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# *The Action of Halothane on Spontaneous Contractile Waves and Stimulated Contractions in Isolated Rat and Dog Heart Cells*

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**The cell length of single, isolated rat and dog heart cells was monitored during exposure to halothane-containing solution to define the cellular mechanism of halothane's negative inotropic effect. Spontaneous contractile waves, which reflect spontaneous Ca release from the sarcoplasmic reticulum (SR) in resting rat heart cells, exhibited a significant increase in frequency and a decrease in amplitude in the presence of halothane 0.27 mM (0.9 vol%) and 0.55 mM (1.7 vol%). Electrically stimulated dog and rat heart cells abruptly exposed to halothane (0.47–0.55 mM or 1.5–1.7 vol%) revealed a transient increase in twitch amplitude (significantly different from control). Twitch amplitude then declined to values significantly below control as halothane exposure continued. This decrease in twitch reached 42 ± 13% (mean ± SD) of control in rat cells and 50 ± 14% in dog cells beating at 60 beats per min. In dog cells the magnitude of the transient increase in twitch amplitude was greater at faster beating rates compared with lower rates in the same cells ($P < 0.01$) and the transient increase was insensitive to verapamil. Halothane 0.55 mM (1.7 vol%) also significantly accelerated the rate of decline in the twitch amplitude of successive beats in rat cells stimulated after a rest interval (negative staircase). The findings regarding spontaneous contractile waves indicate a direct effect of halothane at the SR in resting cells, occurring independently of any changes in the slow inward current. The halothane-induced changes in beating cells can be explained by an enhancement of Ca release from the SR with an eventual reduction of SR Ca stores. (Key words: Anesthetics, volatile: halothane, negative inotropic effect. Heart: contractility; sarcoplasmic reticulum; spontaneous Ca oscillations. Ions: calcium.)**

HALOTHANE has long been known as a potent, direct depressant of myocardial contractility,[1] an action that at times limits the clinical utility of this anesthetic. While the cellular mechanism of halothane's negative inotropic effect remains incompletely understood,[2] halothane has been found to decrease the cytoplasmic Ca transient that occurs with each beat.[3,4] Mechanisms by which such a reduction might occur include blockade of sarcolemmal Ca channels that would reduce Ca entry during the cardiac action potential or a reduction in the amount of Ca released by the sarcoplasmic reticulum (SR). Electrophysiologic studies have indeed revealed that halothane reduces Ca currents across the cardiac muscle cell membrane.[5–7] Experiments involving measurement of the force of contraction in isolated cardiac muscles under conditions designed to discriminate between SR and Ca channel effects indicate that halothane also reduces contractility through actions at the SR.[8–10] Studies using response to caffeine as an index of SR Ca stores in skinned cardiac muscle bundles[11] and in isolated cardiac cells[4,12] reveal that halothane substantially reduces SR Ca. The maximum Ca uptake in isolated cardiac SR is also decreased by halothane,[13] again suggesting a reduction of SR Ca stores. The priority and relative importance of halothane's actions at the sarcolemma (Ca channels) and at the SR have not been resolved. The nature of halothane's action at the SR, whether it involves Ca uptake and/or release or is strictly secondary to decreased transsarcolemmal Ca influx, is also incompletely understood.

The experiments reported here attempt to define halothane's action at the SR in intact and functional cells. To do so, we have used isolated heart cells from two species: the rat provides cells extremely dependent on SR Ca for excitation-contraction coupling,[14] while the dog exemplifies the more typical dual dependence on transsarcolemmal Ca and SR Ca release for activation of contraction. Because we have used many unique properties of rat heart cells in our experiments, a discussion of these attributes follows.

Unstimulated rat heart cells often exhibit spontaneous contractile waves (but not spontaneous contractions) in physiologic extracellular Ca concentrations (*e.g.*, 1 mM).[15] The heart cells of other mammals do not have waves unless exposed to much higher Ca concentrations.[16] Spontaneous contractile waves typically begin at one end of a cell and propagate along the cell's length. The waves represent Ca release from a portion of the SR, which produces localized sarcomere shortening. This local Ca release induces further Ca release from adjacent SR, and nearby sarcomeres shorten while the original region of contraction relaxes.[17] Whether the mechanism responsible for the initiation of waves is the Ca-induced Ca release channel that mediates Ca release from the SR during the action potential is uncertain.[18] However, the mechanism of wave

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propagation is likely Ca-induced Ca release. The frequency of spontaneous contractile waves is directly related to total cell Ca load;[15] that is, it may be a function of both free intracellular Ca concentration and SR Ca content. Another phenomenon unique to the rat occurs when a rat heart cell or heart muscle is stimulated after a rest period. The first beat has greater strength or amplitude than subsequent beats (negative staircase). The greater amplitude of the postrest beat is related to the loading of the SR with Ca during rest, and the negative staircase is due to an unloading or net loss of SR Ca during the first several beats following the rest interval.[19–21]

Rat heart cells thus provide several endpoints (spontaneous contractile waves, postrest contraction amplitude) with which to study the SR Ca load without disrupting the cell and without using caffeine or inhibitors. Furthermore, because spontaneous contractile waves are associated with only a 2–4-mV depolarization from the resting membrane potential,[22] they are not dependent on Ca channels, which are closed at such potentials. The experiments with dog heart cells allow us to extend some of our findings limited by the rat's dependence on SR Ca and to ensure that the results are not unique to the rat. We thus present related observations in two species that are consistent with a specific hypothesis regarding halothane's action at the SR.

## Methods

Isolated rat heart cells were obtained by methods previously described.[22] Briefly, for each experiment, a male Wistar rat was decapitated by guillotine and its heart excised. The aortic root was cannulated, and coronary perfusion was begun by gravity with a nominally Ca-free Earle's salt solution (GIBCO, Grand Island, NY; components: Na, 142 mM; K, 5.4 mM; Cl, 116 mM; $HCO_3$, 26.2 mM; $H_2PO_4$, 1 mM; $MgSO_4$, 0.8 mM; glucose, 5.6 mM). Once cleared of blood, the heart was then perfused for approximately 25 min with collagenase 0.4 g/l (Type I, Sigma Chemical Co., St. Louis, MO) in a 50-$\mu$M Ca Earle's salt solution. Perfusion was discontinued and the ventricles were minced with fine scissors, agitated by pipeting, and strained through nylon mesh. The cells were resuspended in Earle's salt solution containing 200 $\mu$M Ca and then in a 1-mM Ca Earle's salt solution. The cell suspension was then placed in plastic culture dishes and maintained in a 5% $CO_2$ incubator at 37° C while awaiting use. Cells were studied up to 6 h after their isolation. All solutions were bubbled with a 95% $O_2$/5% $CO_2$ gas mixture.

Dog heart cells were obtained in similar fashion, although only a portion of the left ventricular wall was perfused with enzyme as opposed to whole heart perfusion in the rat. Beagle dogs were anesthetized with pentobarbital 25 mg/kg iv. A left thoracotomy was performed and the heart rapidly excised. A portion of the left ventricular free wall supplied by the left anterior descending artery was excised (about 3 g tissue). The artery was then cannulated and perfusion with Ca-free Earle's salt solution was begun. A curved clamp was used to occlude much of the cut surface of the tissue and prevent loss of perfusate from arterial branches. Perfusion with collagenase in 50 $\mu$M Ca Earle's salt solution was then maintained for approximately 40 min. After a brief, final perfusion with 200 $\mu$M Ca Earle's salts to clear the collagenase, the tissue was minced in this latter solution and subsequently handled as per the rat procedure.

Cell length was measured by video dimension analysis during superfusion under controlled conditions. A culture dish containing cells was placed on the stage of an inverted microscope. The dish was perfused with a balanced salt solution containing Na, 137 mM; K, 5 mM; Cl, 144 mM; Ca, 1 mM for rat cells; 2 mM for dog cells; Mg 1.2 mM; $SO_4$, 1.2 mM; HEPES, 20 mM; $p$H adjusted to 7.4. Temperature of the perfusate in the dish was controlled to 36.5 ± 1° C. Single, rod-shaped cells were selected for observation, and their image was monitored with a video camera. Video edge tracking markers were set at the ends of the cell and the distance between them continuously recorded. Electrical stimulation was provided by delivering square wave pulses to platinum wire electrodes at opposite sides of the culture dish. Typical pulse duration was 5 ms and amplitude was set approximately 20% above the threshold for capture.

In one set of experiments, fluorescent signals related to the free intracellular Ca concentration ($Ca_i$) were measured simultaneously with the cell length of single rat cardiac cells as described elsewhere.[23] Briefly, the rat heart cells used for these experiments were loaded with the Ca-sensitive dye indo-1 during an 8-min room temperature incubation with indo-1 acetoxymethyl ester (25 $\mu$M), fetal calf serum (4.5%), and Pluronic-127 (0.1%, BASF, Wyandotte, MI) in an Earle's salt solution. Cells were then resuspended in the HEPES-buffered balanced salt solution described in the preceding paragraph and stored for at least 45 min to allow for intracellular hydrolysis of the indo-1 acetoxymethyl ester. An aliquot of indo-1 loaded cells was then placed in a glass-bottom chamber and allowed to attach to it (5–10 min). The chamber was then perfused with the balanced salt solution and a single cell was selected for observation through an inverted stage Zeiss microscope. Cell length was continuously measured from the bright-field image projected onto a photodiode array. In addition to the visible red light source used to obtain the image for cell length, the cell was excited by light at 350 nm (strobe-driven xenon arc lamp with interference filter). Fluorescent light emitted by the cell at both 490 and 410 nm was collected and the ratio of the

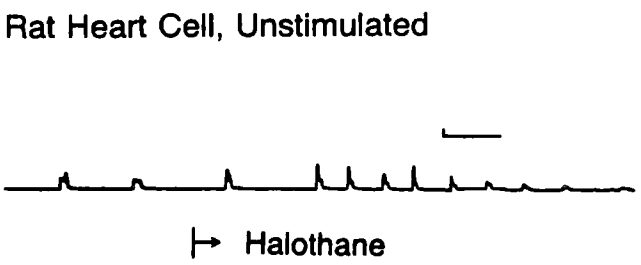


FIG. 1. Cell length of a single unstimulated rat heart cell. Cell shortening is upward. The deflections in the trace represent spontaneous contractile waves. At the time indicated, the superfusion solution was changed to one containing halothane 0.55 mM (1.7 vol%). In this and most subsequent figures, there is a time delay of approximately 7 s between the indicated solution change (valve rotation) and the beginning of the change in solution composition in the culture dish. Calibration bars: 5-s horizontal, 1-$\mu$m vertical.

two signals obtained. This ratio of the indo-1 signals was then used as the index of $Ca_i$.

Halothane was dissolved in aqueous solution, either through mixing of the liquids (and allowance of approximately 1 h for equilibration), or by bubbling the perfusion solution using a calibrated vaporizer. The halothane concentrations at the cells were determined by sampling the solution in the perfused culture dish and determining the halothane concentrations by gas chromatography.

In all of the experiments reported here, each cell served as its own control. Protocols began with superfusion by control solution, then a switch to halothane-containing solution was made, followed by a return to control. Most endpoints measured in halothane were compared with the average of that endpoint in the bracketing controls. Statistical significance was determined by paired $t$ test if one concentration of halothane was involved or by one way analysis of variance and least significant difference tests where two concentrations of halothane were reported. In the latter instance the Bonferroni method was used to correct the critical $t$ value for the two simultaneous comparisons to control. For spontaneous contractile wave properties, the data compared were taken immediately before a solution change and over a comparable time interval beginning 2 min after the solution change. Pairs taken from control-to-halothane transitions and halothane-to-control transitions were pooled, and significance was determined by paired $t$ test. Where required, exponential curve fitting was performed by linear regression of the logarithm of the dependent variable. Comparison of regression line slopes to zero was made by computing the F value from the regression analysis. In the text, results are given as mean ± SD; differences are considered significant at $P < 0.05$.

The rat protocol was approved by both the Animal Care and Use Committee of the Johns Hopkins University School of Medicine and that of the Gerontology Research Center. The dog protocol was approved by the Animal Care and Use Committee of the Gerontology Research Center. The killing of the rats without prior anesthesia was deemed acceptable given the potential alteration of myocardial Ca metabolism by all anesthetics and the speed and reliability of the method of killing.

## Results

When unstimulated rat heart cells were exposed to halothane at concentrations of 0.27 mM (0.9 vol%) and 0.55 mM (1.7 vol%), the characteristics of their spontaneous contractile waves were altered. In many cells, a transient burst of waves occurred upon introduction of halothane (fig. 1). The wave amplitude, (*i.e.*, the cell shortening associated with each wave) was qualitatively similar during the halothane-induced bursts to that in control solution. The wave amplitude then declined as the halothane superfusion was continued. After 2 min of halothane exposure the characteristics of the spontaneous

TABLE 1. Effect of Halothane on Spontaneous Contractile Waves and Stimulated Twitches in Isolated Rat Heart Cells

| | Interwave Period (s) | Wave Amplitude* ($\mu$m) | Twitch Amplitude: Postrest Beat† (% rest length) | Twitch Amplitude at 60 Beats per Min† (% rest length) |
|---|---|---|---|---|
| Control | 18 ± 11 (24) | 0.62 ± 0.19 (24) | 7.5 ± 2.6 (20) | 2.1 ± 1.7 (24) |
| Halothane 0.27 mM‡ (0.9 vol%) | | | | |
| Non-normalized | 15 ± 10 (24) | 0.50 ± 0.16 (24) | 6.2 ± 2.1 (11) | 1.2 ± 0.9 (12) |
| Percent of paired control | 85 ± 14 (24)¶ | 80 ± 14 (24)¶ | 80 ± 16 (11)** | 62 ± 19 (12)¶ |
| Control | 13 ± 7 (16) | 0.54 ± 0.21 (14) | 6.9 ± 1.8 (16) | 1.8 ± 1 (16) |
| Halothane 0.55 mM§ (1.7 vol%) | | | | |
| Non-normalized | 11 ± 5 (16) | 0.20 ± 0.12 (14) | 2.5 ± 1.7 (9) | 0.7 ± 0.8 (9) |
| Percent of paired control | 84 ± 16 (16)¶ | 39 ± 20 (14)¶ | 39 ± 24 (9)¶ | 31 ± 17 (9)†† |

All figures are mean ± SD with the number in parentheses the number of observations or comparisons.

* Measured to "plateau" of wave, typically at its midpoint.

† Twitch amplitudes are measured as the cell shortening during a beat and expressed as a percentage of the cell length prior to contraction.

‡ Concentration inferred from gas chromatographic measurement at twice this halothane dose.

§ Concentration determined by gas chromatography of samples taken from length measurement chamber.

¶ $P < 0.01$ compared with control.

** $P < 0.05$ compared with control.

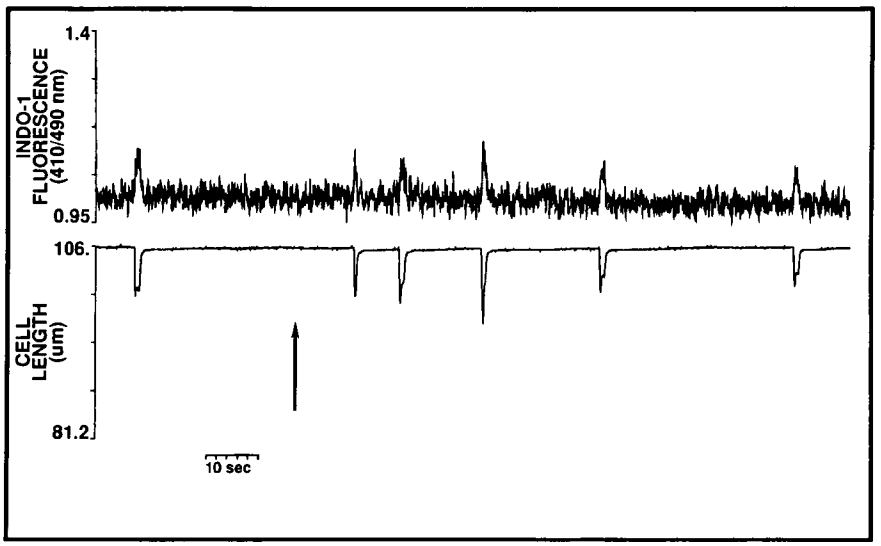


FIG. 2. Simultaneous recording of indo-1 fluorescence ratio (*upper trace*) and cell length (*lower trace*) in a single rat heart cell. At the arrow, perfusate containing halothane 0.65 mM (1.2 vol%) reaches the cell chamber. Cell shortening is downward. The deflections represent spontaneous contractile waves. Calibration bar: 10 s. Temperature: 22° C.

waves remained stable. At this steady-state in the two concentrations of halothane noted above, the wave frequency was increased compared with that in control solution and the cell shortening associated with each wave was decreased (table 1). The resting length (that between waves) of these unstimulated cells was unchanged by halothane. To determine whether the intracellular free Ca concentration ($Ca_i$) remained constant during the exposure of unstimulated heart cells to halothane, indo-1 fluorescent signals were monitored in several cells. The ratio of indo-1 fluorescence increased during spontaneous contractile waves, but there was no discernable change in the baseline $Ca_i$ between waves in six of six unstimulated cells when halothane (0.60–0.65 mM or 1.1–1.2 vol% at room temperature) was introduced (fig. 2).

If the switch to halothane-containing solution (0.47–0.55 mM or 1.5–1.7 vol%) was made while rat or dog heart cells were stimulated to beat, a transient increase in the extent of shortening with each beat (*i.e.*, twitch amplitude) was regularly observed (fig. 3 and table 2). At a halothane concentration approximately half of those cited above, a transient increase in twitch amplitude was also observed in cells from both species. Sufficient replications were not performed at this lower concentration for quantitative analysis. After the transient increase, the twitch amplitude decayed to values below control, as would be expected in halothane. Upon reaching a steady-state at 60 beats per min, rat cells had 42 ± 13% of control twitch amplitude in halothane 0.55 mM (1.7 vol%) and dog cells 50 ± 14% of control in halothane 0.47 mM (1.5 vol%). In some rat cells stimulated at relatively low rates (≤30 beats per min), spontaneous contractile waves occurred between stimulated beats immediately following the switch into halothane 0.55 mM or 1.7 vol% (fig. 4). The switch into halothane 0.47 mM (1.5 vol%) produced a spontaneous contractile wave between stimulated beats even in several dog heart cells, which did not exhibit waves at any other time in the protocol. When these interbeat waves appeared in either rat or dog cells, the transient increase in twitch amplitude was either absent or aborted.

The prior addition of verapamil in concentration sufficient to decrease extent of shortening approximately

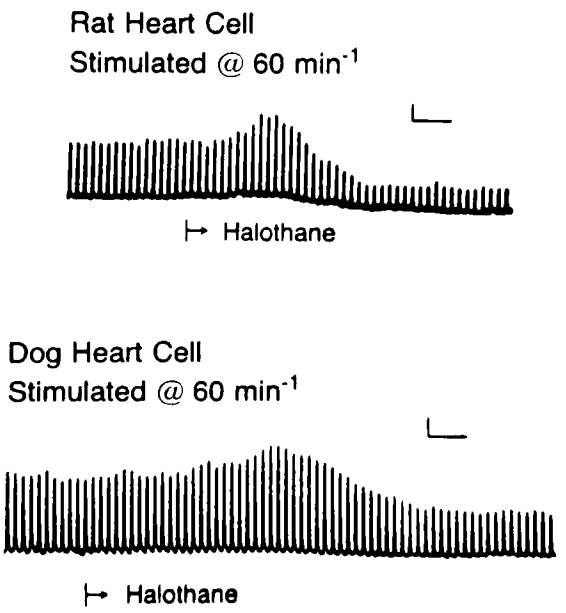


FIG. 3. Cell length tracings of a single rat heart cell (*upper trace*) and a single dog heart cell (*lower trace*) stimulated at 60 beats per min. The upward deflections represent the cell shortening with each contraction. At the time indicated, the superfusion solution was changed to one containing halothane (0.55 mM or 1.7 vol%: *upper trace;* 0.47 mM or 1.5 vol%: *lower trace*). Calibration bars: 5-s horizontal, 1-μm vertical.

TABLE 2. Twitch Amplitude Before and After Introduction of Halothane (0.47–0.55 mM or 1.5–1.7 vol%) in Cells Beating at 60 Beats per Minute

| Species | n | Control Twitch Amplitude | | Twitch Amplitude in Halothane | |
|---|---|---|---|---|---|
| | | Averaged | Maximum | Peak | Steady-State |
| Rat | 10 | 4.2 ± 3.3 | 4.4 ± 3.3 | 5.3 ± 3.6* | 1.9 ± 1.8† |
| Dog | 12 | 3.5 ± 1.3 | 4.1 ± 1.4 | 5.5 ± 2.5* | 1.8 ± 1† |

All values mean ± SD.

Twitch amplitudes are expressed as percent of diastolic length. The maximum control twitch amplitude reflects the largest twitch found in a sample of 30 beats prior to the introduction of halothane.

* Significantly different ($P < 0.05$) from maximum control twitch by paired $t$ test.

† Significantly different ($P < 0.01$) from averaged control twitch by paired $t$ test.

50% from control (range: 0.3–2 μM) in two rat cells and five dog cells did not inhibit the transient increase in twitch amplitude when halothane-containing solution (which also included verapamil) was introduced (fig. 5). At a rate of 60 beats per min, dog cells switched from control solution to halothane 0.47 mM (1.5 vol%) developed a peak increase in twitch amplitude of 155 ± 32% of control (n = 12). In the presence of verapamil, the peak twitch amplitude was 154 ± 35% of the prehalothane value (n = 5). Differences in the peak increase of twitch amplitude due to halothane with and without verapamil were also nonsignificant when only the four cells with pairable observations were analyzed. Change of solutions from control to control or from control to verapamil did not produce increases in extent of shortening.

The magnitude of the transient increase in extent of shortening with each beat and its onset and rate of decay were reproducible over different transitions into halothane in the same cell stimulated at the same rate. To study the effects of beating rate on this transient increase, four dog cells were switched repeatedly into halothane-containing solution with a different stimulation rate for each switch. A transient increase in twitch amplitude occurred at all frequencies studied (range: 6–240 per min; fig. 6). The maximum twitch amplitude during the transient (expressed as a percentage of control twitch amplitude prior to the transient) bore a significant relationship to beating rate, with maximum twitch decreasing as interbeat interval decreased (fig. 7).

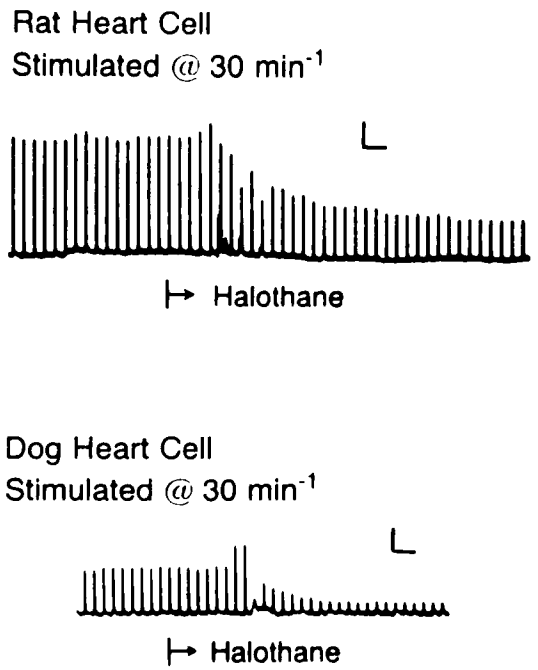


FIG. 4. Cell length tracings of a single rat heart cell (*upper trace*) and a single dog heart cell (*lower trace*). Cell shortening is upward. Each cell was stimulated at 30 beats per min. At the time indicated halothane was introduced (0.55 mM or 1.7 vol%: upper trace; 0.47 mM or 1.5 vol%: lower). The broad upward deflections are spontaneous contractile waves. Calibration bars: 5-s horizontal, 1-μm vertical.

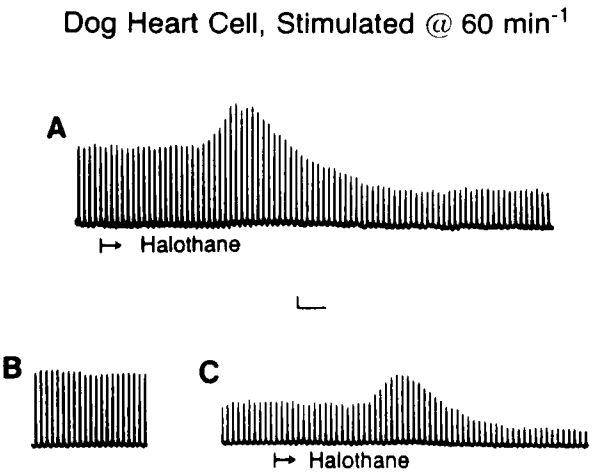


FIG. 5. Cell length tracings from the same dog heart cell stimulated at 60 beats per min. In trace *A*, the superfusion solution was changed to one containing halothane 0.47 mM (1.5 vol%) at the time indicated. The solution was changed back to control between the times of panels *A* and *B*. Between panels *B* and *C*, verapamil 1 μM was added to the superfusion solution. In panel *C*, halothane was reintroduced in the presence of verapamil at the time indicated. Calibration bars: 5-s horizontal, 1-μm vertical.

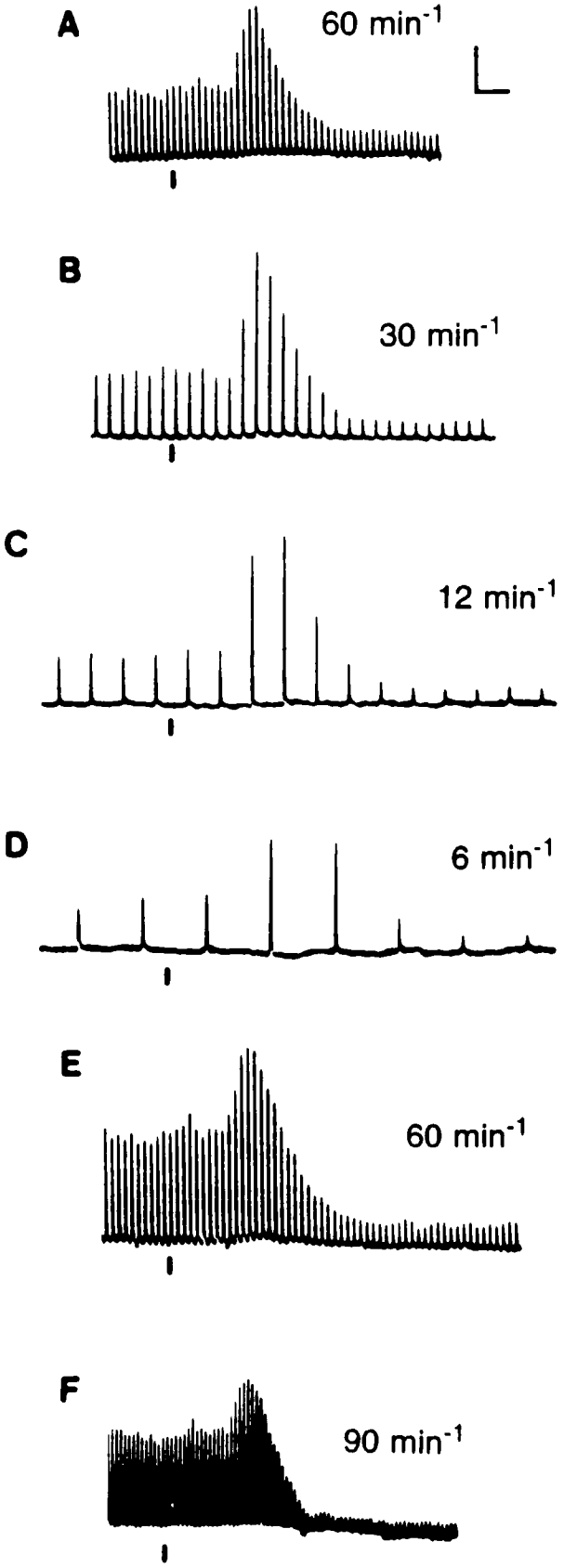


FIG. 6. Cell length tracings of the same dog heart cell stimulated at various rates. The superfusion solution was switched to one containing halothane 0.47 mM (1.5 vol%) at the times indicated by the short vertical bars. A return to control solution occurred between each panel. Calibration bars: 5-s horizontal, 1-$\mu$m vertical.

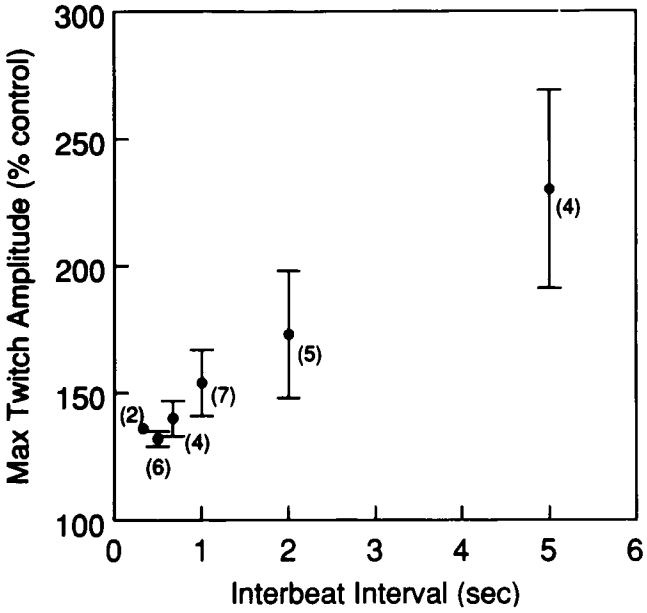


FIG. 7. The maximum twitch amplitude after exposure of beating dog heart cells to halothane 0.47 mM (1.5 vol%) plotted against the interbeat interval. Maximum twitch amplitude is expressed as a percentage of the twitch amplitude immediately before halothane exposure. The data was obtained from four dog heart cells, each stimulated at various rates during the switch into halothane-containing solution. The numbers in parenthesis indicate the number of transitions into halothane used to generate each point. Error bars reflect SEM. The regression line through the data points has a slope of 21 (% units) per s. The regression is significant at $P < 0.01$. The regression is also a significant if frequency is used in place of interbeat interval as the independent variable.

The diastolic length of beating cells did not change significantly from control at the time of the transient increase in twitch amplitude induced by halothane. Some cells shortened slightly at this time, but the change was not sufficiently large or consistent to produce a significant result. As twitch amplitude decreased during continued halothane exposure (0.47–0.55 mM or 1.5–1.7 vol%), the diastolic length of both rat and dog heart cells stimulated at 60 beats per min increased. For rat cells the increase was $0.43 \pm 0.55\%$ ($n = 10$; $P < 0.05$) of baseline diastolic length, and for dog cells the diastolic length increased $0.16 \pm 0.15\%$ ($n = 10$; $P < 0.05$).

The negative staircase of twitch amplitude, which occurs when a rat cell is stimulated after a period of rest, is also altered by halothane (fig. 8 and table 3). Halothane

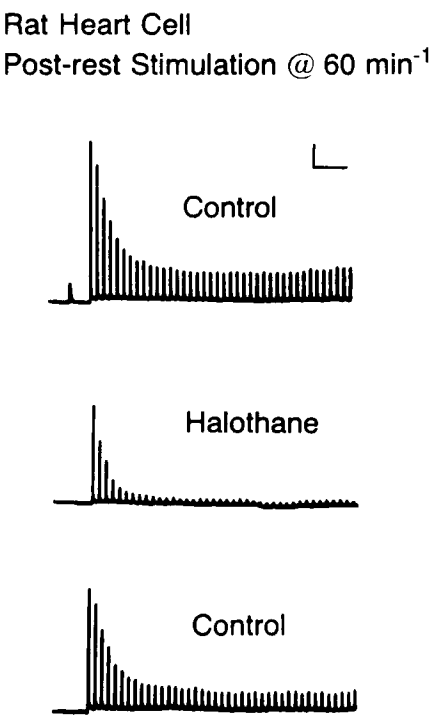


FIG. 8. Cell length tracing of the same rat heart cell in control solution (*upper trace*), halothane (0.55 mM or 1.7 vol%; *middle trace*) and control solution (*lower trace*). In each case, electrical stimulation (at 60 beats per min) was begun after a period of rest, resulting in a negative staircase response. The half times of the exponential curves derived from the first five beats of each train are 2 s (*top*), 1.5 s (*middle*), and 2 s (*bottom*). Calibration bars: 5-s horizontal, 1-μm vertical.

depressed the extent of shortening of both the first beat in the train of stimuli (rested state contraction) and the beat that exhibits the least extent of shortening (here referred to as the nadir beat). Halothane also increased the rate at which twitch amplitude decays from the rested state beat to the nadir beat in the train. To quantitate this rate of fall within a negative staircase, each staircase was fit to an exponential function that decayed to a constant. Nearly all negative staircases in both control and halothane-containing solution were readily fit by such a function. Halothane (0.55 mM or 1.7 vol%) caused a significant decrease in the half time of the decay in cells stimulated at 60 beats per min (table 3). There was a trend toward decreased half time in halothane 0.27 mM (0.9 vol%). These results indicate that halothane hastened the decline of twitch amplitude toward the nadir beat.

## Discussion

The effect of halothane in concentrations of 0.27 mM (0.9 vol%) and 0.55 mM (1.7 vol%) on spontaneous contractile waves in unstimulated rat heart cells indicates that such concentrations of halothane act at the SR independently of any change in sarcolemmal Ca channels which are not active in these resting cells. The lack of change in the cell length between waves suggests that there is no significant halothane-induced change in the baseline free intracellular calcium concentration ($Ca_i$) in resting cells. The absence of an indo-1 fluorescence change (other than increases during spontaneous waves) in unstimulated cells exposed to halothane provides more direct evidence that resting $Ca_i$ does not change. We did not find evidence of a sustained increase in $Ca_i$ as has been recently reported by Katsuoka *et al.* in beating heart cell suspensions.[4] The decrease in wave amplitude at steady state in halothane suggests that the amount of Ca released from the SR during each wave is reduced and is consistent with a reduction of SR Ca stores, a conclusion also reached based on responses to caffeine in other preparations. It should be noted that caffeine, which causes Ca release from the SR, also results in an increased wave frequency and decreased amplitude.[16] If in the presence of halothane there is no change in cytoplasmic Ca and if SR Ca is reduced, then the increase in wave frequency in the presence of halothane indicates that there is an increase in the sensitivity of the spontaneous Ca-release mechanism to total cell Ca, the quantity most closely related to wave frequency.[15] Alternatively stated, the minimum Ca concentration required to trigger this mechanism may be reduced by halothane.

The present results in unstimulated single rat cells must be correlated with previous measurements of cytoplasmic Ca in suspensions of unstimulated rat heart cells. In cell suspensions, halothane induced a transient increase in cytoplasmic Ca due to loss of Ca from the SR.[12] Thus, an apparent discrepancy exists between the transient increase in cytoplasmic Ca in cell suspensions and the constant rest length and baseline indo-1 fluorescence of single cells. A possible resolution follows from the observation that cytoplasmic Ca is increased during a spontaneous contractive wave (fig. 2).[16] The Ca concentrations of the individual

TABLE 3. Half Times of the Decay of the Twitch Amplitude of Successive Beats during Postrest Stimulation at 60 Beats per Minute

| | Half Time (s) | n |
|---|---|---|
| Control | 2.5 ± 0.5 | 11 |
| Halothane (0.27 mM or 0.9 vol%)* | 2 ± 0.5 | 7 |
| Percent of paired control | 85 ± 10 | 7 |
| Control | 1.9 ± 0.6 | 23 |
| Halothane (0.55 mM or 1.7 vol%)† | 1.3 ± 0.7 | 14 |
| Percent of paired control | 71 ± 34‡ | 14 |

* Concentration inferred from gas chromatographic measurement at twice this halothane dose.

† Concentration determined by gas chromatography of samples taken from length measurement chamber.

‡ $P < 0.05$ compared with control.

cells in suspension are averaged by the measurement technique so that a population of cells experiencing a burst of spontaneous contractile waves will appear to have an increase in Ca concentration. Thus, the ability of halothane to produce bursts of waves can account for the transient increase in average cytoplasmic Ca concentration of cells in suspension and is consistent with maintenance of the normal cell length between waves.

The decrease in rat cell rested state contraction amplitude caused by halothane is in agreement with data recently presented by Lynch and Frazer[10] and provides additional evidence for a reduction in SR Ca stores at rest. While results from a variety of preparations support this conclusion, several alternative explanations should be addressed. First, halothane may render the myofilaments less sensitive to Ca and thus reduce the amplitude of rested state contractions, spontaneous contractile waves, and caffeine-induced contractures. Such an effect of halothane does appear to occur[24,25] but may be of relatively small magnitude at clinical halothane concentrations. More importantly though, myofilament changes cannot account for observation that halothane, even at low concentrations, reduces maximal caffeine-induced Ca release—an index of SR Ca content independent of myofilament response.[12] A second alternative is that halothane directly inhibits various forms of Ca release from the SR but does not alter SR Ca stores. This proposal cannot, however, explain the reduction of maximum Ca uptake by halothane in isolated cardiac SR.[13] Neither is it consistent with the ability of halothane itself to cause a cytoplasmic Ca transient which is apparently related to spontaneous Ca release from the SR.[12] The tendency of halothane to produce bursts of spontaneous contractile waves and to increase wave frequency suggests increased Ca release rather than a decrease. The halothane-induced increase in the rate of decay of the potentiated state in rabbit papillary muscle[8] would also be difficult to explain if halothane directly inhibited SR Ca release. Thus, it is most likely that in resting cells, halothane decreases SR Ca content and must do so independently of any changes in sarcolemmal Ca channels.

The consistent transient increase in twitch amplitude found when beating rat and dog heart cells were exposed to halothane (0.47–0.55 mM) confirms reports of similar phenomena in dog Purkinje fibers[26] and guinea pig papillary muscle.[5] This transient increase in twitch appears to require an abrupt exposure to halothane. Cells located distant from the solution inlet on our culture dishes tended to have a less-prominent, and occasionally absent, transient increase in twitch amplitude. In such locations the change in halothane concentration would be expected to be less abrupt as compared with areas near the solution inlet. The need for rapid exposure to halothane may explain why transient increases in contractility are not seen in all cardiac preparations exposed to halothane. In contrast to single isolated cells, all portions of relatively large intact muscle preparations would not be simultaneously exposed to halothane, and the halothane concentration change in the vicinity of interior cells would be gradual due to the limitations of diffusion. Thus, in such preparations the transient increase may not appear due to lack of a sufficiently rapid rise in halothane concentration at the sarcolemmae and/or to a temporal dispersion of transient increases leading to an undetectable increase in total muscle contractility. Given that the transient increase of twitch amplitude by halothane is a reproducible phenomenon now seen in different preparations in several species, significant constraints are placed on possible mechanisms to explain halothane's negative inotropic effect. That is, any such mechanism must be able to account for transient positive inotropy as well as sustained negative inotropy.

What mechanisms might then account for the observed behavior? The absence of significant change in diastolic length during the transient increase in twitch amplitude suggests that a tonic increase in cytoplasmic Ca concentration is not involved. The insensitivity of the transient to verapamil tends to indicate that sarcolemmal Ca channels are not a factor. Because the magnitude of the transient in dog cells increases at lower beating rates, at which rates twitch amplitude is more SR dependent, the SR is probably important in the process. This conclusion is also supported by the observation that spontaneous contractile waves, which are related to Ca release from the SR, could abort the transient. The SR could account for transient positive inotropy if, as suggested also by Luk *et al.*,[26] halothane enhances the release of Ca from the SR with each beat, *i.e.*, Ca-induced Ca release. At the time halothane is first introduced to the cells, the SR stores are normal and thus enhanced Ca release would produce a greater Ca transient and an increased contraction. The SR ultimately becomes depleted of Ca with continued halothane exposure and even enhanced Ca release would produce a Ca transient less than control, as observed by Bosnjak and Kampine[3] and Katsuoka *et al.*[4] The enhancement of SR Ca release could reflect a decreased threshold for Ca-induced Ca release or an increase in Ca flux at the same level of triggering Ca. The former possibility is suggested by analogy to spontaneous Ca release but we cannot distinguish these mechanisms with the present data.

The eventual net loss of SR Ca with continued halothane exposure in beating cells may be related to enhanced release of Ca from the SR or to decreased uptake. The transient increase in twitch amplitude as explained above suggests that increased Ca-induced Ca release with each

beat may account for some of the loss. Spontaneous Ca release from the SR may also be a factor as evidenced by the occurrence of spontaneous contractile waves between beats in some cells exposed to halothane. Additionally, an increase in a Ca leak from the SR, which cannot be directly detected by the methods reported here, could be caused by halothane. A decrease in Ca uptake into the SR could occur secondary to a decrease in the Ca influx into the cell *via* the slow inward current. Also, a direct inhibition of the SR Ca pump by halothane could cause the net loss of SR Ca but this appears unlikely at clinical halothane concentrations based on measurements in isolated SR.[13,27] It is also possible that the volume of the SR could be reduced by halothane, leading to decreased Ca storage and release not necessarily dependent on Ca transport mechanisms.

Halothane's acceleration of the negative staircase of rat heart cells is consistent with the results and interpretation described above. The more rapid decline in twitch amplitude during the negative staircase likely relates to increased net loss of Ca from the SR with each beat, the possible causes for which are the same as those discussed in relation to the development of halothane's negative inotropic effect. The effect on the negative staircase reinforces the point that halothane has the potential to enhance SR Ca release (relative to its Ca load) even when the SR is already partially depleted.

In summary, the present results point toward a direct action of halothane at the SR. The probable mode of action of halothane at the SR is an enhancement of Ca release that contributes to an ultimate reduction in SR Ca stores. This reduction occurs both in resting cells, in which changes in voltage-sensitive Ca currents should not be significant, and in beating cells, in which reduced Ca influx into the cell may play a role. In stimulated cells, the enhancement of Ca release from the SR occurs even in the face of a presumed decrease in Ca current, the trigger for Ca-induced Ca release from the SR. That is, the hypothesis regarding the transient increase in contractility requires that the enhancement of SR Ca release override a decrease in the triggering Ca arriving *via* the Ca channels. At steady-state in the presence of halothane, reduction of the SR Ca load overrides the effect of enhanced Ca-induced Ca release.

Definitive resolution of the relative priority of sarcolemmal and SR actions will require simultaneous direct measurement of the two sites.

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