Effects of sevoflurane on the contractility of ferret ventricular myocardium

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Bartunek, Anna E., and Philippe R. Housmans. Effects of sevoflurane on the contractility of ferret ventricular myocardium. J Appl Physiol 89: 1778–1786, 2000.—Isotonic and isometric variables of contractility and relaxation of isolated ferret right ventricular papillary muscles were measured before and during exposure to incremental concentrations of sevoflurane (0–4.9% vol/vol) (30°C) (n = 9). In a second group of muscles (n = 8), effects of sevoflurane were compared with those of low [Ca\textsuperscript{2+}] (0.45–2.25 mM in steps of 0.45 mM). Sevoflurane caused a reversible concentration-dependent decrease in contractility (ED\textsubscript{50} of developed force 4.6 ± 0.9% vol/vol). When compared with twitches of equal amplitude in low extracellular Ca\textsuperscript{2+} concentration, sevoflurane accelerated both isometric and isotonic relaxation. The myocardial depressant effect of sevoflurane is less than that of isoflurane and results mainly from a decrease of intracellular Ca\textsuperscript{2+} availability. The abbreviated isometric relaxation likely reflects a decrease in Ca\textsuperscript{2+} sensitivity and the faster isotonic relaxation may reflect a mild stimulation of Ca\textsuperscript{2+} uptake by the sarcoplasmic reticulum.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the Mayo Foundation, with protocols completed in accordance with National Institutes of Health guidelines and in accordance with the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council). Adult male ferrets (weighing 1,100–1,500 g and 16–19 wk of age) were anesthetized with pentobarbital sodium (100 mg/kg ip). Suitable papillary muscles were carefully excised and mounted vertically in a temperature-controlled (30°C) muscle chamber that contained a physiological salt solution made up in ultrapure water (Nanopure Infinity, Barnstead, Dubuque, IA) and with the following composition (in mM): 137.5 Na\textsuperscript{+}, 5.0 K\textsuperscript{+}, 2.25 Ca\textsuperscript{2+}, 1.0 Mg\textsuperscript{2+}, 124.5 Cl\textsuperscript{-}, 1.0 SO\textsubscript{4}\textsuperscript{2-}, 20.0 acetate, 10.0 glucose, 5.0 MOPS, pH 7.40, bubbled with 100% O\textsubscript{2}. Experiments were carried out at 30°C and at a stimulus frequency of 0.25 Hz; isolated papillary muscle function is stable for many hours in these conditions. Suitable preparations were selected on the basis of previous studies in guinea pig, rat, and dog ventricle and in human atrium (2, 10, 16–19, 38). The negative inotropic effect has been attributed to a decrease of transsarcolemmal Ca\textsuperscript{2+} influx, and the effect on SR Ca\textsuperscript{2+} release seems to be modest (2, 17, 38). Studies in skinned (37) and intact (23, 24, 28) cardiac fibers provided ample evidence that halothane, enfurane, and isoflurane decrease myofibrillar Ca\textsuperscript{2+} sensitivity to some extent. There is no definitive information on whether sevoflurane may also decrease myofibrillar Ca\textsuperscript{2+} sensitivity, although recently a depressing effect on cross-bridge cycling kinetics was shown in skinned rat cardiac fibers (40). The aim of this study was to evaluate effects of sevoflu-
The first contraction was an isotonic twitch at the preload of relaxation were determined from three types of contraction. The concentration of sevoflurane was measured continuously to zero during the latent period (5). Muscles shortened devoid of agonistic effects in heart muscle (31). Waveforms of sevoflurane concentrations were used: 0.7, 1.4, 2.1, 2.7, 3.4, 4.1, and 4.8% (vol/vol). These concentrations correspond to 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, and 1.75 minimum alveolar concentration (MAC) in the ferret. MAC is an anesthetic half-maximal effective dose (ED50) as defined by Eger et al. (12). One MAC is the concentration of anesthetic at which 50% of the animals respond to a standardized supramaximal stimulus with “gross purposeful muscular movements of body or extremities” (12). It is a measure of anesthetic equipotency. Sevoflurane MAC in the ferret was calculated to be 2.7% (vol/vol) from the following data. The MAC values for isoflurane and halothane in the ferret are 1.52% and 1.01% (vol/vol) (36). The ratio of isoflurane MAC to halothane MAC in the ferret is 1.5, similar to that found in human, dog, and horse (41). Sevoflurane MAC values for human, dog, and horse are reported to be 2.05, 2.36, and 2.31, respectively (1, 32, 42). The halothane MAC values for human, dog, and horse are reported to be 0.75, 0.86, and 0.88, respectively (41). We calculated the MAC value for sevoflurane in the ferret assuming that the relative potency ratio of sevoflurane to halothane is close to that in human (2.73), dog (2.74), and horse (2.63) as well, which brings us to an estimate of 2.7% (vol/vol) in the ferret as used in this study. After the highest concentration, the vaporizer was turned off, and the reservoir bag was emptied. Muscle recovery was then followed under identical conditions, and variables of contraction and relaxation were recorded at 15 min and 30 min of recovery.

In each muscle of group 2 (n = 8), variables of contraction and relaxation and load sensitivity of relaxation were determined in each step of consecutive cumulative concentration-effect experiments, the first for \([Ca^{2+}]_i\) and the second for sevoflurane. The \([Ca^{2+}]_i\)-effect protocol was carried out from 0.45 to 2.25 mM in steps of 0.45 mM. After the \([Ca^{2+}]_i\)-effect experiment, the bathing solution was changed, and the sevoflurane concentration-effect protocol was started with a new (zero-anesthetic) control. The following incremental sevoflurane concentrations were used: 0.7, 1.4, 2.1, 2.7, 3.4, 4.1, 4.8, and 5.4% (vol/vol). Recording of test contractions for analysis of contraction and relaxation was started after 12–

### Table 1. Muscle characteristics during control conditions at \(L_{\text{max}}\)

<table>
<thead>
<tr>
<th>Group 1 (n = 9)</th>
<th>(L_{\text{max}}), mm</th>
<th>CSA, mm²</th>
<th>R, mN/mm²</th>
<th>T, mN/mm²</th>
<th>R/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>5.16 ± 1.02</td>
<td>0.47 ± 0.18</td>
<td>6.17 ± 2.15</td>
<td>35.23 ± 15.19</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Range</td>
<td>4.00–7.00</td>
<td>0.18–0.81</td>
<td>3.70–9.32</td>
<td>19.78–63.78</td>
<td>0.12–0.24</td>
</tr>
<tr>
<td>Group 2 (n = 8)</td>
<td>Mean ± SD</td>
<td>5.35 ± 0.56</td>
<td>0.49 ± 0.18</td>
<td>8.11 ± 2.41</td>
<td>62.34 ± 17.90</td>
</tr>
<tr>
<td>Range</td>
<td>4.64–6.42</td>
<td>0.31–0.86</td>
<td>5.23–12.70</td>
<td>34.86–86.93</td>
<td>0.11–0.19</td>
</tr>
</tbody>
</table>

\(L_{\text{max}}\), length at which twitch active force is maximal; CSA, cross-sectional area; R, resting tension; T, total tension.
15 min, when twitch height in a particular [Ca\(^{2+}\)]\(_o\), or sevoflurane concentration was stable.

This study was carried out in identical conditions as those previously published (24, 26, 27), except for minor differences in the physiological salt solution used. The current study used the organic pH buffer MOPS, a convenient and reliable alternative to the more traditional bicarbonate-CO\(_2\) buffer. MOPS buffer is widely used in muscle tissue studies because it does not require bubbling with an O\(_2\)-CO\(_2\) mixture, and MOPS does not affect smooth muscle contractility (30). MOPS does not affect cardiac muscle contractility in the conditions of our experiments, because 1) peak developed force and other measures of contractility in control conditions are similar to those found in previous studies in which we used a bicarbonate-CO\(_2\) buffer system, and 2) muscles maintained their contractile performance for many hours in these conditions.

Statistical analysis. The relationship between contractile variables and sevoflurane concentration was subjected to least squares linear regression analysis (11) for each individual muscle. Student’s t-tests followed for subsequent calculations. Effect sizes with repeated-measures ANOVA followed by pairwise comparisons when applicable with Student’s t-tests and Bonferroni correction. For each muscle and for each contractile variable (DF, DL, MUVS), two values were obtained for the slope of the linear regression between percent change from control [Ca\(^{2+}\)]\(_o\) and sevoflurane concentration. Boxplots in isometric twitches (Fig. 1D). Sevoflurane slightly decreased the duration of isotonic shortening (TDL) (Fig. 1D). Sevoflurane slightly increased TMUVS of zero-load clamped twitches only at two concentrations (Fig. 1B). Sevoflurane decreased the maximal +dF/dt and −dF/dt in isometric twitches (Fig. 2A), and peak velocities of shortening and of lengthening in isotonic twitches at the preload of L\(_{max}\) all in a concentration-dependent fashion (Fig. 2B). Except for those listed below, variables of contraction and relaxation returned to control values 15 and 30 min after sevoflurane was discontinued. The variables −dF/dt, +dF/dt, and −V had recovered to values above control (−V in 7 out of 9 muscles, −dF/dt in 8 out of 9 muscles, and +dF/dt in all muscles). Relaxation of ferret papillary muscle is sensitive to load during control conditions, and sevoflurane had no significant effect on load sensitivity of relaxation. Table 3 lists the ED\(_{50}\) values of sevoflurane for several variables calculated from least squares linear regression. The ED\(_{50}\) value for MUVS is larger than that for DF and DL (P < 0.001). The ED\(_{50}\) value (DF) for sevoflurane was statistically significantly larger than that for halothane (P < 0.001), enfurane (P < 0.001), and isoflurane (P < 0.02) obtained in identical experimental conditions (26). The statistical analysis of sevoflurane was performed with one-way ANOVA followed by pairwise comparisons when appropriate with Student’s t-test with Bonferroni correction.

TPF, RTH, and load sensitivity of relaxation were displayed as a function of peak developed force, and least squares linear regression in each individual muscle was carried out. Slope values within [Ca\(^{2+}\)]\(_o\) and sevoflurane were tested for statistically significant differences from zero using a one-sample t-test. Slope values of [Ca\(^{2+}\)]\(_o\) vs. sevoflurane experiments were compared for differences by Student’s paired t-test. A similar procedure was applied to differences between [Ca\(^{2+}\)]\(_o\) and sevoflurane concentration for TDL and −V as a function of DL and for TMUVS as a function of MUVS. P < 0.05 was taken as the level for statistical significance of differences.

RESULTS

Table 2 summarizes the absolute values of variables of contraction and relaxation at the onset of the experiments of group 1 (n = 9) and group 2 (n = 8) muscles. Effects of sevoflurane on contraction and relaxation. Figures 1 and 2 illustrate the key observations relating to the concentration-effect experiments. Sevoflurane caused a reversible concentration-dependent decrease in DF, DL, and MUVS (Fig. 1, A and B). Sevoflurane abbreviated TPF and RTH (Fig. 1C). Sevoflurane slightly decreased the duration of isotonic shortening (TDL) (Fig. 1D). Sevoflurane slightly increased TMUVS of zero-load clamped twitches only at two concentrations (Fig. 1B). Sevoflurane decreased the maximal +dF/dt and −dF/dt in isometric twitches (Fig. 2A), and peak velocities of shortening and of lengthening in isotonic twitches at the preloads of L\(_{max}\), in all a concentration-dependent fashion (Fig. 2B). Except for those listed below, variables of contraction and relaxation returned to control values 15 and 30 min after sevoflurane was discontinued. The variables −dF/dt, +dF/dt, and −V had recovered to values above control (−V in 7 out of 9 muscles, −dF/dt in 8 out of 9 muscles, and +dF/dt in all muscles). Relaxation of ferret papillary muscle is sensitive to load during control conditions, and sevoflurane had no significant effect on load sensitivity of relaxation.

Table 3 lists the ED\(_{50}\) values of sevoflurane for several variables calculated from least squares linear regression. The ED\(_{50}\) value for MUVS is larger than that for DF and DL (P < 0.001). The ED\(_{50}\) value (DF) for sevoflurane was statistically significantly larger than that for halothane (P < 0.001), enfurane (P < 0.001), and isoflurane (P < 0.02) obtained in identical experimental conditions (26). Comparison of effects of sevoflurane with those of decreased [Ca\(^{2+}\)]\(_o\). Figure 3 illustrates the concentration-effect relationships between variables of contractility and relaxation, and variables calculated from least squares linear regression. Slopes were statistically significant for the variables of contractility and relaxation in 9 of 9 muscles, and sevoflurane concentration for TDL and −V as a function of DL and for TMUVS as a function of MUVS. P < 0.05 was taken as the level for statistical significance of differences.

Table 2. Variables of contraction and relaxation at the onset of the experiment

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 9)</th>
<th>Group 2 (n = 8)</th>
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<tbody>
<tr>
<td>DF, mN/mm(^2)</td>
<td>29.06 ± 13.50</td>
<td>54.23 ± 16.04</td>
</tr>
<tr>
<td>+dF/dt, mN-mm(^{-2})-s(^{-1})</td>
<td>163.36 ± 69.91</td>
<td>300.56 ± 78.84</td>
</tr>
<tr>
<td>−dF/dt, mN-mm(^{-2})-s(^{-1})</td>
<td>110.92 ± 43.43</td>
<td>177.99 ± 45.95</td>
</tr>
<tr>
<td>TPF, ms</td>
<td>332 ± 44</td>
<td>357 ± 52</td>
</tr>
<tr>
<td>RTH, ms</td>
<td>239 ± 54</td>
<td>280 ± 48</td>
</tr>
<tr>
<td>DL, L/L(_{max})</td>
<td>0.12 ± 0.01</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>TDL, ms</td>
<td>308 ± 29</td>
<td>327 ± 37</td>
</tr>
<tr>
<td>MUVS, L(_{max})/s</td>
<td>1.49 ± 0.34</td>
<td>2.08 ± 0.40</td>
</tr>
<tr>
<td>TMUVS, ms</td>
<td>60 ± 9</td>
<td>55 ± 7</td>
</tr>
<tr>
<td>−V, L(_{max})/s</td>
<td>1.84 ± 0.53</td>
<td>3.33 ± 0.81</td>
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</table>

Values are means ± SD. DF, developed force; +dF/dt, maximal rate of rise of force; −dF/dt, maximal rate of fall of force; TPF, time to peak force; RTH, time from peak force to half isometric relaxation; DL, peak shortening in fraction of L\(_{max}\) (L/L\(_{max}\)); TDL, time to peak shortening; MUVS, maximal unloaded velocity of shortening; TMUVS, time to maximal unloaded velocity of shortening; −V, maximal velocity of lengthening.
tion (DF, DL, and MUVS) and \([Ca^{2+}]_o\) (Fig. 3A) and sevoflurane concentration (Fig. 3B). Sevoflurane is most depressant on DF, less on DL, and least on MUVS. Almost the same order of sensitivity is found when these variables are measured in \([Ca^{2+}]_o\)-effect experiments in the same muscles: DF is decreased most, and MUVS and DL are decreased to the same extent in lower \([Ca^{2+}]_o\). Changes in \([Ca^{2+}]_o\) or in sevoflurane concentration had effects on DF, DL, and MUVS that were statistically different among these variables. Force development changed more than did DL and MUVS with changes either of \([Ca^{2+}]_o\) or of sevoflurane concentration. Differences between DL and MUVS were statistically significant for changes in sevoflurane concentration but not for changes in \([Ca^{2+}]_o\).

Because of the similarities between the \([Ca^{2+}]_o\) and the sevoflurane concentration-effect curve, it is conceivable that most if not all of the anesthetic action can be related to changes of intracellular calcium availability. To test this hypothesis, the anesthetic potency ratio \(W = \text{slope (sevoflurane)/slope (log([Ca^{2+}]_o))}\) values were measured for each muscle for DF, DL, and MUVS and tested for significant differences among the variables. The \(W\) ratios for DF, DL, and MUVS were \(-0.319 \pm 0.088, -0.323 \pm 0.106, \text{and} -0.271 \pm 0.070\) respectively (means \(\pm SD, n = 8\) each). The potency ratios \(W\) (absolute values) for DF and DL were significantly greater than those of MUVS \((P < 0.001)\). This quantifies that sevoflurane has a greater effect on DF and DL than on MUVS when compared with changes in \([Ca^{2+}]_o\). When compared with similar values of potency ratios obtained for halothane, enfurane, and isoflurane in identical experimental conditions \((24)\), sevoflurane’s potency ratios \(W\) were not significantly different from those of isoflurane. But sevoflurane’s potency ratio for DF and DL was significantly smaller in absolute values than that of halothane and of enfurane \((P < 0.001)\) and was also smaller for MUVS than that of halothane \((P < 0.001)\) and enfurane \((P < 0.01)\).

We next determined whether sevoflurane had specific effects on the time course of contraction and relaxation other than those that may be a consequence of its effect on contraction amplitude. Variables of time course were plotted as a function of contraction amplitude for changes both in \([Ca^{2+}]_o\) and in sevoflurane concentration. In isometric twitches, TPF was decreased at small contraction amplitudes by sevoflurane but increased at lower \([Ca^{2+}]_o\) (Fig. 4B). RTH was decreased at small contraction amplitudes by sevoflurane yet was unchanged at lower \([Ca^{2+}]_o\). Figure 4B illustrates a typical example of the abbreviation of isometric relaxation by sevoflurane when compared with \([Ca^{2+}]_o\) at equal contraction amplitudes.

TDL and maximal velocity of relaxation (velocity of lengthening) were plotted as a function of contraction amplitude. In isotonic twitches at the preload of \(L_{max}\), TDL was prolonged by lowering \([Ca^{2+}]_o\) but not by sevoflurane (Fig. 5A). Maximal velocity of relaxation was decreased in sevoflurane and in low \([Ca^{2+}]_o\). At equal contraction amplitude, maximal velocity of relaxation was higher in sevoflurane than in low \([Ca^{2+}]_o\) (Fig. 5B). Figure 5C shows a typical example of the earlier and more rapid isotonic relaxation (lengthening) in sevoflurane vs. low \([Ca^{2+}]_o\) for a same extent of lengthening. TMUVS plotted as a function of MUVS was increased by lowering \([Ca^{2+}]_o\) \((P < 0.001)\) or by sevoflurane \((P < 0.001)\) with no differences between sevoflurane and low \([Ca^{2+}]_o\) at equal MUVS (not
and isoflurane (1.32 ± 0.66) obtained in identical conditions (26). This indicates that the negative inotropic effect of sevoflurane is less than that of halothane, enfurane, or isoflurane.

These findings are in contrast to results of in vitro experimental studies (10, 15, 17, 29, 38) that reported that the myocardial depressant effect of sevoflurane is similar or even slightly greater than that of isoflurane. Yet, in the isolated working rat heart (43) and in isolated canine ventricular muscle strips (18), sevoflurane depresses myocardial function less than does isoflurane. The negative inotropic effect of sevoflurane is more pronounced in heavily loaded (isometric) than in lightly loaded (zero-load-clamped) contractions (Table 3). This observation was extended and confirmed in group 2, muscles in which we compared the slopes of sevoflurane concentration-effect curves with the low-[Ca$^{2+}$]$_o$-effect curves. The slopes of the [Ca$^{2+}$]$_o$-effect curves differed in the same order (DF > DL = MUVS) as the slopes in the sevoflurane effect curves DF > DL > MUVS. On the basis of this analysis, the negative inotropic effect of sevoflurane can be accounted for by a decrease of intracellular calcium availability, which is in agreement with observations that sevoflurane decreases transsarcolemmal Ca$^{2+}$ influx (2, 18–20, 29, 38). In an effort to discern subtle differences between sevoflurane’s effect in heavily loaded vs. lightly loaded contractions, the anesthetic potency ratios $W = \text{slope (sevoflurane)}/\text{slope (log[Ca$^{2+}$]$_o$)}$ for DF, DL, and MUVS were calculated. The absolute values for the anesthetic potency ratio $W$ were lower for MUVS than for DF or DL. The fact that MUVS is less sensitive to sevoflurane than is DF suggests that sevoflurane might also decrease Ca$^{2+}$ sensitivity. The negative inotropic effect of sevoflurane is more pronounced in isometric conditions when the native myofibrillar Ca$^{2+}$ sensitivity is high (21, 25), whereas in unloaded contractions the native myofibrillar sensitivity is low. Sevoflurane’s potency ratios did not differ from those of isoflurane but differed from those of halothane and enfurane. Therefore, the relative contribution of a decreased myofibrillar Ca$^{2+}$ sensitivity to the negative inotropic effect of sevoflurane may be similar to that of isoflurane.

Sevoflurane abbreviated both TPF and RTH. The acceleration of isometric relaxation is not necessarily a consequence of the concomitant decrease in peak force, because RTH is unchanged in control conditions over the range of extracellular concentrations 0.45–2.25 mM. Starting from the same peak force either in low [Ca$^{2+}$]$_o$ vs. sevoflurane (not shown).

Sevoflurane had no statistically significant effects on load sensitivity of relaxation in group 1 muscles ($n = 9$) and group 2 muscles ($n = 8$). When compared at equal amplitudes of the isometric twitch, there were no statistically significant differences between load sensitivity of relaxation in low [Ca$^{2+}$]$_o$ vs. sevoflurane (not shown).

**DISCUSSION**

Sevoflurane exerts a reversible concentration-dependent negative inotropic effect that is less than that of halothane, enfurane, and isoflurane. The negative inotropic effect of sevoflurane cannot be entirely explained by decreased intracellular Ca$^{2+}$ availability. This is the first study that suggests that sevoflurane decreases myofibrillar Ca$^{2+}$ sensitivity in intact ventricular muscle. ED$_{50}$ values (in MAC) for sevoflurane for DF, DL, and MUVS are higher (Table 3) than those for halothane (0.85 ± 0.03, 0.94 ± 0.11, and 1.13 ± 0.10), enfurane (0.86 ± 0.12, 0.96 ± 0.19, and 1.34 ± 0.28) and isoflurane (1.32 ± 0.33, 1.44 ± 0.25, and 1.97 ± 0.66) obtained in identical conditions (26). This indicates that the negative inotropic effect of sevoflurane is less than that of halothane, enfurane, or isoflurane.

| Table 3. Sevoflurane concentrations in % (vol/vol) and in MAC required to decrease the amplitude of contractile variables by 50% (ED$_{50}$) |
|----------------|----------------|----------------|
|                | % (vol/vol)    | MAC            |
| ED$_{50}$ for  |                |                |
| DF             | 4.58 ± 0.92    | 1.70 ± 0.34    |
| DL             | 5.30 ± 1.23    | 1.96 ± 0.46    |
| MUVS           | 6.94 ± 1.84*   | 2.57 ± 0.68*   |

Values are means ± SD. ED$_{50}$, half-maximal effective dose; MAC, minimum alveolar concentration. *$P < 0.001$ for comparison with DF and DL.
or in sevoflurane, force declined faster in the presence of sevoflurane. There is strong evidence that isometric relaxation in cardiac muscle is controlled by the contractile proteins themselves, whereas cell re-lengthening rate in isolated myocytes (similar to isotonic lengthening in this study) is limited by the rate of decrease of the intracellular Ca$^{2+}$ transient (3, 44). The rate-limiting step for isometric relaxation is therefore in the kinetics of contractile proteins. A faster isometric relaxation, as seen with sevoflurane, would then most likely result from 1) a decreased affinity in tropo-nin C for Ca$^{2+}$ in its low-affinity Ca$^{2+}$-specific site; 2) an effect on the thin filament troponin-tropomyosin complex that results in a decreased gain of force per Ca$^{2+}$ bound to troponin; 3) an increase in cross-bridge turnover, resulting in a shorter average cross-bridge cycle duration; or any combination of these effects. A recent study compared mechanisms of sevoflurane and halothane on cross-bridge cycling kinetics in neonatal and adult skinned rat cardiac fibers (40). The rate of force redevelopment after a release-stretch cycle was decreased by sevoflurane. When interpreted in a two-state cross-bridge model, this finding suggests a decrease in the cross-bridge apparent attachment rate with no change in the cross-bridge detachment rate (40). This would keep cross bridges attached in the force-generating state for a shorter period of time and shorten the average cross-bridge lifetime, and fewer cross-bridges would be attached at any given time. Consequently, these changes will become manifest as a faster isometric relaxation in intact fibers as observed in this study. Currently it is not known whether sevoflurane has additional effects on further mechanisms listed above. Preliminary data (4) on the intracellular Ca$^{2+}$ transient in intact muscle fibers support the view that sevoflurane decreases myofibrillar Ca$^{2+}$ sensitivity.

Sevoflurane decreased maximal velocity of lengthening (relaxation) ($V_L$) in a concentration-dependent and reversible manner. Sevoflurane caused isotonic lengthening to proceed faster than the amplitude-matched low-[Ca$^{2+}$]$_o$ control so that the maximal velocity of lengthening in sevoflurane-exposed twitches was

Fig. 3. Concentration-effect graphs (means ± SE) of DF, DL, and MUVS as a function of [Ca$^{2+}$]$_o$, (A) and of sevoflurane concentration (B) in 2.25 mM [Ca$^{2+}$]$_o$. Results (means ± SE) are from the same muscles ($n = 8$). Slopes of linear relationship between [Ca$^{2+}$]$_o$ or sevoflurane concentration and DF, DL, and MUVS, respectively, were tested for significant differences with one-way repeated-measures ANOVA. Pairwise comparison between DF, DL, and MUVS was carried out with Student's t-test with Bonferroni correction. ***$P < 0.001$ (DF vs. DL); **$P < 0.001$ (DF vs. MUVS); ###$P < 0.001$ (DL vs. MUVS).

Fig. 4. A: dependence of TPF and of RTH on peak DF in isometric twitches in two different conditions: changes in [Ca$^{2+}$]$_o$ from 0.45 to 2.25 mM in 0.45 mM increments and of sevoflurane concentration in 0.6 or 0.7% increments in 2.25 mM [Ca$^{2+}$]$_o$. Values (means ± SE) in the graph are from the same muscles ($n = 8$). Levels of significance ($P$) above or below data points reflect differences of slopes from zero for each of the four data groups (one-sample t-tests); NS, not significant. Slopes of the relationship of TPF vs. DF and of RTH vs. DF in sevoflurane were different from those in different [Ca$^{2+}$]$_o$ (Student's paired t-tests). B: representative single-muscle example of specific effects of sevoflurane on isometric relaxation. Two isometric twitches were superimposed, one in sevoflurane 4.9% (vol/vol) in 2.25 mM [Ca$^{2+}$]$_o$, and one in 0.90 mM [Ca$^{2+}$]$_o$. From the same contraction amplitude, the muscle relaxed faster in the presence of sevoflurane than in low [Ca$^{2+}$]$_o$. Muscle characteristics: length at maximal twitch force ($L_{max}$), 5 mm; mean cross-sectional area, 0.42 mm$^2$, ratio of resting to total force at $L_{max}$, 0.24.
Fig. 5. Dependence of time to peak shortening (A) and of maximal velocity of isotonic relaxation (B) during preloaded isotonic twitches on contraction amplitude (peak shortening) at various \([\text{Ca}^{2+}]_o\), 0.45–2.25 mM in 0.45 mM increments and at various sevoflurane concentrations [0.4–5.4% (vol/vol) in 0.6 or 0.7% increments] in 2.25 mM \([\text{Ca}^{2+}]_o\). Values (means ± SE) are from the same muscles (n = 8). Levels of significance (P) above or below data points reflect differences of slopes from zero for each of the four data groups (one-sample t-tests); NS, not significant. Slopes of the relationship of TDL vs. DL and of −V vs. DL in sevoflurane were different. C: representative single-muscle example of specific effects of sevoflurane on relaxation in preloaded isotonic twitches. From a same-peak shortening amplitude, muscle relaxed sooner and faster in the presence of sevoflurane (2.25 mM \([\text{Ca}^{2+}]_o\)) than in low \([\text{Ca}^{2+}]_o\). Muscle characteristics: length at \(L_{\text{max}}\), 6.43 mm; mean cross-sectional area, 0.48 mm²; ratio of resting to total force at \(L_{\text{max}}\), 0.22.

higher than in low \([\text{Ca}^{2+}]_o\). When \([\text{Ca}^{2+}]_o\) is decreased, maximal isotonic relaxation velocity and load sensitivity of relaxation are decreased (45). This results predominantly from delayed isotonic lengthening and slowed \(\text{Ca}^{2+}\) uptake by the sarcoplasmic reticulum (SR) in low \([\text{Ca}^{2+}]_o\) (34). If the intracellular free \([\text{Ca}^{2+}]_o\) is the same either in low \([\text{Ca}^{2+}]_o\) or in normal \([\text{Ca}^{2+}]_o\) plus anesthetic, then \(\text{Ca}^{2+}\) uptake rate by the SR is enhanced in the presence of anesthetic (7), a drug-specific effect that partially overrides the decreased SR uptake rate at low intracellular \([\text{Ca}^{2+}]_o\). On the other hand, if intracellular free \([\text{Ca}^{2+}]_o\) is higher in control \([\text{Ca}^{2+}]_o\) plus anesthetic than in low \([\text{Ca}^{2+}]_o\) alone, either a greater stimulation of SR \(\text{Ca}^{2+}\) uptake in the former condition and/or an anesthetic-induced decrease in myofibrillar \(\text{Ca}^{2+}\) sensitivity will cause the muscle to relax faster. It is therefore possible that sevoflurane stimulates SR \(\text{Ca}^{2+}\) uptake to some extent. Several investigations show that there is either no or a modest depressing effect on SR \(\text{Ca}^{2+}\) release contributing to the overall negative inotropic effect exerted by sevoflurane (2, 17, 38). Yet there is evidence that sevoflurane might increase SR \(\text{Ca}^{2+}\) content. In rat ventricular myocytes, partial recovery of contraction during isoflurane and sevoflurane application and greater contractions immediately after washout, both more pronounced in sevoflurane, were completely abolished by pretreatment with ryanodine (10). An increased SR \(\text{Ca}^{2+}\) content would be consistent with a stimulation of SR \(\text{Ca}^{2+}\) uptake reflected by the greater −\(V\) in sevoflurane than in the low \([\text{Ca}^{2+}]_o\) control. We also observed a recovery significantly above control values of the variables \(+dF/dt\), −\(dF/dt\), and −\(V\) immediately after washout of sevoflurane, a phenomenon not seen in isoflurane, enfurane, and halothane (26). DF and DL showed a tendency to increase above control values after washout, yet the increase did not reach statistical significance. We did not follow the recovery over a period longer than 30 min and therefore do not know whether the values would return to control values, but one might speculate that our observations reflect in part the same mechanism as that observed in the investigation of Davies et al. (10).

Load sensitivity of relaxation is often used in cardiac muscle mechanics to characterize physiological or pharmacological effects on relaxation. Load sensitivity of relaxation is affected by \([\text{Ca}^{2+}]_o\) (14), osmolality (13), species (6), hypoxia (45), and volatile anesthetics (27) such as halothane, enfurane, and isoflurane. Load sensitivity changes as a result of changes of the time course of the isotonic twitch in reference to the isometric twitch in identical experimental conditions. With halothane and enfurane, and to a lesser extent with isoflurane, isometric relaxation was abbreviated more than was isotonic lengthening, and a decrease in load sensitivity of relaxation resulted. With sevoflurane, isometric and isotonic relaxation are abbreviated to approximately the same extent, and this could well explain the lack of changes of load sensitivity of relaxation and the observation that load sensitivity was unchanged in some muscles, increased in a few muscles, and decreased slightly in other muscles.

In summary, sevoflurane exerts concentration-dependent, reversible negative inotropic effects on ferret ventricular myocardium. These depressant effects are less than those seen in equianesthetic concentrations of isoflurane, enfurane, or halothane. The negative inotropic effects result from a combination of a decrease in intracellular \(\text{Ca}^{2+}\) availability and a decrease in myofibrillar \(\text{Ca}^{2+}\) sensitivity. The precise locus of
action for either mechanism will require further study. Sevoflurane might also slightly increase the rate of $\text{Ca}^{2+}$ removal from the cytoplasm as reflected by a faster and earlier isotonic relaxation when compared with amplitude-matched twitches in low $[\text{Ca}^{2+}]_{i}$.\footnote{This work was supported by grant GM-36365 (to P. R. Housmans) from the National Institutes of Health, Bethesda, MD, and by the Mayo Foundation, Rochester, MN.}

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