Depressant Effects of Volatile Anesthetics upon Rat and Amphibian Ventricular Myocardium: Insights into Anesthetic Mechanisms of Action

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To clarify the mechanisms by which volatile anesthetics may depress myocardial contractility, the depressant effects of equivalent concentrations of isoflurane, enfurane and halothane were compared in rat and frog ventricular myocardium, preparations which differ markedly in excitation-contraction coupling. In Tyrode solution, right ventricular papillary muscles from rat exhibited very large, rapidly developing contractions after rest, with a subsequent negative force-frequency relation as the stimulation rate was increased to 0.1, 0.25, 0.5, 1, 2, and 3 Hz. The large contractions after rest and at 0.1 Hz were depressed by 0.75% halothane and 1.7% enfurane to about 60% of control, but less so by 1.3% isoflurane (~0.8 MAC). Halothane at 1.5% was more depressant than 2.5% isoflurane at all stimulation rates, while 3.5% enfurane caused intermediate depression (~1.6 MAC). Contractions in frog ventricular strips were studied in Ringer solution following rest and at stimulation rates of 0.1, 0.25, 0.5, and 1 Hz, in the absence and presence of equivalent anesthetic concentrations. At 0.1 to 1 Hz, isoflurane was less depressant than equivalent concentrations of halothane. Enfurane (1.7%) was less depressant than 0.75% halothane at 0.1 and 0.25 Hz; 3.5% enfurane was more depressant than 2.5% isoflurane at 1 Hz. Anesthetic effects on sustained contractions were also studied in frog ventricular strips that were superfused for 4–5 min with 40, 60, 80, and 100 mM K Ringer solution. Contractions induced by 80 and 100 mM K solution were depressed more by halothane (to 60% of control) than by isoflurane or enfurane (approximately 85% of control). However, only enfurane depressed the contractions at 1 Hz more than the sustained contractions in 100 mM K Ringer. The Ca²⁺ for activating contractions in rat ventricle is derived largely from the sarcoplasmic reticulum, the intracellular Ca²⁺ accumulation and release organelle. In contrast, Ca²⁺ for activating contractions in the frog ventricle originates primarily from the external medium. These results suggest that halothane is more potent than isoflurane in reducing the amount of Ca²⁺ rapidly released from the sarcoplasmic reticulum (as observed in rat), as well as in depressing entry of extracellular Ca²⁺ to activate myofibrils (as in frog). Enfurane appears to have intermediate potency with actions distinct from halothane and isoflurane. The greater potency of halothane may also be due in part to greater direct depression of actin-myosin ATPase. (Key words: Anesthetics; volatile; enfurane; halothane; isoflurane. Animal: frog; rat. Heart: force-frequency relation; myocardial depression.)

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Received from the Department of Anesthesiology, University of Virginia Medical Center, Charlottesville, Virginia. Accepted for publication October 31, 1988. Supported by NIH Grant R01-GM31144. Presented in part at the Workshop on Subcellular Mechanisms of Anesthetics in Muscle, Augusta, Georgia, September 22–24, 1988.

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STUDIES EMPLOYING a variety of experimental techniques have shown that the volatile anesthetics depress cardiac contractility by several different mechanisms. The volatile anesthetics can depress ATPase activity of the myofibrils (actin and myosin), but at concentrations which correspond to very high or excessive clinical levels. The volatile anesthetics appear to interfere with excitation-contraction coupling in myocardium by reducing the rapid increase in intracellular [Ca²⁺], which is initiated by depolarization and which activates the myofibrils to produce tension. Electrophysiologic studies suggest that the volatile anesthetics depress slow channel mediated Ca²⁺ flux into the myocyte, with halothane being more potent than isoflurane. In skinned fibers, Ca²⁺ release from the sarcoplasmic reticulum has also been shown to be depressed by the volatile anesthetics, with halothane again being more potent than isoflurane. Finally, the overall effect of the volatile anesthetics in isolated sarcoplasmic reticulum vesicles prepared from cardiac tissue is to decrease the maximal amount of Ca²⁺ accumulated.

The present study was undertaken to define how altered Ca²⁺ entry or altered sarcoplasmic reticulum function may contribute to the depressant actions of halothane, isoflurane, and enfurane in cardiac tissue. For this study, the markedly different mechanisms of excitation-contraction coupling exhibited by rat and frog were exploited to elucidate anesthetic mechanisms. The sarcoplasmic reticulum in rat myocardium is very highly developed. It shows the highest sensitivity for Ca-induced Ca release from the sarcoplasmic reticulum, and appears to be the nearly exclusive source for activator Ca. During rest, little Ca²⁺ is lost from ventricular cells. Therefore, following a period of rest, a substantial internal store of Ca²⁺ is available and its release accounts for a very large rested state contraction upon depolarization. In contrast to mammalian tissues, the frog ventricular tissue is notable for extremely sparse sarcoplasmic reticulum, which contributes little or no Ca²⁺ to activate tension development under most circumstances. When depolarized, Ca²⁺ for frog myocardial tension development arises from the extracellular milieu. Comparison of contractile depression caused by halothane, isoflurane, and enfurane on these divergent ventricular muscle types can provide insight into how these anesthetics depress contractility in myocardial tissue from human and other mammalian species.
Materials and Methods

RAT MYOCARDIUM EXPERIMENTS

The techniques employed to study rat ventricular myocardium were those previously employed in guinea pig. After the rats were anesthetized with diethyl ether, each heart was removed and right ventricular papillary muscles were excised and superfused at 37°C with modified Tyrode solution (composition in mM: Na, 143; K, 4.7; Cl, 128; Ca, 2.5; Mg, 2.0; SO4, 2.0; HCO3, 25; glucose, 11; EDTA, 0.1), which had a measured free Ca2+ activity of 1.30 mM (Nova 2 ionanalyzer, Nova Biomedical, Inc.). Solution circulated through the chamber (8 ml·min−1) from heated reservoir containers through which 95% O2/5% CO2 was bubbled, maintaining pH at 7.45 ± 0.5. To elicit a propagated action potential, muscles were field stimulated with a 0.5–1 msec voltage step approximately 10% above threshold. Isometric contraction tension was measured at the minimum muscle length and rest tension which developed maximum active tension. Preparations were equilibrated for 1 h in the chamber with intermittent short periods of stimulation at 0.1 to 3 Hz to verify muscle integrity and define performance.

At the termination of each experiment, the cross-sectional area (CSA) of each muscle was estimated from the weight, length, and muscle density (1.04 g · ml−1 measured value).

The following protocol of stimulation was employed: after 10–15 min rest, a rested state (RS) contraction was elicited, followed sequentially by stimulation rates of 0.1, 0.25, 0.5, 1.0, 2.0, and 3.0 Hz. A period of rest of at least 10 min was required to produce an unchanging rest response. Following rest, rat ventricular muscle shows extremely rapid tension development with substantial peak tension, as shown in figure 1A (tracing RS). With each increase in stimulation frequency, peak tension decreased over 30 s to a stable and unchanging response, demonstrating a negative force-frequency relation (fig. 1A). Figure 1B characterizes the peak tension and the maximum rate of tension development (dT/dt-max) of rat papillary muscle standardized for CSA as a function of the stimulation rate. The force-frequency relation is similar to that previously reported for this [Ca2+]i. Higher, more physiologic stimulation rates were not employed due to the potential for hypoxia in the core of these superfused preparations.

Following control measurements, muscles were exposed to an anesthetic equilibrated solution for 20 min.
prior to the identical stimulation protocol, which was then repeated again after 20–30 min in anesthetic free solution. In spite of the prior 1-h equilibration period, rat papillary muscle tended to show a decline in developed tension, which was present at all stimulation rates, but largest for the rested state contraction. The typical recovery of contractile performance following wash-out of anesthetic was to about 80% of control, and appeared to represent a previously described decline in the muscle performance, rather than any continuing anesthetic effect. Nine of 29 muscles were exposed to a second anesthetic following recovery from the first anesthetic; in the remainder of cases, the experiment was terminated following one anesthetic exposure.

In contrast to rat ventricular myocardium, other mammalian species employed in studies of anesthetics, such as guinea pig and rabbit, show a positive force-frequency relation. That is, there are increases in developed tension and dT/dt-max with increasing stimulation rate. For comparison with rat, the superimposed tension tracings at various stimulation rates and the average observed guinea pig force-frequency relation for peak tension and dT/dt-max are shown in figure 1A and B (from experiments in ref 6).

**Frog Myocardium Experiments**

Frog hearts were removed after the frogs (*Rana pipiens*) had been sedated with methohexital and pithed. The ventricle was sliced into longitudinal strips (1–2 mm wide), which were placed in a chamber (volume = 0.8 ml) and superfused with Ringer solution (composition in mM: Na, 100; K, 3.0; Cl, 80; Ca, 1; Mg, 1.2; SO₄, 1.2; HCO₃, 25; glucose, 2.5) equilibrated with 95% O₂-5% CO₂, which had a measured free Ca²⁺ activity of 0.82 mM. Experiments were performed at constant room temperature (20–22° C). For each muscle strip, the cross-sectional area (CSA) was estimated from the weight, length, and muscle density (1.04 g·ml⁻¹ measured value).

Both force-frequency (A) and potassium contracture studies (B) were performed on the frog ventricular muscle. **Force-frequency Studies.** After 20 min rest, a rested state contraction was elicited, followed by stimulation at rates of 0.1, 0.25, 0.5, and 1 Hz. At each stimulation rate, a steady-state contractile response was achieved before proceeding to the next higher rate. Frog ventricular strips showed a modest positive force-frequency relation or "staircase" with increases in peak tension at 0.1, 0.25, and at 0.5 Hz. At 1 Hz, the peak tension would decline, and resting tension would begin to increase; therefore, stimulation at higher frequencies was not attempted. The physiologic heart rate of these frogs at room temperature (20–22° C) was 50–60 beats·min⁻¹ (0.87–1 Hz) as measured by a doppler flow probe placed against the thorax.

In figure 1A, contractions of frog ventricular strips at the different stimulation rates are superimposed and are shown at the same time scale as those for rat and guinea pig contractions. The average force-frequency response for peak tension and dT/dt-max, standardized for the estimated CSA, is shown in figure 1B. Due to the sustained nature of its contraction, frog ventricle achieves a greater estimated peak tension for its CSA. However, rat ventricle has the highest rate of tension development for its CSA. Frog ventricular muscle showed modest decline in contractile performance during the course of an experiment: control contractions at 0.1 up to 1 Hz decreased about 10% per hour, while the rested state contraction frequently showed a more marked decline following the first one or two series of stimulations. During the course of an experiment, sufficient recovery to near control performance permitted multiple anesthetic exposures.

**Potassium Contracture Studies.** Frog ventricular strips were pinned in a chamber with a nonrecirculating superfusion system in which four different Ringer solutions containing 40, 60, 80, or 100 mM K (prepared by substitution for the equivalent mM of Na) could be applied or washed off within 10 s. Throughout the study, electrical stimulation was maintained at 0.1 Hz in order to monitor contraction amplitude and muscle integrity. Each high [K⁺] solution was applied for 4–5 min, which was sufficient to achieve a stable contracture and eliminate the stimulated contractions. Then the contracture-producing (high [K⁺]) solution was washed off the strips with normal Ringer ([K⁺] = 2.5 mM) until the 0.1 Hz contraction had returned to a constant (precontracture) level. Another contracture-producing (high [K⁺]) solution was then applied. The order of solution application did not appear to alter the amplitude of the contracture observed for each solution, and the order usually employed was 100, 80, 60, and 40 mM K⁺ (typical control responses are shown in figure 7, left panel). Following control application of the four contracture-producing solutions, normal Ringer equilibrated with either 1.5% halothane, 2.5% isoflurane, or 3.5% enflurane was applied. Once the 0.1 Hz contractions had been depressed to a constant degree, the contracture solutions (equilibrated with the same anesthetic concentration) were applied in an identical sequence with intervening washout applications of normal Ringer (also equilibrated with anesthetic). The anesthetic was then discontinued and contractures were again elicited in the same sequence. For the study of a single anesthetic, these experiments typically lasted 5–7 h, during which the 0.1 Hz contraction amplitude occasionally declined compared to the initial control. However, following anesthetic application the contracture amplitudes remained stable, and recovered to 90–105% of control.

The anesthetic concentrations employed (0.75 and 1.5% halothane; 1.3 and 2.5% isoflurane; 1.7 and 3.5%
TABLE 1. Gas Concentrations in Solutions

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Gas Conc</th>
<th>Tyrode Solution at 37°C</th>
<th>Ringer Solution at 20°C</th>
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</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>0.75%</td>
<td>0.20</td>
<td>0.34</td>
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<tr>
<td></td>
<td>1.5%</td>
<td>0.38</td>
<td>0.62</td>
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<tr>
<td>Isoflurane</td>
<td>1.3%</td>
<td>0.22</td>
<td>0.48</td>
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<tr>
<td></td>
<td>2.5%</td>
<td>0.42</td>
<td>0.88</td>
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<tr>
<td>Enflurane</td>
<td>1.7%</td>
<td>0.40</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>3.5%</td>
<td>0.76</td>
<td>1.38</td>
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enflurane) were those previously used in the study of guinea pig myocardium. In rat, these concentrations represent approximately 0.75 and 1.5 MAC for halothane and enflurane and 0.85 and 1.7 MAC for isoflurane. In all studies, anesthetics were equilibrated with solution in a reservoir by passing the O₂/CO₂ through a calibrated, temperature-compensated vaporizer for at least 20 min before application to the preparation. The resulting concentrations in solutions are shown in table 1. It is clear that, in the cooler solution, the concentrations of anesthetics are substantially higher due to the higher fluid/gas partition coefficient. The concentrations observed were 72–82% of those estimated from the published fluid/gas partition coefficients, presumably due in part to loss into the atmosphere and perfusion tubing. Following control measurements of contractility, the anesthetic equilibrated solution was applied for 15 min before recordings were made. Anesthetics were washed off for at least 25 min with anesthetic-free solution before recovery measurements were recorded.

DATA ANALYSIS

For statistical comparison, peak tension and rate of tension development (dT/dt) during anesthetic exposure were expressed as percent of the mean control-recovery value. Peak contracture tensions, both in the absence and presence of anesthetic, were standardized to mean control-recovery contracture tension in 100 mM K Ringer, and expressed as a percent of that value. By averaging the control and recovery, the effect of decline in muscle tension development, particularly prominent in rat muscle, was minimized. Anesthetic effects and variations among stimulation frequencies were compared with analysis of variance employing Duncan’s Multiple Range test. All values are expressed ± SEM and significance was assumed for P < 0.05.

RESULTS

ANESTHETIC EFFECTS ON RAT MYOCARDIAL CONTRACTIONS

The superimposed contractions elicited from a rat papillary muscle after rest and up to 3 Hz stimulation during control, 3.5% enflurane exposure, and a recovery period are shown in figure 2. There is reversible depression caused by enflurane at each stimulation rate, although the contractions during the recovery period did not return to control level. As previously noted, this latter effect probably represents the intrinsic behavior of excised rat papillary muscle.

Figure 3 illustrates the average depression of rat ventricle caused by approximately 0.8 or 1.6 MAC isoflurane, halothane, and enflurane exposure. At 1–3 Hz, neither 0.8 or 1.6 MAC isoflurane cause significant depression of tension. Isoflurane (1.3%) was significantly less depressant (P < 0.05) than 0.75% halothane after rest and at 0.1 Hz, and less depressant than 1.7% enflurane after rest. The greater depression by 1.5% halothane (P < 0.01) compared to 2.5% isoflurane persisted at all stimulation rates; 3.5% enflurane was less depressant than 1.5% halothane at 0.1 to 1 Hz, but was more depressant than 2.5%
MYOCARDIAL DEPRESSION IN RAT AND FROG

![Graph showing the average depression in peak tension developed by rat papillary muscle at various stimulation rates in the presence of approximately 0.8 or 1.6 MAC anesthetic concentrations of enflurane, halothane, and isoflurane. At each rate, the peak tension in the presence of anesthetic was expressed as the percent of the average control and recovery peak tension at that rate (RS = rested state). In each case n = 6, except for 1.5% halothane n = 8. Halothane (1.5%) was significantly less depressant (P < 0.01) at 2–3 Hz than at RS to 0.25 Hz. *P < 0.05, **P < 0.01 different from isoflurane at the given frequency. †P < 0.05 enflurane different from halothane at the given frequency.]

Isoflurane at 2–3 Hz. At these higher concentrations (approximately 1.6 MAC), all three anesthetics showed decreasing depression with increasing stimulation rate, but the effect was most prominent with halothane. Anesthetic effects on the maximum rate of tension development (dT/dt-max) were very similar to effects on peak tension, with differences between anesthetics at the same stimulation rates.

ANESTHETIC EFFECTS ON FROG MYOCARDIAL CONTRACTIONS

Figure 4 shows the superimposed contractions of frog ventricular muscle following rest (RS) and at 0.1 up to 1 Hz stimulation. Control and intervening recovery responses are shown in the upper panels, while contractions in the presence of 2.5% isoflurane, 1.5% halothane, and 3.5% enflurane (administered in that order) are shown in the lower panels. In this muscle strip, halothane and enflurane appear more depressant than isoflurane. Figure 5 depicts the average depression of peak tension caused by equivalent concentrations of volatile anesthetics. Halothane was more depressant than isoflurane or enflurane: at 0.25 and 1 Hz, 1.3% isoflurane was less depressant than 0.75% halothane, while 1.7% enflurane was less depressant than halothane at 0.1–0.25 Hz (P < 0.05). At the higher concentrations as employed in the experiment of figure 4; 2.5% isoflurane was less depressant than 1.5% halothane (at 0.5–1 Hz) and was also less depressant than 3.5% enflurane at 1 Hz; enflurane (3.5%) was less depressant than 1.5% halothane a 0.1 Hz (P < 0.05).

In addition to the differences demonstrated among the anesthetics, contractile depression by all three anesthetics was, to some degree, frequency dependent. Halothane (0.75 and 1.5%) caused greater depression of contractions at 0.1–0.25 Hz than at 0.5–1 Hz rates. Contractions at 0.1 Hz were depressed more by isoflurane (1.3 and 2.5%) and enflurane (3.5%) than were those at 0.5–1 Hz. The rested state contractions were less depressed than the contractions at 0.1–0.25 Hz. However, the depressant effect on rested state contractions also showed a large standard error, probably due to the frequent variation observed in control and recovery values during the course of an experiment. The anesthetic effects upon dT/dt-max paralleled very closely the changes in peak tension.

A consistently observed difference in anesthetic effect in frog myocardium was the alteration in the pattern of relaxation observed in the presence of enflurane, but not seen with isoflurane or halothane. In figure 6, the 0.25 and 0.5 Hz tension tracings from figure 4 are superimposed for the control and anesthetic-exposed conditions. The period of tension development was prolonged by enflurane, resulting in an altered rate of decline in tension and a late “shoulder” of tension. This effect was quantified by determining the 80% relaxation time, which is the time required for the muscle to relax to 20% of peak tension. In the presence of 3.5% enflurane, the 80% relaxation times at 0.25 and 0.5 Hz increased to 463 ± 14 and 432 ± 17 msec, significantly higher than the average control-recovery values of 437 ± 17 and 402 ± 19 msec, respectively (P = 0.015, P < 0.001 by paired t test). In contrast,
Fig. 4. Anesthetic effects on frog ventricular tension responses. Steady-state contractions from the same muscle strip (estimated CSA = 1.90 mm²) at each stimulation rate are superimposed. The stimulation frequency in Hz is indicated beside each tension tracing (RS = rested state). The contractions were elicited under the following situations in the order: control; 2.5% isoflurane; recovery from isoflurane and control for halothane exposure; 1.5% halothane; recovery from halothane and control for enfurane exposure; 3.5% enfurane. Recovery of peak tension from enfurane (not shown) was to 85–89% of the preceding control/recovery value.

Fig. 5. Average depression of peak tension in frog ventricular muscle contractions by approximately equivalent anesthetic concentrations. At each rate, the peak tension in the presence of anesthetic was expressed as the percent of the average control and recovery peak tension that at rate (RS = rested state). Left panel, average depression caused by 1.3% isoflurane (n = 7), 0.75% halothane (n = 10), and 1.7% enfurane (n = 10). Right panel, average depression caused by 2.5% isoflurane (n = 9), 1.5% halothane (n = 9), and 3.5% enfurane (n = 10). Enflurane (3.5%) and isoflurane (1.3, 2.5%) caused significantly greater depression at 0.1 Hz compared to 0.5–1 Hz; halothane (0.75, 1.5%) was significantly more depressant at 0.1–0.25 Hz compared to 0.5–1 Hz. *P < 0.05 different from halothane at the given frequency. †P < 0.05 enfurane different from isoflurane at the given frequency.
the times to 80% relaxation in the presence of 1.5% halothane and 2.5% isoflurane did not differ from control at rates of 0.25–0.5 Hz (range: 92–99% of control-recovery). At 0.1 Hz stimulation, the 80% relaxation times in the presence of isoflurane and halothane were reduced to 93 ± 2 and 87 ± 4% of the control-recovery value (P = 0.007, 0.017, respectively), while enflurane had no effect (104 ± 2% of control, P = 0.121). Consequently, 3.5% enflurane relaxation times at 0.1 to 0.5 Hz rates were significantly longer than those for 2.5% isoflurane and 1.5% halothane (values expressed as percent of the control-recovery values, compared by ANOVA employing Duncan’s multiple range test).

**ANESTHETIC EFFECTS ON CONTRACTURES IN FROG MYOCARDIUM**

Figure 7 shows the tension developed by a frog ventricular muscle strip with exposure to contracture-producing 40, 60, 80, and 100 mM K⁺ Ringer solutions. On the time scale shown, the “spikes” represent the contractions due to electrical stimulation at 0.1 Hz, which was continued throughout. The depolarization caused by the increased extracellular [K⁺] prevents continued action potential propagation and ultimately eliminates the contractions caused by the underlying 0.1 Hz electrical stimuli. The sustained contracture tension which develops increases with the [K⁺] applied. The exposure to approximately 3.5% enflurane resulted in marked depression of the 0.1 Hz stimulated contractions, but only modest depression of the contractures.

In figure 8, the average sustained contracture tensions are plotted for each [K⁺]. Contracture tension with each high K⁺ solution in the absence or presence of anesthetic is expressed as a fraction of the 100 mM K⁺ contracture tension. For five muscle strips in each group, the mean control-recovery contracture tensions and the mean contracture tensions in the presence of anesthetic are shown. Halothane depressed contracture tension at all K⁺ concentrations, and was significantly (P < 0.01) more depressant than enflurane or isoflurane upon the contractures in 80 and 100 mM K⁺.

As illustrated for enflurane in figure 8, all three anesthetics depressed the contractions at 0.1 Hz significantly more than the contractures in 100 mM K⁺ Ringer. However, the sustained contracture tension in high K⁺ solu-
Contractions and contractions at 0.5–1 Hz (fig. 5) were depressed to the same extent by halothane (~60% of control) or by isoflurane (~85% of control). In contrast, enflurane depressed the contractions at 1 Hz (68 ± 3% of control) significantly more (P < 0.005) than it depressed the contractions (84 ± 3% of control).

**Discussion**

The cellular processes in ventricular myocardium responsible for delivery of Ca²⁺ to activate myofibrils and cause contractions differ markedly between the rat and frog. However, these cellular processes are expressed to

**FIG. 7.** Contractures of frog ventricular muscle strips due to application of 40, 60, 80, and 100 mM K solution. Contractures are shown for control and in the presence of 3.5% enflurane. The vertical lines represent contractions at steady stimulation rate of 0.1 Hz, which was maintained throughout the experiment. Estimated CSA of the muscle was 0.50 mm².

**FIG. 8.** Average contracture tension of frog ventricular strips in the absence (averages of control and recovery) and presence of 2 MAC anesthetic (n = 5). All contracture tensions were expressed as a percent of the average of the control and recovery contracture tension in 100 mM K Tyrode. Halothane depressed contractures in 80 and 100 mM K⁺ significantly more than either isoflurane or enflurane (P < 0.01). *P < 0.05 difference between anesthetic and control-recovery value at each K concentration.
varying degrees in human and other mammalian myocardium. Therefore, volatile anesthetic effects upon myocardium from such divergent species as rat and frog provides insight into how the anesthetics interfere with excitation-contraction coupling in general.

The sarcoplasmic reticulum is the intracellular membrane system within muscle cells which envelops the myofibrils and actively accumulates Ca\(^{2+}\) by means of a Ca-Mg-ATPase (Ca-pump) to bring about relaxation. Upon depolarization, the sarcoplasmic reticulum releases its store of Ca\(^{2+}\) into the myoplasm via a distinct Ca-release channel, thereby activating contraction.\(^{28,29}\) This Ca-release channel of the sarcoplasmic reticulum is distinctly different in location, structure, and function from the Ca channels of the surface membrane that are blocked by nifedipine, verapamil, and diltiazem. Ryanodine is a plant alkaloid that binds to the Ca-release channel with high specificity, inhibiting rapid Ca\(^{2+}\) flux through the channel and instead inducing a continuous low conducting state.\(^{28}\) Thus, even during rest, Ca\(^{2+}\) passes out of the sarcoplasmic reticulum, causing a gradual depletion of Ca\(^{2+}\). Ryanodine causes almost total depression of rat ventricular contractions after rest and depression is almost as profound at higher stimulation rates,\(^{14}\) an effect observed in rat papillary muscles as employed in this laboratory (unpublished data). Furthermore, the sarcoplasmic reticulum of rat myocardium is extensive, occupying 7.3% of the myofibrillar volume,\(^{12}\) while physiological evidence also attests to its prominent role in rat ventricle. Fabiato\(^{13}\) has extensively studied the function of sarcoplasmic reticulum in skinned myocardial cells from the atria and ventricles of a variety of species. Rat ventricular muscle is the most sensitive in demonstrating Ca-induced Ca-release from the sarcoplasmic reticulum, a positive feedback process that is thought to control Ca\(^{2+}\) release and subsequent tension development. Consequently, in rat myocardium, the sarcoplasmic reticulum release of Ca\(^{2+}\) is almost exclusively responsible for activating contraction, particularly the large rested state contraction.

In contrast to the large cellular volume occupied by the sarcoplasmic reticulum in rat, this organelle is extremely small in frog myocardium.\(^{16,17}\) It constitutes approximately 0.5% of the myofibrillar volume,\(^{17}\) or \(\frac{1}{15}\)th the volume that sarcoplasmic reticulum occupies in rat. Muscle contractions studied by optical methods employing polarized light reveal a distinct birefringence signal preceding contraction in both skeletal and mammalian ventricular muscle. This signal has been attributed to activation of the sarcoplasmic reticulum and release of Ca\(^{2+}\); such a signal is absent in frog ventricular muscle.\(^{30}\) Also, in contrast to rat ventricle, ryanodine has little effect upon frog ventricular contractions.\(^{14}\) Some authors suggest that the sarcoplasmic reticulum supplies no activator Ca\(^{2+}\) in frog ventricle;\(^{18}\) other workers have suggested a small contribution under well-defined circumstances.\(^{19}\) The preponderance of evidence suggests that the sarcoplasmic reticulum plays little role in frog ventricular tension development or relaxation. Physical considerations also suggest that, in frog heart, internal Ca\(^{2+}\) stores are not required. Due to the narrow diameter of frog myocytes (\(\sim 5 \mu m\)) combined with their prolonged action potential depolarization (\(\sim 500\) msec), sufficient extracellular Ca\(^{2+}\) can enter the cell from outside and diffuse throughout the cell to activate the myofibrils over 500–800 msec. In contrast, rat myocytes are 15–20 \(\mu m\) in diameter, and are only able to rapidly develop peak tension within 50–60 msec because the 0.5–1 \(\mu m\) diameter myofibrils are enveloped by the sarcoplasmic reticulum, which can rapidly release its large intracellular store of Ca\(^{2+}\).

Halothane caused marked depression of rat ventricular contractions, the effect being most prominent after rest, when the contraction is greatest due to the accumulation of Ca\(^{2+}\) into the sarcoplasmic reticulum during rest. Furthermore, halothane was significantly more potent than isoflurane in depressing the tension development in rat ventricle caused by the sarcoplasmic reticulum-dependent Ca\(^{2+}\) release. This conclusion agrees with that of Komai and Rusy,\(^{31,32}\) who studied anesthetic effects on rabbit papillary muscle potentiated state contractions, in which a brief period of 2 Hz stimulation is followed by 2 s rest, after which a final potentiated contraction is elicited. Increased amounts of extracellular Ca\(^{2+}\) enter the cell during the high stimulation rate and are accumulated by the sarcoplasmic reticulum during the brief rest interval. The subsequent contraction is potentiated due to this increased intracellular Ca\(^{2+}\) store. Such potentiation is inhibited by ryanodine. Halothane caused significantly greater depression of the enhanced potentiated state contraction than did isoflurane, suggesting that, in the presence of halothane, less Ca\(^{2+}\) is released by the sarcoplasmic reticulum.\(^{92}\) Previous studies by Su et al. have examined the effect of anesthetics upon Ca\(^{2+}\) release from sarcoplasmic reticulum by examining tension development in skinned rabbit cardiac fibers, in which Ca\(^{2+}\) release was activated by application of caffeine.\(^{7,8}\) These series of experiments suggested that isoflurane was less potent than halothane in depressing the amount of Ca\(^{2+}\) released from the sarcoplasmic reticulum of skinned rabbit cardiac myofibrils.

The studies by Blanck and Thompson\(^{9,10}\) and Casella \textit{et al.}\(^{11}\) in isolated cardiac sarcoplasmic reticulum vesicles suggest that anesthetic effects on Ca\(^{2+}\) uptake are a function of time, pH, and ATP concentration. Under normal intracellular conditions, there was modest enhancement in the rate of Ca\(^{2+}\) uptake caused by the volatile anesthetics. However, overall, anesthesia depressed the maximal amount of Ca\(^{2+}\) accumulated by the (isolated) sarcoplasmic reticulum.\(^{11}\) Such an effect would be consistent with an enhanced efflux of Ca\(^{2+}\) from the vesicles.
In a number of studies, transient enhancement of myocardial contractions has been observed upon halothane application, also consistent with briefly enhanced release of Ca\(^{2+}\) from the sarcoplasmic reticulum, prior to depletion of Ca\(^{2+}\) from the organells. Likewise, submaximal stimulation of Ca\(^{2+}\) release from the loaded sarcoplasmic reticulum of skinned fibers employing 2 mM caffeine was enhanced by halothane, but less so by isoflu- rane. Although the evidence is circumstantial, the decreased sarcoplasmic reticulum Ca\(^{2+}\) release inferred for halothane may result from an enhanced release or leak of Ca\(^{2+}\) from the sarcoplasmic reticulum, resulting in a relative depletion of this Ca\(^{2+}\) store. Such an effect in myocardium would also correlate with results from skeletal muscle in which halothane appears to enhance release of Ca\(^{2+}\) from isolated sarcoplasmic reticulum ves- icles.

This present study appears to contradict prior work from this laboratory on guinea pig papillary muscle in which isoflurane and enflurane caused greater depression of a late-peaking rested-state contraction. The depression of late-peaking tension was attributed to an alteration in sarcoplasmic reticulum Ca-release by isoflurane and enflurane. Subsequent study suggests that the late-peaking tension may represent an alternate type of sarcoplasmic reticulum Ca-release, which is inhibited by local anes- thetics.

Since frog myocardial contractions depend very largely on extracellular Ca\(^{2+}\) entry to activate myofibrils, volatile anesthetic depression of contractility could result from anesthetic depression of Ca\(^{2+}\) entry. The greatest depression might be anticipated in rested state contractions, when any residual intracellular Ca should be removed from the cell. Actually, the anesthetics caused highly variable depression of rested state contractions, which probably in part reflected unavoidable variations in these con- tractions with time. Such highly variable anesthetic depression was not observed at greater stimulation rates and maximum anesthetic depression occurred at 0.1 Hz. At roughly equipotent concentrations, halothane was more potent than isoflurane, while enflurane was typically intermediate in potency. Previous electrophysiologic studies in guinea pig ventricle suggested also that halothane is a more potent inhibitor of Ca\(^{2+}\) entry than isoflurane based on its depression of slow action potential rate of rise. In such partially depolarized cells, the action potential rate of depolarization represents Ca\(^{2+}\) entry through a slow calcium channel, presumably the L-type, nifedipine-sensitive slow channel.

It should be appreciated that only part of the extra- cellular Ca\(^{2+}\) enters the frog myocyte through the voltage- activated Ca channel. Substantial Ca\(^{2+}\) movement also is mediated by the Ca\(^{2+}\) exchange pathway(s). The best defined mechanism is the Na-Ca exchange, in which three Na\(^{+}\) are passively exchanged for one Ca\(^{2+}\) according to the balance of the electrochemical gradients for each ion. The process is electrogenic and sensitive to membrane potential so that, at rest, Ca\(^{2+}\) is eliminated from the cell by Na\(^{+}\) entering into the cell; during depolarization (i.e., the plateau) Ca\(^{2+}\) enters the cell and Na is removed. Thus, the Na-Ca exchange pathway is responsible in large part for Ca\(^{2+}\) elimination from the cell during diastole, and serves as a means of Ca\(^{2+}\) entry during the plateau of the action potential. A decreased external [Na\(^{+}\)] or an in- creased intracellular [Ca\(^{2+}\)] reduces the transmembrane gradient for Na. Such reductions in the Na electrochem- ical gradient shift the electrochemical balance toward less Ca\(^{2+}\) removal and greater Ca\(^{2+}\) entry. During more rapid rates of stimulation, more Na\(^{+}\) enters the cell, which results in an increased intracellular [Ca\(^{2+}\)]; this, in turn, increases Ca\(^{2+}\) entry, leading to larger contractions at greater stimulation rates. Increased Ca\(^{2+}\) entry by this pathway can explain the decreased depression by the anesthetics of frog myocardial contractions as the stimulation rate was increased to 0.5–1 Hz, presuming the anesthetics have a modest effect upon Na-Ca exchange pathway.

In the case of the contractures mediated by K depolarization, a reduction in external [Na\(^{+}\)] in the solution was necessary for substitution of K\(^{+}\). As with a rise in internal [Ca\(^{2+}\)], a reduction in external [Na\(^{+}\)] also causes less Ca\(^{2+}\) to be eliminated from the cell and enhances its entry by the Na-Ca exchange mechanism. In the setting of these sustained contractures, the continuous depolarization combined with low external [Na\(^{+}\)] markedly enhances Ca\(^{2+}\) entry through the Ca channel as well as the Na-Ca exchange. The sustained K\(^{+}\) contracture, as well as contractions at 1 Hz, were depressed by halothane (1.5%) to ~60% of control, compared to depression by 2.5% isoflurane to ~85% of control. The continuing contractile depression after Ca\(^{2+}\) entry was maximized may, in part, reflect anesthetic depression of Ca\(^{2+}\) entry, which persists for some minutes, as well as direct depression of the actin-myosin ATPase by halothane and isoflurane. Consistent with the above results, halothane was also two to three times more potent than isoflurane in depressing ATPase activity in isolated myofibrillar preparations.

Enflurane (3.5%) depressed peak tension of 1 Hz con- tractions to 68% of control, only slightly less depressant than 1.5% halothane. In contrast, 3.5% enflurane de- pressed 80 and 100 mM K contractures to 84% of control, which was similar to the depression by 2.5% isoflurane. Thus, unlike halothane and isoflurane, enflurane did not cause similar depression in these two settings (1 Hz con- tractions or sustained 80–100 mM K contractures) that enhance Ca\(^{2+}\) entry. Also, unlike halothane and isoflu- rane, enflurane delayed relaxation and prolonged the contraction in frog myocardium, an effect not observed.
in rat heart. Such a depression of peak tension with a delay in relaxation could result from depression of Ca\(^{2+}\) entry by enfurane\(^5\) combined with a counteracting depression of Ca\(^{2+}\) removal from the myofibrils. Alteration by enfurane of Ca\(^{2+}\) removal from the myofibrils, specifically from troponin, could occur due to a direct action upon the troponin-C-Ca\(^{2+}\) interaction, or because of altered Ca\(^{2+}\) fluxes. For example, if the period of Ca\(^{2+}\) entry is prolonged or if Ca\(^{2+}\) removal from cell is slowed, then a more sustained period of contraction might also result.

It has been suggested that the relative contribution of external Ca entry to myofibrillar activator Ca for ventricular tissue is in the order: frog > fetal (human, cat, rabbit) > rabbit ~ guinea pig > cat > dog > ferret > rat.\(^{14}\) The importance of the sarcoplasmic reticulum would be in the reverse order. However, in many mammalian ventricular muscles, the contribution of extracellular space versus sarcoplasmic reticulum as primary sources of activator Ca\(^{2+}\) may be dependent on stimulation rate. Specifically, in guinea pig, ryanodine markedly depresses rapid tension development only at higher stimulation rates, suggesting the sarcoplasmic reticulum is a substantial source of Ca\(^{2+}\) at these rates.\(^{39}\) The depression of guinea pig heart contractions at 2–3 Hz by halothane\(^6\) is similar to the depression of rat heart rested state contractions observed for similar anesthetic concentrations. In each setting, isoflurane was significantly less depressant than halothane.

Comparison of these results to human myocardium is difficult, since the force-frequency relation of normal isolated human ventricular myocardium is not reported. Segments of viable human ventricular trabeculae adjacent to scarred and infarcted endocardium show a positive force-frequency relation,\(^{40}\) similar in behavior to ventricular myocardium from many mammalian species except rat. Such tissues also showed a frequency dependent difference in anesthetic depression, 0.75% halothane being more depressant than 1.3% isoflurane at higher stimulation rates. In human myocardium stimulated at 0.5–1 Hz, the magnitude of depression caused by 0.75% halothane (~60% of control) or 1.3% isoflurane (~80% of control) was similar to that observed for guinea pig heart at 2–3 Hz,\(^5\)\(^,\)\(^40\) as well as for rat heart following rest or at 0.1 Hz, situations in which the sarcoplasmic reticulum supplies the activator Ca\(^{2+}\).

In summary, halothane caused greater depression of tension development in both rat and frog than did isoflurane at an equivalent anesthetic dose, while the depression caused by enfurane was intermediate. These results suggest that halothane causes more depression of Ca\(^{2+}\) release from the sarcoplasmic reticulum than isoflurane, as demonstrated by its effect in rat papillary muscle. Halothane is also a more potent inhibitor of extracellular Ca\(^{2+}\) entry, as demonstrated by the depression of contractility in frog ventricle.

The authors wish to thank Drs. David E. Longnecker and Dan Lawson for review of and suggestions regarding the manuscript.

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