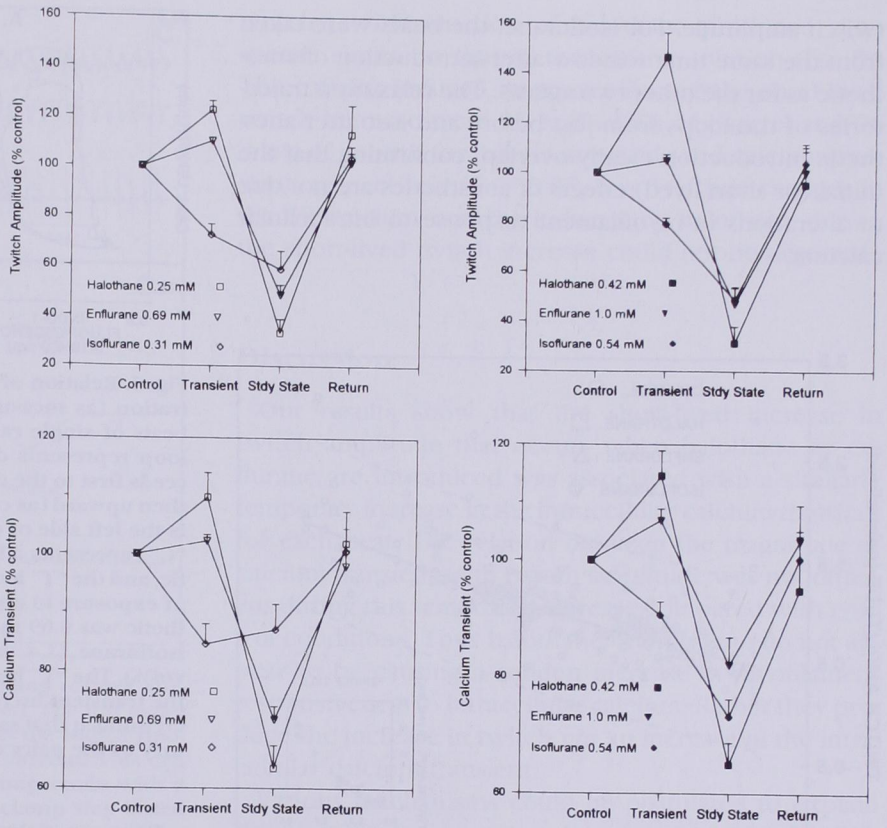


**Fig. 3. Summary of the response of the twitch amplitude (*upper*) and the intracellular calcium transient (*lower*) to volatile anesthetics. The points designated "transient" originate from the time immediately after anesthetic introduction; "steady-state" points are derived from beats after several minutes of exposure to the anesthetic; and "return" represents points after the washout of anesthetic. Data points are mean + SEM. All differences between any pair of anesthetics at the "transient" points were significant ($P < 0.05$). The numbers of experiments done with each anesthetic were as follows: 9 with 0.25 mM halothane; 15 with 0.69 mM enflurane; 10 with 0.31 mM isoflurane; 8 with 0.42 mM halothane; 10 with 1 mM enflurane; and 18 with 0.54 mM isoflurane.**

such a same-cell comparison is shown in figure 2, and these tests confirmed the differences observed in the separate-cell runs. Statistical tests were done only for the responses that occurred immediately after the anesthetics were introduced. The anesthetics were compared with respect to the twitch amplitude and the calcium transient, and each pairwise comparison between anesthetics was significantly different, except for the calcium transient in cells exposed to halothane and enflurane.

We then tried to determine if the initial changes in twitch amplitude could be ascribed entirely to the parallel changes in calcium transient amplitude. The relation between the calcium transient (as measured by changes in the indo-1 fluorescence ratio) and twitch amplitude therefore was studied. In the absence of anesthetic, a range of twitch responses was generated by varying the extracellular calcium concentration. A nonlinear relation between the height of the calcium transient and the twitch amplitude was found. This relation was the same for beats just after the introduction of anesthetic as for those in control solution. This identity could be documented in a single cell or by normalizing the data and comparing many cells (fig. 4).

This analysis of the relation between twitch amplitude and intracellular calcium transient is commonly used but may be deficient. The minimum cell length achieved during a beat (on which the twitch amplitude depends) and the peak of the calcium transient do not occur simultaneously. Thus an analysis of the relation between these two end points may not reflect the actual relation between cell length and intracellular calcium. A phase-plane analysis that converts each beat to a loop on the cell length *versus* indo-1 ratio plane overcomes this shortcoming. Furthermore, it has been shown that during the relaxation phase of a contraction, a quasi-equilibrium exists between cell length and intracellular calcium.[28] Agents that change the cell length at a given intracellular calcium change the relaxation trajectory on the phase-plane loop. Figure 5 illustrates a series of loops that compare the beats of cells just before and immediately after anesthetic exposure. With halothane and enflurane, the beats after introduction of anesthetic are taken from the time of the short-lived increase in

twitch amplitude. For isoflurane, the beats were taken from the same time window after introduction of anesthetic as for the other two agents. The relaxation trajectories of the loops from just before and just after anesthetic introduction clearly overlap, confirming that the initial (or short-lived) effects of anesthetics are not due to alterations in myofilament response to intracellular calcium.

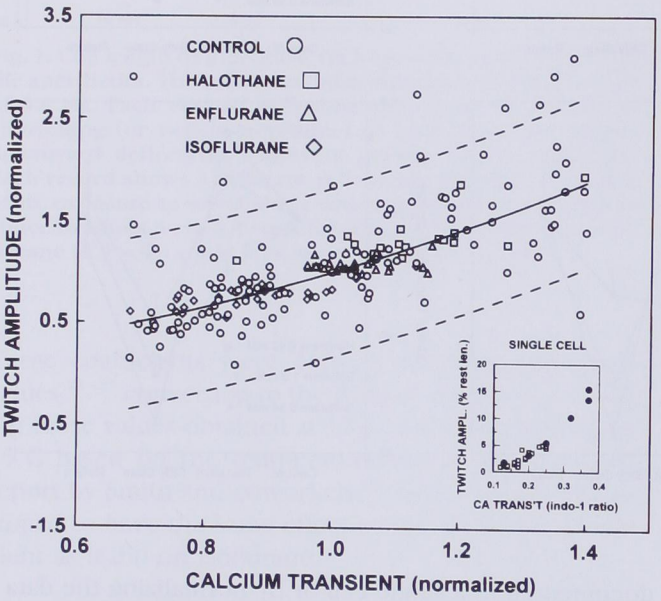


**Fig. 4. Relation of twitch amplitude to the magnitude of the intracellular calcium transient, with a comparison of beats in control solution of various calcium concentrations to beats that occurred just after introduction of anesthetic. Data are normalized for each cell so that in control solution with [Ca] = 1 mM, the twitch amplitude and the peak of the calcium transient both equal 1. Control points were obtained by varying the [Ca] in the bathing solution from 0.5 to 5 mM. The solid curve represents the best power curve ($y = ax^b$) fit to the control points; the dotted curves are the 95% confidence limits for this fit. The data points identified with the anesthetics (halothane: □; enflurane: △; isoflurane: ◇) were obtained from beats just after the introduction of the agent at the time of the short-lived increase in twitch for halothane and enflurane, or in the comparable time window for isoflurane. All anesthetic data points were obtained with an extracellular [Ca] of 1 mM. Anesthetic concentrations were as follows: 0.25 or 0.40 mM halothane (0.5 or 0.8 vol%); 0.69 or 1 mM enflurane (1.3 or 1.9 vol%); and 0.31 or 0.54 mM isoflurane (0.8 or 1.4 vol%). The inset shows the relation of the magnitude of the intracellular calcium transient with each beat and the twitch amplitude in a single cardiac cell. The cell was first exposed to solutions of varying calcium concentrations (points shown as solid dots). Then the superfusate was changed from control solution ([Ca] = 1 mM) to one containing 0.40 mM halothane ([Ca] also 1 mM). The beats during the short-lived increase in twitch amplitude and during the early course of the decline of twitch in halothane are represented as open squares.**

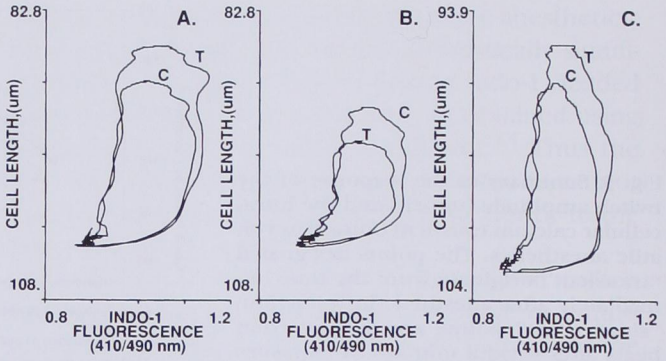


**Fig. 5. Relation of cell length to intracellular calcium concentration (as measured by the indo-1 ratio) during individual beats of single rat heart cells. The lower-left corner of each loop represents diastole. During contraction, the trace proceeds first to the right (as intracellular calcium increases) and then upward (as cell shortening occurs). The relaxation phase is the left side of each loop. For each panel, the loop marked "C" represents a beat just before the introduction of anesthetic, and the "T" loop comes from a beat just after the beginning of exposure to anesthetic-containing solution. In *A*, the anesthetic was 0.69 mM enflurane (1.3 vol%); in *B*, it was 0.54 mM isoflurane (1.4 vol%); in *C*, it was 0.40 mM halothane (0.8 vol%). The "T" beats in halothane and enflurane demonstrate the transient increase in twitch, whereas twitch is already decreasing at that same time in isoflurane. The relaxation trajectory of the pairs of beats match for each anesthetic.**

Because it thus appeared that the different responses to abrupt anesthetic exposure primarily involved the intracellular calcium transient, the calcium current was measured in several cells during exposure to halothane or isoflurane. Although the small number of cells studied renders quantitative analysis impractical, in each cell the response of the calcium current was a decline in the presence of the anesthetics. There is no hint of a biphasic response in the calcium current such as that seen in the twitch amplitude. In these cells beating on the basis of voltage-clamp steps, the changes in twitch amplitude followed the same patterns as in cells stimulated extracellularly (figs. 6 and 7). The transient increase in twitch on exposure to halothane often occurred before there was even a measurable change in the calcium current. Conversely, the decrease in twitch on exposure to isoflurane happened at a time when the decrease in calcium current was scarcely apparent. Therefore, sudden changes in the calcium current on exposure to halothane or isoflurane are unlikely to explain the transient twitch amplitude phenomena.

We also examined, for the case of abrupt transition to halothane-containing superfusate, the SR calcium content. These experiments used the property of caffeine, in millimolar concentrations, to cause a release

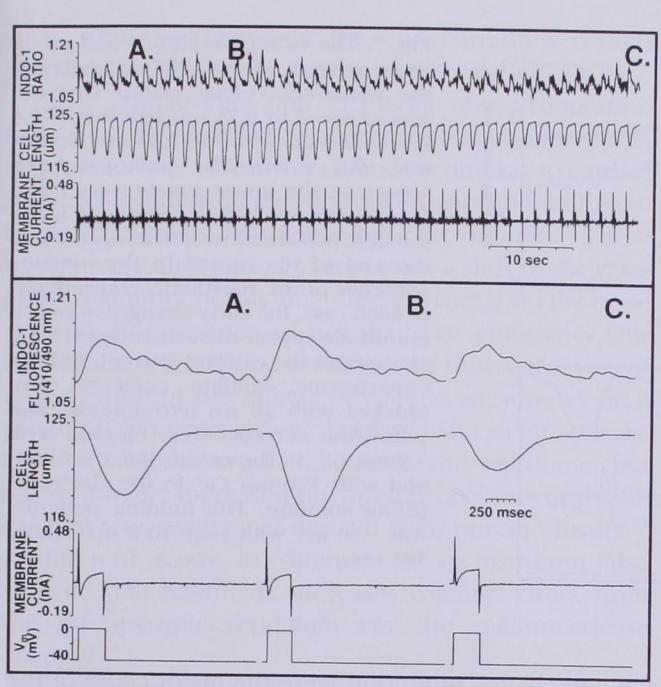


**Fig. 6. A single rat heart cell under voltage-clamp control during exposure to 0.42 mM halothane (0.8 vol%). The top panel contains a record of intracellular calcium (as the indo-1 fluorescence ratio), cell length, and membrane current. This cell was controlled in the whole-cell voltage-clamp mode with a holding potential of −40 mV. Each voltage clamp step lasted 200 ms to a potential of 0 mV (as shown at the bottom of the lower panel). Transient outward current was blocked by 4-aminopyridine (2 mM) in the bathing solution. The lower panel displays the same end points on an expanded time scale. The three beats shown in the lower panel each represent the average of four adjacent beats and originate from the area of the upper panel marked with the corresponding letter (*A:* in control solution, *B:* during the temporary increase in twitch after halothane introduction, and *C:* after approximate 50 s of exposure to halothane).**

of calcium from the SR into the cytoplasm. Caffeine (15 mM) was used to cause a transient release of the bulk of the releasable SR calcium. This calcium release could be measured as a contraction and as an intracellular calcium transient. Thus these end points are an indirect measure of the SR calcium content. The response of cells to a caffeine bolus was blunted at a steady state in halothane (0.25–0.42 mM), with the caffeine-induced twitch height reduced to 58 ± 13% (SD) of control and the caffeine-induced calcium transient height to 73 ± 15% of control ($P < 0.01$, n = 5, for both end points). This action was reversible with washout of halothane. This result indicates that halothane reduced SR calcium content, as demonstrated previously.[13–19] Caffeine pulses during the time of the temporary increase in twitch on introduction of halothane produced a response similar to that in the control solution (fig. 8). The caffeine-induced responses during the short-lived twitch increase were 95 ± 8% (SD) of control and 96 ± 6% of control for twitch amplitude and calcium transient, respectively ($P > 0.05$, n = 5). Thus changes in the SR calcium content induced by halothane during the short-lived twitch increase could not be detected.

## Discussion

Our results show that the short-lived increase in twitch amplitude that occurs when halothane or enflurane are introduced was associated with a similarly temporary increase in the intracellular calcium transient for each beat. The relation between the magnitude of calcium transients and twitch amplitude was not different during this temporary increase compared with control conditions. Thus halothane or enflurane do not appear to be causing a sudden increase in myofilament responsiveness to intracellular calcium. Rather they produce the increase in twitch *via* an increase in the intracellular calcium transient.

Various mechanisms could be postulated to explain the short-lived increase in calcium transients and twitch amplitude. Our results eliminate many of the possible mechanisms. Because this phenomenon was observed in cells under voltage-clamp control, in which contractions are the result of set steps in membrane potential, changes in the action potential duration are not an explanation. Neither could potassium currents account for the increase in twitch amplitude, because potassium channels were blocked during the voltage-clamp experiments. Measurements of the calcium current further revealed that the increase in twitch occurred when this current was not different from control and may have been decreasing. Thus the short-lived increase in twitch amplitude and calcium transient cannot be ascribed to increased calcium influx *via* calcium channels.

Our results do not directly address the role of sarcolemmal sodium–calcium exchange as a possible explanation for the short-lived increase in twitch immediately after halothane or enflurane introduction. Halothane, enflurane, and isoflurane have been shown to inhibit sodium–calcium exchange in isolated rat heart cells.[29] This exchange is thought to function primarily as a calcium extrusion mechanism, driven by the inwardly directed sodium gradient. Inhibition of the exchange could mediate a positive inotropic effect by

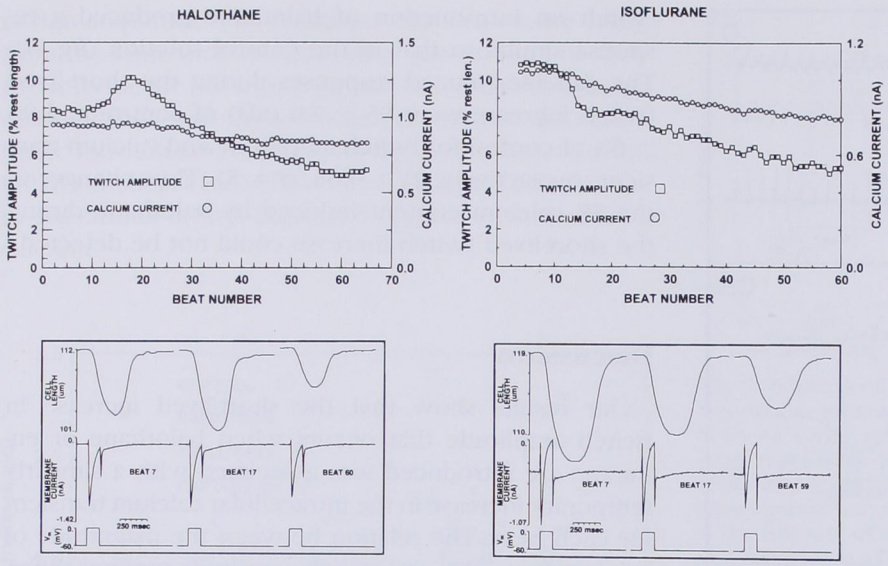


**Fig. 7. The twitch amplitude and membrane current of two different cells under whole-cell voltage clamp. One cell was exposed to 0.25 mM halothane (0.5 vol%) and the other to 0.31 mM isoflurane (0.8 vol%). The enlarged beats shown in the lower panels were taken from times just before anesthetic introduction, just after introduction, and near the end of the record in the ongoing presence of the anesthetic, respectively. In each case, the early changes in twitch amplitude appear to be dissociated from changes in the calcium current. In both experiments, sodium currents were blocked with 20 μM tetrodotoxin, and potassium currents were blocked with 4.9 mM $Cs^+$ in the extracellular solution and with 120 mM $Cs^+$ in the electrode-filling solution. The holding potential was −60 mV with steps to 0 mV lasting 105 ms.**

decreasing calcium extrusion while the membrane potential is at or near its resting level, thereby increasing the calcium filling of the SR. Theoretically, such an effect could be temporary if an unrelated negative inotropic effect of slower onset later superseded it. However, several results virtually rule out sodium-calcium exchange as the primary mechanism responsible for the short-lived increase in twitch amplitude. First, we found no suggestion of an increase in the SR calcium content during the increase in twitch amplitude after halothane introduction. Second, isoflurane is intermediate to halothane and enflurane in its potency for inhibiting sodium-calcium exchange (comparing equianesthetic concentrations).[29] Thus, if sodium-calcium exchange inhibition were the major cause of the temporary increase in twitch, then isoflurane should also produce the phenomenon. Our results clearly indicate that it does not.

With most sarcolemmal mechanisms thus eliminated from consideration, our attention turns to the SR as the most likely source of the short-lived increase in twitch and calcium transient after halothane and enflurane introduction. Because there was no evidence of increased SR calcium content at the time of halothane introduction, it appears that halothane (and probably enflurane as well) caused more of the SR calcium to be released with each beat. In contrast, the introduction of isoflurane did not increase twitch amplitude or calcium tran-

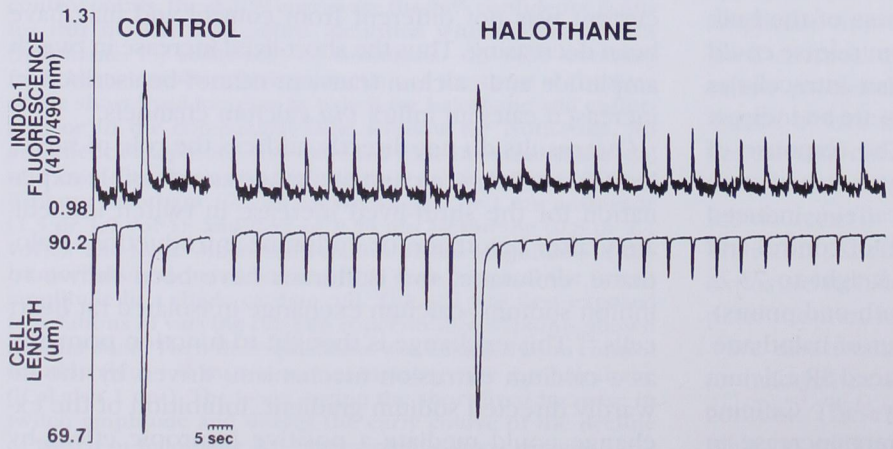


**Fig. 8. Simultaneous measurements of intracellular calcium and cell length in a cell beating at 0.25 Hz and exposed at two points to pulses of caffeine (15 mM). At the left, the caffeine pulse was applied during superfusion with control solution. In the larger (right) segment, the superfusion solution was changed to one containing 0.42 mM halothane (0.8 vol%). During the short-lived increase in twitch amplitude, the caffeine pulse was applied. In each case the caffeine pulse was delivered in lieu of the regular electrical stimulus.**

sient. Thus isoflurane (at the concentrations tested) probably lacks any such effect on SR calcium release.

These conclusions find elements of corroboration from work involving several other types of experimental preparations. A study of ryanodine binding to cardiac SR vesicles indicated that both halothane and enflurane increased binding, whereas isoflurane did not.[30] These results were understood to represent a shift of the ryanodine receptor (SR calcium-release channel) to the open state in the presence of halothane or enflurane. Our results also correspond closely with those of a recent investigation of the effects of volatile anesthetics on a planar bilayer preparation of isolated SR calcium-release channels. In this work, halothane and enflurane, but not isoflurane, increased the open probability of the SR calcium-release channel derived from porcine hearts.[31] A different study in skinned rat myocardium also showed that halothane increases calcium efflux from the sarcoplasmic reticulum *via* the calcium-release channel.[32]

However, in the skinned myocardium study,[32] the increased calcium efflux involved a mechanism other than calcium-induced calcium release, although it did occur through SR calcium channels sensitive to ruthenium red. Our study did not directly address which channel population or gating mechanism may be altered by halothane and enflurane. It does indicate that in intact and beating rat heart cells, the stimulation of SR calcium release was manifested primarily during the calcium transient associated with cardiac contraction. Because calcium-induced calcium release is the primary mode of calcium release from the SR during regular contraction, we conclude that halothane and enflurane probably stimulate calcium-induced calcium release. This conclusion must remain tentative because it is based on association rather than direct demonstration. The halothane- and enflurane-induced calcium release from the SR of quiescent rat heart tissue and cells (see fig. 7D of Herland and colleagues[32] and Wheeler and associates[16]) may involve a different mechanism than that most apparent in beating cells.

An increase in the SR calcium release with each beat can account for the short-lived increase in twitch and the eventual reduction in the SR calcium content. However, it cannot by itself explain the negative inotropic effect caused by continued exposure to the anesthetics. If enhanced SR calcium release were the only action of halothane and enflurane, the reduction in SR calcium content at steady state would be effectively offset by enhanced SR calcium release with each beat, and the resultant intracellular calcium transient would be comparable to that without anesthetic. Of course, a wide variety of other work has shown that enhanced SR calcium release is not the only volatile anesthetic effect that affects the contractility of heart cells. Mathematical models of cardiac contraction thus may prove useful in relating the various subcellular actions of the anesthetics to their negative inotropic effect at steady state. Recently, a three-compartment model of cardiac cellular calcium[33] has been applied to halothane's effects on rat atrial contractions.[34] Halothane's actions on mechanical restitution, post-extrasystolic potentiation, and decay of post-rest potentiation were modeled by changing the values of four of the six model parameters.[34] When this model was applied to the experimental situation addressed in this article, no transient positive twitch response could be produced by shifting the parameter values from control to those associated with halothane. It is important to note that one of the key assumptions of the model is that all of the calcium in the release compartment (presumably the junctional SR) is released with each beat.[33] Because our results suggest that halothane and enflurane increase the amount of calcium released from the SR without increasing its content (implying that not all of the calcium is released under control conditions), the model was modified to allow for only a portion of the release-compartment calcium to be released with each beat. As would be expected, a sudden increase in the fractional release parameter caused a transient increase in "twitch," which decayed to a steady-state value slightly less than the original. When the increase in release fraction is included with the other parameter perturbations ascribed to halothane, a temporary increase in "twitch" is produced by the model, followed by a decline in contraction amplitude to a steady-state value substantially less than control. Thus it appears that in at least one model system, a provision for increased fraction of calcium released from the release compartment is necessary to describe mathematically the short-lived increase in twitch produced by halothane and enflurane. Because the increase in SR calcium release was absent in the case of isoflurane, this difference may contribute to the lesser negative inotropic effect of that anesthetic. However, a firm conclusion must await a model that incorporates all of the varied effects of each drug on intracellular calcium metabolism.

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