Comparison of the In Vitro Myocardial Depressant Effects of Isoflurane and Halothane Anesthesia

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The myocardial depressant effects of isoflurane and halothane were compared using feline right ventricular papillary muscles bathed in Krebs-bicarbonate solution. In experiment 1 muscles were stimulated by field electrodes (0.2 Hz) to obtain control measurements of developed tension (dT) and maximal rate of tension development (dF/dt) prior to exposing the papillary muscles to four concentrations of either isoflurane (4.0%, 2.0%, 1.0%, 0.5%) or halothane (2.0%, 1.0%, 0.5%, 0.25%). Repeat measurements of dT and dF/dt were recorded after 20 min at each concentration. Isoflurane and halothane both caused dose-dependent depression of dT and dF/dt, but at 0.5%, 1.0%, and 2.0%, halothane was significantly more depressant than isoflurane (P < 0.01 for dT and dF/dt). Quadratic equations were fitted to the dose–response data by least squares analysis (R² > 0.985 for both anesthetics), and the isoflurane and halothane concentrations that decreased dT to 90%, 70%, 50%, and 30% of control were determined to compare the relative myocardial depressant potency of isoflurane and halothane by linear regression analysis. This potency relationship is described by the equations: isoflurane concentration = −0.005 + 1.445 (halothane concentration). In experiment 2 papillary muscle responses at two similar cardiodepressant concentrations of isoflurane (1.25% and 2.0%) or halothane (0.8% and 1.35%) were compared at stimulus frequencies of 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, and 2.0 Hz. The concentrations of isoflurane and halothane were selected from the data obtained in experiment 1 and represent the anesthetic concentrations that diminish muscle function to approximately 70% and 50% of control. After control measurements of dT were obtained at all stimulus frequencies, the muscles were exposed to either isoflurane or halothane for 20 min. Repeat measurements of dT at the different stimulus rates were obtained. At lower stimulus rates (range, 0.05–1.0 Hz) halothane and isoflurane caused comparable depression of dT, but both anesthetics demonstrated some degree of frequency dependence in that their depressant effects diminished as muscle stimulation rates increased. However, at high muscle stimulation rates (2.0 Hz) isoflurane was significantly less depressant than halothane (P < 0.001). The observed frequency dependence of both halothane and isoflurane suggest that the negative inotropic effects of both agents may involve inhibition of extracellular Ca²⁺ influx; however, the depressant effects of isoflurane are more effectively attenuated by stimulus conditions that enhance extracellular Ca²⁺ influx when muscle stimulation rates approach the physiologic range. (Key words: Anesthetics, volatile; halothane; isoflurane. Heart: contractility; papillary muscles; rate response. Myocardium: force–frequency effect; isometric contraction.)

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potencies of isoflurane and halothane. The effect of similar cardiodepressant concentrations of both anesthetics were then compared at varying muscle stimulation rates (frequency effect) to assess whether the depressant actions of isoflurane and halothane are differentially attenuated by alterations in the frequency of muscle stimulation.

Methods

Adult cats (1.5–2.5 kg) were anesthetized with intra-peritoneal sodium pentothal (35 mg/kg). Once the animal was asleep and had no response to painful stimuli, the heart was removed and transferred to a dish containing aerated Krebs-bicarbonate solution. One or two thin right ventricular papillary muscles (mean cross-sectional area of 1.14 ± 0.08 mm²) were excised and suspended in a jacketed muscle bath containing Krebs-bicarbonate solution (concentration in mM: Na⁺, 143; K⁺, 4.5; Mg²⁺, 0.5; Ca²⁺, 2.5; Cl⁻, 124; H₂PO₄⁻, 1.2; HCO₃⁻, 24; glucose, 11). The solution was oxygenated with 95% O₂ plus 5% CO₂, and maintained at 31°C and pH 7.4. The non-tendinous end of the muscle was held by a clip secured to the bottom of the muscle bath. The tendinous end was attached by a silk suture to a force transducer (Grass FT03) fixed to a micrometer used to alter papillary muscle length by known amounts. The muscle was stimulated at 0.2 Hz with square-wave pulses 1 ms in duration by field electrodes placed parallel to the long axis of the muscle. Stimulus voltage was maintained at 10% above threshold values. Each muscle was initially stretched to 0.5 g resting tension and allowed to contract isometrically for 30 min. After contractile force had stabilized and initial length-tension curve was established, the muscle was then rinsed and allowed to contract at original resting tension. A second isometric length–tension relation was determined by stretching the muscle by known increments from a length at which active force development was first apparent to the point at which active force first declined from its maximum. The muscle length was then fixed at peak active force development (Lmax), and the muscle was allowed to stabilize for 45 min prior to any experimental intervention. Control measurements of developed tension (dt), and the electronically derived maximal rate of tension development (dF/dt) were then obtained (Gould Differentiator). Measurements of dt were expressed in terms of muscle cross-sectional area (g/mm²) as determined from the wet weight and length measured at Lmax and as the percent of control. Measurements of dF/dt were expressed only as the percent of control. This muscle stabilization sequence was performed prior to both experiments 1 and 2.

Experiment 1: Dose–Response

After obtaining control measurements of dt and dF/dt papillary muscles were exposed to four concentrations of either isoflurane (4.0%, 2.0%, 1.0%, and 0.5%; n = 5) or halothane (2.0%, 1.0%, 0.5%, and 0.25%; n = 4) beginning with the highest and ending at the lowest concentration. This range of concentrations was selected on the basis of pilot data, which showed that the low concentrations produced a small but consistently discernible depressant effect on papillary muscle contractility, whereas the high concentrations reduced muscle tension by a minimum of 50%. During the experiment the anesthetics were delivered into the muscle bath by the use of calibrated vaporizers (100F and 100H Ohio Medical) connected through the CO₂ + O₂ delivery line. The accuracy of the vaporizers and the delivered anesthetic concentrations were monitored by mass spectrometry (Perkin Elmer MGA 1100A). Each anesthetic concentration was delivered into the bath for a minimum of 20 min to allow muscle function to stabilize at a new steady state. Measurements of dt and dF/dt were then obtained. The anesthetic was then discontinued, the bath medium was replaced with fresh solution, and papillary muscle function returned to stable baseline values within 20–30 min. After complete muscle recovery measurements of dt and dF/dt were repeated and compared to the preanesthetic control measurements prior to administration of the next anesthetic concentration. This sequence was repeated until the papillary muscle had been exposed to all four concentrations of either isoflurane or halothane.

Each papillary muscle served as its own control. Muscle responses to each anesthetic concentration were compared to the muscle recovery control measurements of dt and dF/dt that immediately preceded the administration of that particular concentration. The data from each anesthetic group were analyzed to assess the significance of the anesthetic, the concentration, and anesthetic by concentration interaction effects, using analysis of variance for repeated measures. This analysis was performed at the concentration levels common to both anesthetics (0.5%, 1.0%, and 2.0%). Polynomial equations were fitted to the dose–response data obtained for each anesthetic by using a least squares analysis to detect a functional relationship between papillary muscle response and anesthetic concentration level.

Experiment 2: Frequency Response

Papillary muscles were randomly assigned to either the halothane (n = 7) or isoflurane (n = 6) group. After baseline measurements of dt were obtained at 0.2 Hz each muscle was sequentially stimulated at rates of 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, and 2.0 Hz. Each stimulation rate was maintained until a steady state was observed; then repeat measurements of dt were obtained. Peak tension had a tendency to decline when faster stimulation rates (1.0 and 2.0 Hz) were maintained for more than 60 s; therefore,
muscle performance was measured in the stable period that preceded this decline in function. This phenomenon has been observed by others\(^8\) and may represent hypoxia of the muscle core or fatigue. After stimulation at 2.0 Hz the muscle was rinsed and allowed to return to baseline tension at the control stimulation rate (0.2 Hz).

Papillary muscles were then exposed to two concentrations of either halothane (0.80% and 1.35%) or isoflurane (1.25% and 2.0%) administered in random order. The selection of these anesthetic concentrations was based on the data obtained in experiment 1 and represent the concentrations of each agent that depress muscle function to approximately 70% (halothane 0.80%, isoflurane 1.25%) or 50% (halothane 1.35%, isoflurane 2.0%) of control. Each anesthetic concentration was administered for 20 min at baseline stimulation rates (0.2 Hz) to allow muscle function to stabilize prior to initiating the sequence of increasing stimulation frequency as outlined above. Measurements of dt were obtained at all stimulation frequencies for each anesthetic concentration.

In experiment 2 each papillary muscle served as its own control. Muscle responses to each anesthetic were compared to control measurements of muscle performance at each stimulation frequency, and muscle responses at the anesthetic concentrations depressing muscle function 70% and 50% were compared between anesthetics. The data were analyzed as a three-factor repeated measures design with the factors defined as the anesthetic (halothane, isoflurane) with repeated measures at each frequency level (0.05–2.0 Hz), and anesthetic concentration at two levels (70% and 50%). The results of the overall analysis of variance indicated a significant two-factor interaction; therefore, the data were analyzed separately for each anesthetic concentration level. Halothane versus isoflurane simple effects were then tested at each frequency level after combining the appropriate error terms and calculating the adjusted degrees of freedom for each of the separate analyses.\(^{11}\) Alterations in dt for each anesthetic concentration at the different stimulation frequencies were expressed as the percent of control (mean ± SD).

### Results

Baseline papillary muscle function remained stable throughout the experimental period. There were no significant differences between preanesthetic control measurements of dt and dF/dt, and those recorded after complete muscle recovery between anesthetic doses. The stability of the experimental preparation is also indicated from the data describing the changes in dt (percent of control) in response to each anesthetic concentration (table 1). The consistent magnitude of the standard deviations (1.4–2.9) and the narrow spread from minimum to maximum muscle responses (2.9–6.9) across anesthetic agents and concentrations indicate the stability of the muscle preparation over the experimental period.

In experiment 1 halothane and isoflurane both caused a dose-dependent depression of dt and dF/dt (table 1), but at common anesthetic concentrations (0.5%, 1.0%, and 2.0%) halothane was a significantly more potent myocardial depressant than isoflurane (P < 0.01 at each concentration) (table 2). Alterations in dt in response to each anesthetic concentration were similar to those noted for dF/dt (table 1). A general quadratic polynomial was fitted to the dt data obtained for each anesthetic for the purpose of profiling a dose–response curve.

The quadratic equations provide excellent fit over the range of anesthetic concentrations studied (R\(^2\) > .985 for
both halothane and isoflurane). The coefficients of the fitted curves for isoflurane and halothane are shown in Table 3, and the curves, as well as the observed mean depression values, are plotted in Figure 1.

The quadratic equations were subsequently used to examine the relationship between the relative potency of halothane and isoflurane with regard to their cardiodepressant effects. This was performed by determining the least squares linear regression of isoflurane concentrations onto the corresponding halothane concentrations based on the calculated 90%, 70%, 50%, and 30% depressant levels for both anesthetics. The coordinates for these pairs (at potencies of 90%, 70%, 50%, and 30% depression) and the estimated line are plotted in Figure 2. The results of the regression analysis demonstrate that the potency of isoflurane’s depressant effect is approximately linearly related to the potency of halothane, and can be described by the equation: isoflurane concentration = -0.005 + 1.445(halothane concentration). The statistics for the linear fit are $R^2 = 0.994$, $S_{y,x} = 0.095$, and the standard error of the estimated slope = 0.078. It is apparent from the regression analysis that these two anesthetics exert comparable myocardial depressant effects over the range of 30–90% depression at isoflurane concentrations that are approximately 1.45 times greater than those of halothane.

**Frequency Response**

Alterations in muscle stimulation frequency attenuated the myocardial depressant effects of both halothane and isoflurane. Anesthetic-induced alterations in $dt$ (expressed as percent of control) over the seven frequencies at the 70% and 50% depressant concentrations of isoflurane and halothane are plotted in Figure 3. The myocardial depressant effects of both isoflurane and halothane were attenuated by increases in muscle stimulation rates (frequency effect) at low and high anesthetic concentrations. This frequency-dependent decrease in anesthetic-induced myocardial depression did not differ significantly between the halothane and isoflurane groups at either concentration until high muscle stimulation rates (2.0 Hz) were obtained. At 2.0 Hz halothane still caused significant depression of $dt$, whereas isoflurane did not depress $dt$ significantly from control. This differential effect in the frequency dependence of these two anesthetics at high muscle stimulation rates (2.0 Hz) was significant for both the 70% ($P = 0.001$) and 50% concentrations ($P < 0.0001$).

**Discussion**

The volatile anesthetics are myocardial depressants; yet in clinical studies isoflurane has been associated with less myocardial depression than halothane.\(^{13,12}\) It has been suggested that the systemic vasodilation that accompanies isoflurane administration may preserve myocardial func-

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**Table 3. Polynomial Dose–Response Data for $dt$**

<table>
<thead>
<tr>
<th>Anesthetic</th>
<th>Intercept</th>
<th>Linear</th>
<th>Quadratic</th>
<th>$S_{xx}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>105.69</td>
<td>-43.02</td>
<td>2.64</td>
<td>2.57</td>
<td>.991</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>112.23</td>
<td>-59.64</td>
<td>4.01</td>
<td>2.38</td>
<td>.994</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Dose–response data for alterations in $dt$ (percent of control) following halothane (•) or isoflurane (□) administration with corresponding least squares quadratic fits. Data points are given as mean values ± SD.
tion by off-loading the ventricle, thereby allowing the compromised myocardium to normalize its contractile performance. Others have suggested that the negative inotropic effects of isoflurane may be attenuated by sympathetic nervous activity in the intact heart. In an effort to more accurately assess the direct myocardial effects of these two agents, Horan et al. compared several indices of myocardial contractility during halothane and isoflurane anesthesia in the dog. Left ventricular dP/dt, maximum aortic acceleration, and peak left ventricular power were all significantly greater during isoflurane anesthesia. Stroke volume decreased significantly, but cardiac output was maintained by a compensatory increase in heart rate. In a similar in vivo study halothane and isoflurane caused comparable depression of myocardial performance, but halothane alone increased left ventricular end-diastolic pressure, thereby suggesting that halothane was a slightly greater in vivo myocardial depressant than isoflurane.

In vitro studies of papillary muscle function permit the direct assessment of anesthetic-induced alterations in myocardial contractility without the confounding influence of secondary peripheral vascular or neurohumoral changes on myocardial performance. Previous studies have shown that halothane and isoflurane are comparable in vitro myocardial depressants at similar MAC anesthetic concentrations. In the present study we first compared the negative inotropic effects of halothane and isoflurane at common anesthetic concentrations (0.5%, 1.0%, and 2.0%) in order to determine a linear regression equation that would describe the relative cardiodepressant potency of these two anesthetics. From this analysis isoflurane and halothane exert comparable in vitro myocardial depressant effects at isoflurane concentrations that are approximately 1.45 times greater than those of halothane when muscle performance is compared at low stimulation rates. This relationship is similar to that described by Lynch who noted that these two anesthetics were comparable in vitro depressants at low muscle stimulation rates when compared at anesthetic concentrations of 1:1.7 for halothane and isoflurane, respectively.

It is recognized that contractile force can be altered by changing the rate and/or pattern of myocardial muscle stimulation. The positive inotropic effect that is observed during an increase in muscle stimulation rate (positive staircase) predominantly reflects an increase in the transsarcolemmal (extracellular) influx of Ca²⁺. The contribution of enhanced intracellular Ca²⁺ release by the SR during the positive staircase has not been clearly delineated; however, previous studies suggest that SR-mediated Ca²⁺ release does not significantly contribute to the positive inotropy that accompanies an increase in muscle stimulation rates. This interpretation is supported by the observation that ryanodine (an inhibitor of SR-mediated Ca²⁺ release) does not alter frequency-dependent positive inotropy. Although several studies have shown that isoflurane and halothane are comparable in vitro myocardial depressants at low muscle stimulation rates, it was recently demonstrated that isoflurane is significantly less depressant than halothane when myocardial muscle performance is compared at high muscle stimulation rates. Like Komai and Rusy, we observed that the negative inotropic actions of both halothane and isoflurane are progressively attenuated by increasing muscle stimulation rates (positive staircase). However, at high muscle stimulation rates (2.0 Hz) isoflurane’s negative inotropic effects are completely reversed, whereas halothane continues to exert a significant myocardial depressant effect. Lynch also noted that isoflurane’s negative inotropic effects are significantly less than those of halothane when myocardial muscle performance is compared at high stimulation frequencies; yet at lower frequencies he did not observe any frequency-dependent attenuation of halothane’s negative inotropic effect. These discrepant observations regarding halothane’s frequency-dependence are unexplained but may reflect species differences or temperature-related variations in myocardial muscle responsiveness. However, in an earlier study Lynch et al. demonstrated that halothane decreased the amplitude and maximum rate of rise of the slow action potential. This response suggests that halothane exerts an inhibitory action on the influx of extracellular Ca²⁺ and is consistent with our results demonstrating that halothane’s negative
inotropic effect can be partially attenuated by stimulus conditions that enhance extracellular Ca\(^{2+}\) influx.

It is apparent from our results and that of other investigators\(^8,^9\) that the myocardial depressant effects of isoflurane are significantly more attenuated than those of halothane when myocardial muscle performance is compared at high muscle stimulation rates. Because the depressant actions of isoflurane are completely attenuated by stimulus conditions that enhance extracellular Ca\(^{2+}\) influx, it suggests that the negative inotropic action of isoflurane is more directly mediated by inhibition of transsarcolemmal Ca\(^{2+}\) influx. In the present study we did not assess papillary muscle performance under conditions that would selectively alter SR-mediated Ca\(^{2+}\) release; therefore, we cannot comment on the relative depressant potencies of either halothane or isoflurane on extracellular or intracellular Ca\(^{2+}\) metabolism.

The mechanisms underlying the myocardial depressant
effects of the volatile anesthetics are not clearly understood, and there is considerable controversy regarding the extent to which isoflurane and halothane alter myocardial extracellular and intracellular Ca\(^{2+}\) metabolism. This controversy reflects the inherent difficulties in inferring the subcellular site of anesthetic-induced myocardial depression based on studies of in vitro papillary muscle function. This problem was recently addressed in a comprehensive review\(^{17}\) that presented many of the limitations of interpretation and application of the observations from in vitro myocardial muscle studies to infer the subcellular site of action involved in anesthetic-induced myocardial depression. Although it appears that the mechanism underlying the myocardial depressant effects of isoflurane and halothane differ, further delineation of the cellular processes that control and alter myocardial contractility are necessary to determine the sites at which the volatile anesthetics exert their primary negative inotropic effects.

References


