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## Differential Effects of Halothane and Isoflurane on Contractile Force and Calcium Transients in Cardiac Purkinje Fibers

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**Background:** Halothane and isoflurane have been shown to differentially effect mechanisms of contraction in working myocardial fibers. The aim of this study was to compare effects of halothane and isoflurane on contractile force (CF) and  $Ca^{2+}$  transients in canine cardiac Purkinje fiber preparations.

**Methods:** Fiber preparations ( $n = 29$ ) were superfused with Krebs-Ringer solution and stimulated at 40–60 pulses/min at 30°C in the absence and presence of 0.55% and 1.01% isoflurane or 0.27% and 0.78% halothane. Isometric tension (CF), and intracellular  $Ca^{2+}$  transients (luminescence, L) were measured after microinjecting the  $Ca^{2+}$  sensitive photoprotein aequorin into Purkinje fibers. Peak CF and peak L, rate of rise (slope) of CF and L, time to attain peak CF and L, and duration of CF and L at half-peak CF and L, were measured at 5 mM extracellular  $CaCl_2$ . Changes in peak CF and peak L also were measured during incremental increases in  $CaCl_2$  from 3.6 to 9.0 mM.

**Results:** Both anesthetics depressed peak CF and peak L and the rate of increase in peak CF and L in a concentration-dependent fashion, and effects of halothane were greater than those of isoflurane. Time to attain peak L and duration of L at half-peak L was decreased or unchanged by isoflurane and was increased by halothane, whereas time to attain peak CF and duration of CF at half-peak CF was shortened by both. The change in peak CF response as a function of the change in peak L with increasing extracellular  $CaCl_2$  was attenuated similarly by both halothane and isoflurane.

**Conclusions:** Halothane depresses peak CF and  $Ca^{2+}$  transients and prolongs  $Ca^{2+}$  transients more than does isoflurane at equivalent minimum alveolar concentration in Purkinje fibers. This suggests  $Ca^{2+}$  concentration is differentially altered by anesthetics in this tissue. Peak CF at equivalent peak  $Ca^{2+}$  transients, however, appears to be attenuated similarly by both anesthetics. These differences in anesthetic effect are qualitatively similar to those found in cardiac tissue of other species. These findings add to our understanding of effects of volatile anesthetics on contractile properties and myoplasmic  $Ca^{2+}$  in cardiac Purkinje fibers. (Key words: Anesthetics, volatile: halothane; isoflurane. Animal: dog. Drugs: aequorin. Heart: calcium transients; contractility; Purkinje fiber. Ions: calcium.)

STUDIES in isolated hearts<sup>1</sup> and papillary muscles<sup>2-5</sup> have demonstrated that halothane, at molar or minimum alveolar concentration (MAC) multiples equivalent to isoflurane, depresses cardiac contractility more than does isoflurane. In guinea pig right ventricular papillary muscle, halothane decreases contractile force (CF) and intracellular  $Ca^{2+}$  transients more than does isoflurane.<sup>6</sup> Moreover, the smaller depression of  $Ca^{2+}$  transients by isoflurane is accompanied by a decrease in myofibrillar  $Ca^{2+}$  sensitivity by isoflurane but not by halothane. Equivalent anesthetic concentrations of halothane and isoflurane similarly reduce peak transsarcolemmal  $Ca^{2+}$  currents both in isolated canine ventricular cells<sup>7</sup> and in Purkinje cells.<sup>8</sup> This suggests that intracellular  $Ca^{2+}$  concentration or sensitivity to  $Ca^{2+}$  in ventricular cells is affected differentially by isoflurane and halothane.

The effect of anesthetics on  $Ca^{2+}$  transients in Purkinje cells has not been reported. Halothane and isoflurane, in individual or comparative studies, have been shown (1) to slow conduction in canine His-Purkinje tissue,<sup>9,10</sup> (2) to decrease action potential duration and refractoriness in canine Purkinje fibers,<sup>11-13</sup> and (3) for halothane in the same tissue, to decrease delayed afterdepolarization induced by triggered activity,<sup>12</sup> and to decrease conduction velocity in a dose-dependent

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fashion, albeit moderately at 1.5 MAC.<sup>14</sup> Because volatile anesthetics variably alter the flux of many ions across the sarcolemma and sarcoplasmic reticulum (SR), it is difficult to determine whether or how much a change in intracellular free calcium  $[Ca^{2+}]_i$ , as estimated by  $Ca^{2+}$  transients, contributes to the above effects. It is well known that  $Na^+$  and  $K^+$  currents are involved mainly in determining conduction velocity, resting membrane potential, and the probability of dysrhythmia development. Even so, a decrease in  $[Ca^{2+}]_i$  may slow conduction by increasing the rate of early repolarization in the presence of "high-resistance coupling" between ventricular cells,<sup>15</sup> and may underlie the reduction by halothane in post-coronary occlusion fibrillation thought to be caused, at least in part, by SR calcium overload and consequent afterdepolarizations.<sup>16</sup>

For this study, we compared effects of isoflurane and halothane on altering  $Ca^{2+}$  transients while measuring CF in canine Purkinje fiber preparations. We believed that such an investigation was important to confirm or contradict similar studies undertaken in other cardiac tissues and species. Measurements of  $Ca^{2+}$  transients were used to indicate availability of  $[Ca^{2+}]_i$ . Differential decreases in the rate or peak of  $Ca^{2+}$  availability, whether due to differences in inward  $Ca^{2+}$  current, sarcolemmal  $Ca^{2+}$  storage or release mechanisms, or  $Ca^{2+}$  sensitivity by the contractile apparatus, could underlie some of the observed differences of these volatile anesthetics on automaticity and impulse propagation in Purkinje fibers.<sup>17</sup>

### Materials and Methods

These experiments were approved by the Medical College of Wisconsin Animal Care Committee and conform with standards set forth in the National Institutes of Health *Guide for Care and Use of Laboratory Animals*. Twenty-nine adult mongrel dogs were anesthetized with halothane, and the hearts were rapidly removed and washed in cold oxygenated Krebs solution. The left ventricle was opened, and a thin, muscle segment (width 2–3 mm), with attached Purkinje fibers (average cross-sectional area of 1.2 mm<sup>2</sup>), was removed and carefully mounted horizontally in a single low-volume, high-flow chamber by securely pinning the muscle segment to the Sylgard floor (Dow Corning, Midland, MI) and fixing the free-running Purkinje fiber segment to the arm of the force displacement transducer (FT03C, Grass, Quincy, MA) with an 8-0 Prolene

surgical suture. The preparation was superfused continuously with modified Krebs solution containing (mM): NaCl 137, KCl 3.8, NaHCO<sub>3</sub> 12, CaCl<sub>2</sub> 5, NaH<sub>2</sub>PO<sub>4</sub> 1.8, MgCl<sub>2</sub> 0.5, glucose 5.5, and EDTA 0.05, with a pH of 7.4. The superfusate solution was equilibrated in a large reservoir with a 97% O<sub>2</sub>–3% CO<sub>2</sub> mixture, maintained at 30 ± 0.5°C with a heated circuit and delivered at a constant rate of 6 ml/min *via* an occlusive roller pump. Anesthetics were introduced to the superfusate reservoir through agent specific vaporizers (halothane, Fluotec 3, Cyprane Lt., Richmond, England, and isoflurane, Forane, Ohio Medical Products, Madison, WI) placed in the gas-delivery circuit. Superfusate samples were collected through a catheter placed at the level of the tissue in the chamber. pH, PCO<sub>2</sub> and PO<sub>2</sub> were measured (ABL3 Radiometer, Copenhagen, Denmark) at 38°C. Anesthetic concentrations in the bath were measured by head space analysis using a gas chromatograph with a flame ionization detector.

The Purkinje fiber strands were directly stimulated (A350 D, WP Instruments, New Haven, CT) with a bipolar electrode at intervals between 1,000 and 1,500 ms (1–0.5 Hz or 40–60/min). To assure adequate oxygen delivery to the central core of Purkinje fibers, these pacing rates were maintained only for short periods during data collection. During equilibration periods, fibers were stimulated at an interval of 3,000 ms.

Highly purified aequorin, a  $Ca^{2+}$ -sensitive bioluminescent protein, was obtained from the laboratory of J. R. Blinks (Rochester, MN) and prepared as noted previously.<sup>6,18,19</sup> Under biologic conditions, this luminescent jellyfish protein emits a blue light in the presence of  $Ca^{2+}$ . The light signal (L) results from interaction of free intracellular  $Ca^{2+}$  with aequorin present in cytosol.<sup>20–22</sup> The aequorin light signal is qualitatively similar in overall magnitude and time course as Fura 2 fluorescence for estimating intracellular cytoplasmic  $Ca^{2+}$  concentration.<sup>23</sup> Aequorin solution was filtered (0.1 μm), drawn into fine-tipped microinjection-glass electrodes, and pressure-injected into several regions of Purkinje fibers while superfused temporarily in a low  $Ca^{2+}$  concentration (0.25 mM). After closure of the light-tight box, the light emitted by aequorin was detected by a photomultiplier cathode (EMI bi-alkali 9635A, Plainview, NY) with a high quantum efficiency in the blue range of the spectrum (aequorin, 469 nm) and a low dark current. The photomultiplier cathode was equipped with a slide-type shutter and a plexiglass light guide (tip diameter 13 mm) located

about 1 mm above the preparation. Successive L and CF were amplified and averaged (100–160 beats at 40–60 beats/min) to obtain satisfactory  $\text{Ca}^{2+}$  signal-to-noise ratios.

#### *Experimental Protocol*

At the beginning of each experiment, a series of isometric contractions was recorded at several resting lengths. A final length was adjusted to a point at which the stimulated tension was 90–95% of that maximally achieved. Contractile force and L were measured simultaneously in the presence and absence of two concentrations each of halothane and isoflurane. Contractile force and L also were measured during 1.8-mm step increases in extracellular  $\text{Ca}^{2+}$  concentration between 3.6 and 9.0 mM. Delivered concentrations of anesthetics (calibrated vaporizer dial settings), which correspond approximately to 0.57 and 1.15 MAC for the dog,<sup>24</sup> were 0.5% (low) and 1.0% (high) halothane and 0.75% (low) and 1.5% (high) isoflurane. Calculation of effective vapor pressure (in vol%), as noted previously,<sup>25</sup> from measured bath concentrations of isoflurane ( $0.14 \pm 0.005$  and  $0.26 \pm 0.011$  mM) and halothane ( $0.09 \pm 0.003$  and  $0.26 \pm 0.007$  mM) and the respective saline/gas partition coefficients at 1 atm and 30°C gave values of 0.55% and 1.01% for isoflurane and 0.27% and 0.78% for halothane. Effective vapor pressures were lower than delivered vapor pressures because of anesthetic volatilization from the bath. Anesthetic was not measurable in the bath after a 10-min washout. Superfusate temperature was monitored with a digital thermometer and maintained at  $30 \pm 0.5^\circ\text{C}$  with a servocontrolled heated bath. Anesthetic-free control measurements were recorded after a 20-min equilibration and after two consecutive recordings were similar. After the initial period of equilibration, the following recordings were made: (1) control (0% anesthetic), (2) low or high halothane or low or high isoflurane, and (3) anesthetic-free post-control. Both the concentration and order of anesthetic administered were randomized.

#### *Analysis of Signals and Statistical Analysis*

Averaged data (100–160 beats) of both CF and L were displayed, stored on computer disks, and analyzed with a custom-written computer program (9000 computer, Hewlett-Packard, Palo Alto, CA) and plotted (7475A plotter, Hewlett-Packard). Several components of the isometric muscle tension (CF) and L measurements were analyzed: (1) peak CF and peak L; (2) slopes of

onset of CF and L measured at 50% of peak responses; (3) time to peak signal (TTP), a duration measured from the stimulus artifact to the peak CF (TTP<sub>CF</sub>) or the peak L (TTP<sub>L</sub>); (4) duration of CF or L when signals exceeded 50% of peak CF (T-CF<sub>50</sub>) or 50% of peak L (T-L<sub>50</sub>); and (5) percentage change in peak CF as a function of percentage change in peak L at extracellular  $\text{CaCl}_2$  concentrations of 3.6, 5.4, 7.2, and 9.0 mM.

For individual studies, CF was normalized for the cross-sectional area of the muscle and expressed as milli-Newtons per square millimeter ( $\text{mN} \cdot \text{mm}^2$ ), whereas L was expressed in terms of photomultiplier current in nanoamperes (nA). All mean data are displayed only as a percentage change from the control for each anesthetic at its low or high concentration for several reasons. Absolute light emission values were not compared among the experiments because many of the physical parameters are expected to be constants only within a given experiment.  $[\text{Ca}^{2+}]_i$  was not expressed directly as a function of  $\text{Ca}^{2+}$  transients because fractional light emission ( $L/L_{\text{max}}$ ) was not measured.  $\log L/L_{\text{max}}$  as a function of  $\log [\text{Ca}^{2+}]_i$  has a linear slope of 2.5, but this relationship is not linear at the lower (diastolic) range of  $[\text{Ca}^{2+}]_i$ .<sup>21</sup> Because the relationship of CF as a function of  $[\text{Ca}^{2+}]_i$  is nonlinear, the effects of anesthetics on altering the percent increase in peak light emission and peak CF with increases in extracellular  $\text{CaCl}_2$  concentration were expressed only as the effects varied from the nonanesthetic controls at the same extracellular  $\text{CaCl}_2$  concentration.

Data are displayed as mean  $\pm$  SEM. Data were analyzed by one-way analysis of variance followed by Fisher's least significant difference test. A linear regression analysis program was used to determine and compare slopes of peak CF as a function of peak L. A probability level of 0.05 or smaller was used to indicate statistical significance.

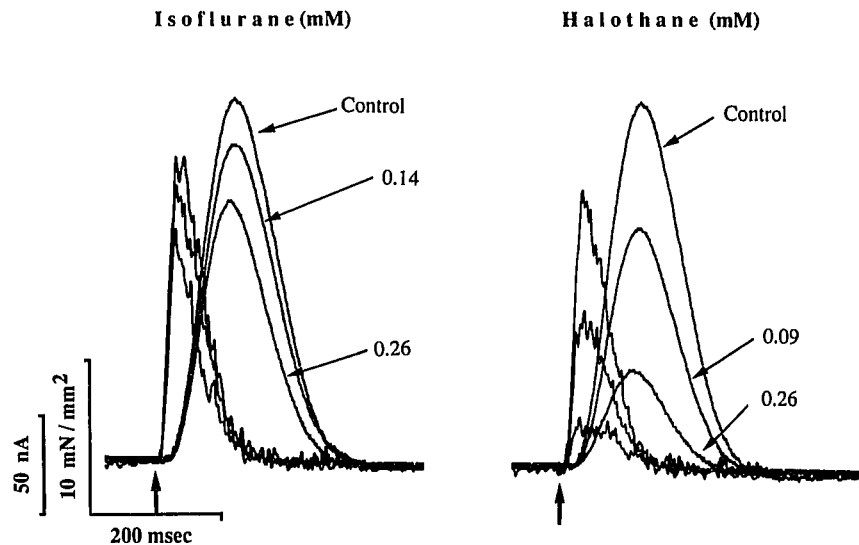
## Results

### *Effects of Anesthetics on CF and $\text{Ca}^{2+}$ Transients*

Figure 1 shows representative sequential tracings of effects of 0 (control), 0.14 and 0.26 mM isoflurane, and 0, 0.09, and 0.26 mM halothane on isometric CF (tension in  $\text{mN}/\text{mm}^2$ ) and  $\text{Ca}^{2+}$  transients (L in nA). On a molar basis, halothane appears to depress the magnitude, slope, and TTP<sub>CF</sub> more than isoflurane, to depress the magnitude and slope of L more than isoflurane, and to shift the TTP<sub>L</sub> to the right for halothane

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Fig. 1. Representative tracings of isometric contractile force (smooth signal,  $\text{mN}/\text{mm}^2$ ) and aequorin light signal (rough signal,  $\text{nA}$ ) from a canine Purkinje fiber preparation showing the effects of mean effective fractions of 0.55% and 1.01% (0.14 and 0.26 mM) isoflurane and 0.27% and 0.78% (0.09 and 0.26 mM) halothane. Each trace represents an average of 100–160 signals at a pacing rate of 1 Hz. Arrows indicate the pacing stimulus from which time measurements were made.

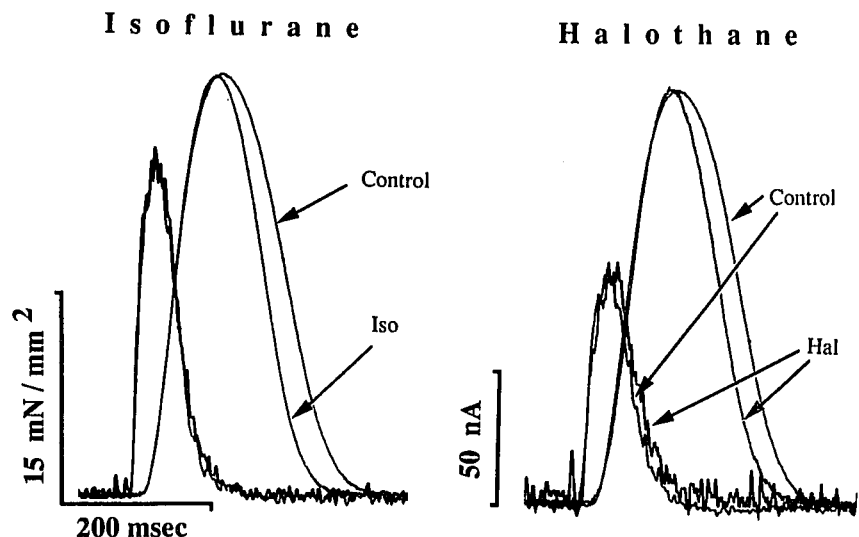


but not for isoflurane. In Figure 2, peak CF and L obtained at the high concentrations of isoflurane and halothane were electronically amplified to the peak levels observed in the absence of anesthetic so that other characteristics of the signal waveforms could be visually compared. These tracings attempt to show that  $\text{TTP}_{\text{CF}}$  and duration of CF at  $\text{T-CF}_{50}$  were decreased by both anesthetics, and that  $\text{TTP}_{\text{L}}$  and duration of L at  $\text{T-L}_{50}$  were increased only by halothane.

Figure 3 summarizes the effects of isoflurane and halothane on peak CF and peak L expressed as a percentage of the controls, normalized to 100%, for each

anesthetic. Isoflurane, and more so halothane ( $P < 0.05$ ), decreased both variables in a dose-dependent fashion. At the high concentration of either anesthetic, peak CF decreased more than the peak L. Figure 4 summarizes the effects of isoflurane and halothane on the rates of CF development and light emission, measured at half-peak amplitude for CF and L and expressed as percentage of controls. Similar to their concentration-dependent effects on peak CF and peak L, isoflurane and halothane effectively reduced the positive slopes of these variables in a concentration-dependent fashion. Contractile force and L slopes were decreased more by

Fig. 2. Tracings of isometric contractile force (smooth signal) and aequorin light signal (rough signal) from a Purkinje fiber preparation. Signals during exposure to the high concentration of each anesthetic were electronically increased to the level of control signals to facilitate comparison of their time courses. Contractile force and L calibrations refer to nonanesthetic controls. In the presence of isoflurane and halothane, respectively, tracings were amplified 1.35 and 2.5 times for L and 1.65 and 3.3 for CF to match peak control signals.



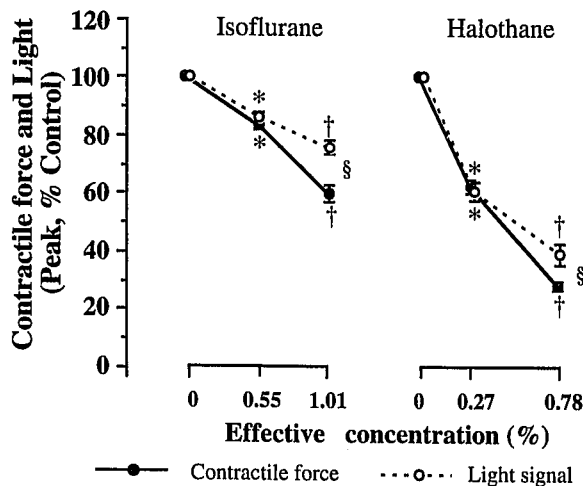


Fig. 3. Effects of isoflurane ( $n = 20$ ) and halothane ( $n = 18$ ) on peak contractile force (CF) and aequorin light signals (L) in Purkinje fibers. Values are expressed as a percentage of anesthetic free controls normalized to 100%. \*Low anesthetic concentration versus no anesthetic. †High versus low anesthetic concentration. §Percent change in CF versus percent change in L at a given anesthetic concentration ( $P < 0.05$ ). Differences in responses to isoflurane and halothane for CF and L are noted in the text. Light signals are shifted to the right of CF for clarity of presentation. Note the more marked effects of halothane on both signals and the greater decrease in CF than in L at the high concentrations.

halothane than by isoflurane ( $P < 0.05$ ) at both the low and high concentrations.

Figure 5 displays the effects of isoflurane and halothane on  $TTP_{CF}$  and  $TTP_L$ . These measurements were made from the onset of the stimulus artifact to the time of peak CF and L, without a change of gain, and were expressed as percentage of controls (100%). Both anesthetics, but more so halothane ( $P < 0.05$ ), shortened  $TTP_{CF}$ . Isoflurane slightly decreased  $TTP_L$ , but the high concentration of halothane significantly lengthened  $TTP_L$  while it shortened  $TTP_{CF}$ . Figure 6 shows similarly that  $T-CF_{50}$  decreased in a dose-dependent fashion and to a greater extent with halothane than with isoflurane and that  $T-L_{50}$  increased markedly with halothane but was unchanged with isoflurane.

Figure 7 expresses percentage change in peak CF as a linear function of percentage change in peak L in five preparations when extracellular  $CaCl_2$  was increased incrementally from 3.6 to 5.4, 7.2, and 9.0 mM. For analysis, raw peak CF and peak L were normalized to 100% at 3.6 mM  $CaCl_2$  (control) in the absence and in the presence of the lower concentration of isoflurane or halothane. This normalization procedure was performed to standardize the reductions in L and CF by

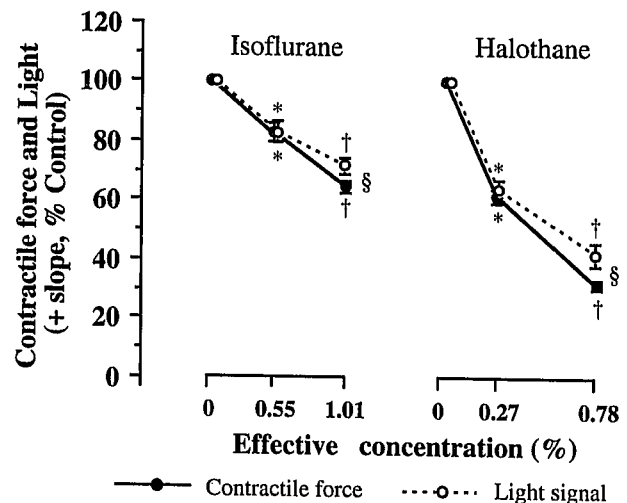


Fig. 4. Effects of two fractions of isoflurane and halothane on the maximal slope of contractile force and light signal measured at the half-peak responses. Data presented as in figure 3.

the two anesthetics to their nonanesthetic control with increases in  $[Ca^{2+}]_i$  at a given extracellular  $CaCl_2$  concentration. Linear regression analysis indicated that a onefold increase in the peak L was associated with about a 1.7-fold increase in peak CF in the absence of anesthetic, but only 0.7- and 0.5-fold increases in peak CF with halothane and isoflurane, respectively. The control

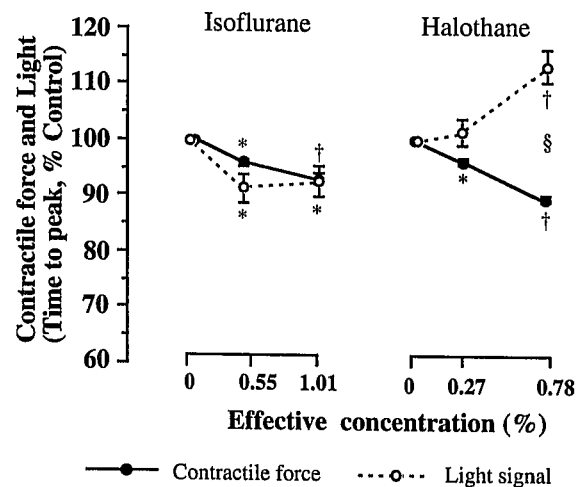


Fig. 5. Effects of isoflurane and halothane on time-to-peak contractile force ( $TTP_{CF}$ ) and time-to-peak light signal ( $TTP_L$ ) measure from the stimulus artifact to the peak response. Data presented as in figure 3. Note that  $TTP_{CF}$  decreased with both isoflurane and halothane but that  $TTP_L$  increased with the high concentration of halothane and decreased with isoflurane.

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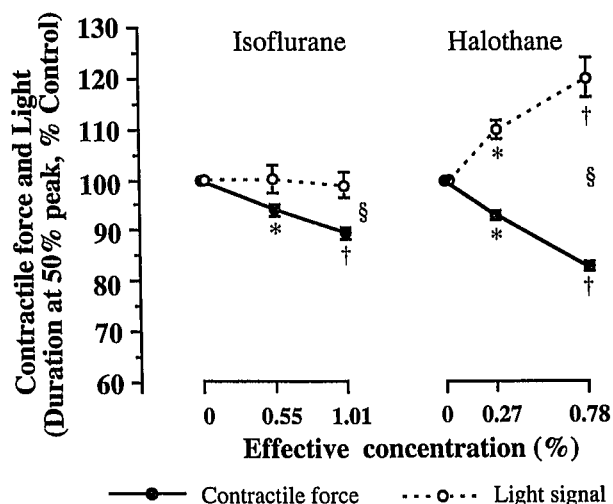


Fig. 6. Effects of isoflurane and halothane on duration of contractile force (CF) and light emission (L) measured from the 50% rise to the 50% fall of CF (T-CF<sub>50</sub>) and L (T-L<sub>50</sub>). Data presented as in figure 3. Note greater changes with halothane and the dose-dependent increase in T-L<sub>50</sub> coupled with a dose-dependent decrease in T-CF<sub>50</sub> with halothane compared with isoflurane.

slope was significantly greater ( $P < 0.05$ ) than the two anesthetic slopes, which did not differ significantly from each other.

## Discussion

These results in canine Purkinje fibers at equivalent MAC fractions demonstrate that (1) halothane depresses CF and Ca<sup>2+</sup> transients more than isoflurane, (2) halothane increases the time to attain peak Ca<sup>2+</sup> transients and increases the duration of Ca<sup>2+</sup> transients more than does isoflurane, and (3) both anesthetics decrease CF as a function of Ca<sup>2+</sup> transients. More specifically, we found that both anesthetics decreased peak CF and the rate of CF development, shortened the time to develop peak CF, and shortened the duration of CF (at T-CF<sub>50</sub>), and attenuated the increases in peak CF for a given increase in Ca<sup>2+</sup> transients with a stepwise increase in extracellular CaCl<sub>2</sub>. These contractile effects were accentuated by halothane more than by isoflurane. In contrast, halothane prolonged the time to attain peak Ca<sup>2+</sup> transients and prolonged the duration of Ca<sup>2+</sup> transients (at 50% of peak Ca<sup>2+</sup> transients), whereas isoflurane had little effect on these measurements. These differential effects of halothane and isoflurane are not likely to depend solely on anesthetic concen-

trations because differences in these variables persist even when the low concentration of halothane is contrasted to the high concentration of isoflurane.

Most evidence indicates that anesthetics alter availability of [Ca<sup>2+</sup>]<sub>i</sub> for the contractile mechanism.<sup>26,27</sup> Intracellular Ca<sup>2+</sup> is a two-compartment system such that influx of Ca<sup>2+</sup> through the sarcolemma slightly raises free intracellular, *i.e.*, myoplasmic Ca<sup>2+</sup>, which then triggers a much larger release of Ca<sup>2+</sup> from the SR into the myoplasm.<sup>28,29</sup> The release of SR Ca<sup>2+</sup> results in an augmented increase in myoplasmic Ca<sup>2+</sup> concentration during systole, and an increase in SR Ca<sup>2+</sup> occurs during diastole upon reuptake of myoplasmic Ca<sup>2+</sup>. A possible mechanism<sup>27</sup> for halothane's negative inotropic effect is based on observations that halothane increases Ca<sup>2+</sup> permeability of the SR<sup>26</sup> and decreases SR Ca<sup>2+</sup> uptake.<sup>30</sup> Thus, the major effect of volatile anesthetics may be to decrease the amount of Ca<sup>2+</sup> available for release from SR by directly activating Ca<sup>2+</sup> release channels, which reduces intracellular Ca<sup>2+</sup> stores; this depletion may be enhanced and maintained by depression of inward Ca<sup>2+</sup> current,<sup>7,8</sup> which further decreases Ca<sup>2+</sup> induced release of Ca<sup>2+</sup> from the SR. Halothane may not only deplete SR Ca<sup>2+</sup><sup>26,31</sup> but may also cause a loss of Ca<sup>2+</sup>

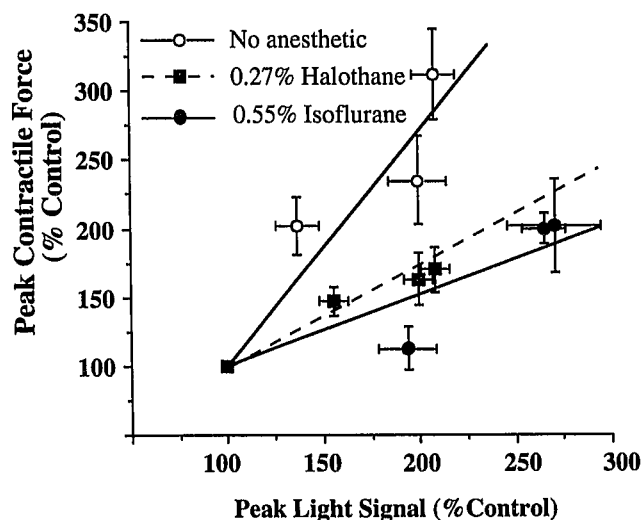


Fig. 7. Percent peak contractile force (CF) and as a function of percent peak light signals (L) in five Purkinje tissue preparations with incremental increases in suffusate CaCl<sub>2</sub> from 3.6 (control) to 5.4, 7.2, and 9.0 mM. Values for both variables obtained at 3.6 mM CaCl<sub>2</sub>, whether in the absence or presence of the low concentration of halothane or isoflurane, were normalized to equal 100%. Note that, in the presence of either anesthetic, peak CF increased less for a given increase in peak L, which suggests that both agents decrease myofibrillar Ca<sup>2+</sup> sensitivity.

from the whole cell because  $\text{Na}^+/\text{Ca}^{2+}$  exchange and, secondarily, the sarcolemmal  $\text{Ca}^{2+}$ -ATPase are very active in pumping  $\text{Ca}^{2+}$  out of the cell between contractions (presuming volatile anesthetics have any effects on the  $\text{Ca}^{2+}$ -ATPase and  $\text{Na}^+/\text{Ca}^{2+}$  exchange mechanism).<sup>32</sup> These effects likely account for the marked decrease in  $\text{Ca}^{2+}$  transients.<sup>6,19,33</sup> Another possibility is that volatile anesthetics directly alter  $\text{Ca}^{2+}$  binding to the contractile apparatus. One biochemical study, however, indicates that halothane has no direct effect on  $\text{Ca}^{2+}$  affinity of troponin C.<sup>34</sup>

We measured several characteristics of CF and L to infer effects of these anesthetics on the mechanisms of contraction. Depression of  $\text{Ca}^{2+}$  transients by anesthetics could result from reduced sarcolemmal influx and/or SR-induced  $\text{Ca}^{2+}$  efflux. A rapid rise in the  $\text{Ca}^{2+}$  transient primarily reflects  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release from the SR, such that equivalent anesthetic levels of halothane may slow SR  $\text{Ca}^{2+}$  release more than isoflurane. An increase in the time to attain peak  $\text{Ca}^{2+}$  transients by halothane but not by isoflurane, with reduced rates of development of CF, suggests that halothane has a more pronounced effect to slow  $\text{Ca}^{2+}$  release from the SR secondary to a lesser  $\text{Ca}^{2+}$  gradient between SR and cytoplasm. The increase in duration of  $\text{Ca}^{2+}$  transients by halothane but not by isoflurane suggests that halothane also slows removal of  $\text{Ca}^{2+}$  from myoplasm to SR. This combination of prolonged  $\text{Ca}^{2+}$  transients with an abbreviated contractile response also could result from a slower rate of  $\text{Na}^+/\text{Ca}^{2+}$  exchange or a greater depression of  $\text{Ca}^{2+}$  affinity for contractile proteins by halothane and by isoflurane. We indirectly tested the latter possibility by examining the relative amount of CF for a given increase in  $\text{Ca}^{2+}$  transients caused by increasing extracellular  $\text{CaCl}_2$ . Our data suggest that halothane and isoflurane similarly reduce  $\text{Ca}^{2+}$  sensitivity.

Depression of  $\text{Ca}^{2+}$  transients by isoflurane and halothane, as reported in the current study on canine Purkinje fibers, confirms our previous studies on cat<sup>17</sup> and guinea pig<sup>6</sup> papillary muscle at the same temperature, that the decline in isometric CF is preceded by a decrease in peak intracellular  $\text{Ca}^{2+}$ . Most studies also show that halothane depresses contractility more than does isoflurane at equianesthetic concentrations.<sup>1-5</sup> The current study confirms in another cardiac tissue model that isoflurane causes less pronounced but more proportional reductions of peak isometric force and  $\text{Ca}^{2+}$  transients than halothane. Our previous study in guinea pig papillary muscle<sup>6</sup> suggested that isoflurane de-

creases responsiveness of contractile proteins to activation by  $\text{Ca}^{2+}$  more than halothane. For Purkinje fibers, results are consistent with an equivalent decrease in sensitivity to  $\text{Ca}^{2+}$  at the myofibrillar apparatus by both agents. We have observed not only a qualitatively greater effect of halothane than isoflurane to depress  $\text{Ca}^{2+}$  transients but also a difference in the CF response to a given increase in  $\text{Ca}^{2+}$  transients during stepwise increases in extracellular  $\text{CaCl}_2$ . This suggests, along with other *in vitro* studies that have examined anesthetic effects on contractile characteristics, that volatile agents exert different cardiac effects by differentially affecting mechanisms for  $\text{Ca}^{2+}$  handling at the SR or other intracellular sites such as the contractile apparatus.<sup>26</sup> Volatile anesthetics also have been reported to decrease  $\text{Ca}^{2+}$  sensitivity in chemically treated cardiac cells.<sup>37</sup> This may be an indirect effect because halothane appears not to directly alter troponin C binding to  $\text{Ca}^{2+}$ .<sup>34</sup>

The mechanisms of the differential effects of halothane and isoflurane on contractile responses are better understood than the effects of these anesthetics to alter action potential characteristics, to slow impulses through the Purkinje system, or to promote pro- or antidysrhythmic effects.<sup>38</sup> The question arises if the observed and putative differences in calcium handling by volatile anesthetics in Purkinje cells have any relationship to conduction abnormalities or the potential for promoting or preventing dysrhythmias. Although volatile anesthetics probably alter many ionic fluxes across extracellular and intracellular membranes,  $\text{Ca}^{2+}$  is a major ion affected by these agents. Halothane shortens the plateau phase and duration of the action potential and its refractoriness in normal Purkinje fibers.<sup>9,12,13</sup> Halothane prolongs conduction through the His bundle in dogs.<sup>10</sup> However, in canine Purkinje fibers, 1.5 MAC halothane (0.36 mM) decreases conduction velocity by 26%, whereas 1.5 MAC isoflurane (0.45 mM) has no significant effect.<sup>14</sup> In normal tissue, action potential conduction depends mainly on  $\text{Na}^+$  ions as well as on  $\text{K}^+$  ions. Although volatile anesthetics can slow conduction in normal Purkinje tissue, especially at supra-clinical concentrations, it is doubtful that decreases in  $\text{Ca}^{2+}$  transients or inward current are wholly or even partially involved in altering conduction velocity in normal cardiac tissue, because any change in extracellular calcium or treatment with calcium channel blockers has little or no effect on intraventricular conduction. However, it has been reported that action potential conduction velocity in isolated guinea pig ven-

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tricular cells can be modulated by the  $\text{Ca}^{2+}$  current in the presence of discontinuous conduction caused by a relatively high resistance coupling between cells.<sup>15</sup> Thus, a decrease in  $[\text{Ca}^{2+}]_i$  by anesthetics might underlie slowed conduction across Purkinje fibers, particularly in scarred tissue with altered conduction pathways. In canine Purkinje cells, halothane and isoflurane equivalently depress both L (long-lasting)- and T (transient)-type  $\text{Ca}^{2+}$  channel current.<sup>8</sup> Suppression of L-type  $\text{Ca}^{2+}$  inward current across Purkinje cell sarcolemma by volatile anesthetics could contribute to a decrease in  $\text{Ca}^{2+}$  accumulation by the SR, as demonstrated by decreased peak  $\text{Ca}^{2+}$  transients, and provide protection against an increase in conduction delay caused by  $\text{Ca}^{2+}$  overload during or after reperfusion. Further studies are required to determine whether the effects of anesthetics on  $\text{Ca}^{2+}$  handling underlie altered conduction in normal, ischemic, and infarcted tissues.

Volatile anesthetics appear to have both pro- and antidysrhythmic effects on canine Purkinje fibers, depending on the conditions of the experiment.<sup>12</sup> Responses to  $\alpha$ - and  $\beta$ -adrenergic agonists form the basis for at least some of their differences in dysrhythmic effects.<sup>38,39</sup> T-type  $\text{Ca}^{2+}$  current depression might affect pacemaking potential and impulse conduction in Purkinje tissue,<sup>40</sup> especially during ischemic and reperfusion which increases  $[\text{Ca}^{2+}]_i$ . The greater depression of peak  $\text{Ca}^{2+}$  transients by halothane compared with isoflurane, coupled with a greater delay in attaining peak transients and a longer duration of transients, could account for part of the differences in the dysrhythmic effects of these anesthetics. Volatile anesthetics have been shown to be antidysrhythmic during reperfusion after coronary artery occlusion<sup>16,41</sup> and during reoxygenation after hypoxic perfusion.<sup>42</sup> At least part of this effect may result from depletion of SR  $\text{Ca}^{2+}$ , which may reduce the degree of calcium overload.<sup>43</sup> Moreover, the greater effect of halothane compared with isoflurane to reduce delayed afterdepolarization with induced triggered activity<sup>12,17,38</sup> could be a result of the greater decrease in  $\text{Ca}^{2+}$  transients by halothane subsequent to depletion of SR  $\text{Ca}^{2+}$ . With less intracellular  $\text{Ca}^{2+}$ , less  $\text{Na}^+$  might enter the cell *via* the 3:1  $\text{Na}^+/\text{Ca}^{2+}$  exchange mechanism and so reduce the depolarizing current, which results in afterdepolarizations. Indeed, depletion of SR  $\text{Ca}^{2+}$  by ryanodine has been shown to abolish late afterdepolarizations.<sup>44</sup>

In conclusion, halothane and isoflurane reduce CF of Purkinje cell myofilaments, at least in part, by reducing intracellular free  $\text{Ca}^{2+}$  concentration. Isoflurane ap-

pears to depress these factors less than halothane does at equianesthetic concentrations. Both agents appear to similarly decrease sensitivity of myofilaments to  $\text{Ca}^{2+}$ . It is difficult to interpret what role changes in  $\text{Ca}^{2+}$  transients might play in conduction of impulses or development of ectopic impulses in Purkinje fibers. Purkinje fibers contribute little to overall myocardial contractile activity, but they play a major role in conduction of impulses from the atrioventricular node to all regions of the ventricles. The clinical implication for the impact of these anesthetics on cardiac Purkinje tissue more likely reflects their relative effects on altering  $[\text{Ca}^{2+}]_i$  than their effects on altering CF.

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