

Effects of Sevoflurane on the Intracellular Ca^{2+} Transient in Ferret Cardiac Muscle

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Background: Sevoflurane depresses myocardial contractility by decreasing transsarcolemmal Ca^{2+} influx. In skinned muscle fibers, sevoflurane affects actin–myosin cross-bridge cycling, which might contribute to the negative inotropic effect. It is uncertain to what extent decreases in Ca^{2+} sensitivity of the contractile proteins play a role in the negative inotropic effect of sevoflurane in intact cardiac muscle tissue. The aim of this study was to assess whether sevoflurane decreases myofibrillar Ca^{2+} sensitivity in intact living cardiac fibers and to quantify the relative importance of changes in myofibrillar Ca^{2+} sensitivity versus changes in myoplasmic Ca^{2+} availability by sevoflurane.

Methods: The effects of sevoflurane 0–4.05% vol/vol (0–1.5 minimum alveolar concentration [MAC]) on isometric and isotonic variables of contractility and on the intracellular calcium transient were assessed in isolated ferret right ventricular papillary muscles microinjected with the Ca^{2+} -regulated photoprotein aequorin. The intracellular calcium transient was analyzed in the context of a multicompartiment model of intracellular Ca^{2+} buffers in mammalian ventricular myocardium.

Results: Sevoflurane decreased contractility, time to peak force, time to half isometric relaxation, and the $[\text{Ca}^{2+}]_i$ transient in a reversible, concentration-dependent manner. Increasing $[\text{Ca}^{2+}]_o$ in the presence of sevoflurane to produce peak force equal to control increased intracellular Ca^{2+} transient higher than control.

Conclusions: Sevoflurane decreases myoplasmic Ca^{2+} availability and myofibrillar Ca^{2+} sensitivity in equal proportions except at 4.05% vol/vol (1.5 MAC), where Ca^{2+} availability is decreased more. These changes are at the basis of the negative inotropic effect of sevoflurane in mammalian ventricular myocardium. (Key words: Calcium; calcium sensitivity; inotropy; myocardium; sarcoplasmic reticulum.)

It is well established that the volatile anesthetic sevoflurane in clinical useful concentrations depresses myocardial contractility *in vitro* because of a decrease in transsarcolemmal Ca^{2+} influx.^{1–6} There is evidence that sevoflurane might also decrease Ca^{2+} sensitivity of the contractile proteins,^{7,8} as was shown for halothane, enflurane, and isoflurane in skinned^{9,10} and intact muscle fibers.¹¹ The purpose of this study was to test the hypothesis that sevoflurane decreases myofibrillar Ca^{2+} sensitivity in intact living cardiac fibers and to quantify the relative importance of changes in myofibrillar Ca^{2+}

sensitivity versus changes in myoplasmic Ca^{2+} availability in the overall concentration-dependent negative inotropic effect of sevoflurane.

Materials and Methods

This study was approved by the Animal Care and Use Committee of the Mayo Foundation, Rochester, Minnesota, 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 aged 16–19 weeks) were anesthetized with sodium pentobarbital (100 mg/kg intraperitoneally), and the heart was quickly removed through a left thoracotomy. During generous superfusion with oxygenated physiologic solution (see below), suitable right ventricular papillary muscles were carefully excised from the beating heart. Papillary muscles were then mounted vertically in a temperature-controlled (30°C) muscle chamber that contains a physiologic salt solution made up in ultrapure water (Nanopure Infinity; Barnstead, Dubuque, IA) and with the following composition: Na^+ 137.5 mM, K^+ 5.0 mM, Ca^{2+} 2.25 mM, Mg^{2+} 1.0 mM, Cl^- 127.0 mM, SO_4^{2-} 1.0 mM, acetate⁻ 20.0 mM, glucose 10.0 mM, 3-(N-morpholino)propanesulfonic acid 5.0 mM, pH 7.40, bubbled with 100% O_2 . Experiments were conducted at 30°C and at a stimulus frequency of 0.25 Hz; isolated papillary muscle function is stable for many hours under these conditions. Suitable preparations were selected on the basis of the following criteria: length at which twitch active force is maximal (L_{max}) more than or equal to 3.5 mm, a mean cross-sectional area less than or equal to 1.2 mm², and a ratio of resting to total force in an isometric twitch at L_{max} less than or equal to 0.30. The tendinous end of each muscle was tied with a thin braided polyester thread (size 6.0 Deknatel Surgical Suture, Fall River, ME) to the lever of a force-length servo transducer.¹¹ The ventricular end of each muscle was held in a miniature Lucite (Dupont, Wilmington, DE) clip with a built-in platinum punctate electrode; two platinum wires were arranged longitudinally, one along each side of the muscle, and served as anode during punctate stimulation. Rectangular pulses of 5-ms duration were delivered by a Grass S88D (Astro-Med, Inc., West Warwick, RI) stimulator at a stimulus interval of 4 s. Stimuli at 10–20% above threshold (range, 4–12 V) were used to minimize the release of endogenous norepinephrine by the driving

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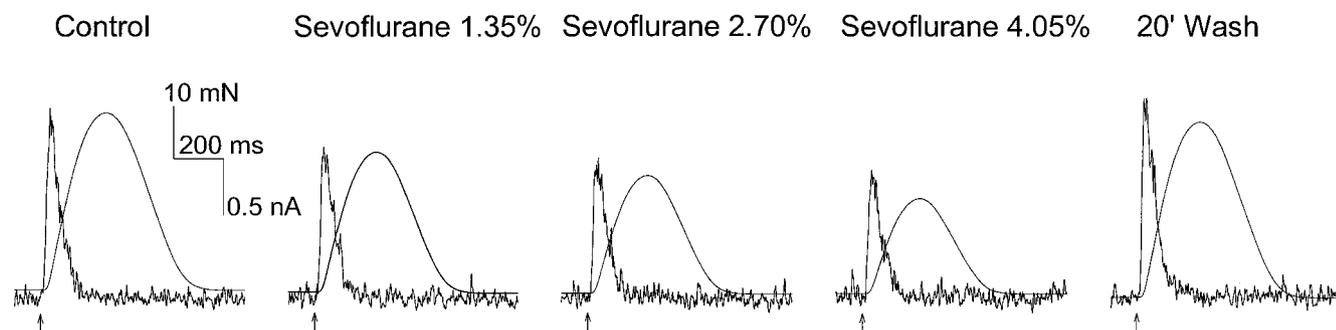


Fig. 1. Aequorin luminescence and force traces as a function of time during a cumulative concentration-effect experiment to sevoflurane in isometric twitches. One hundred twenty-eight twitches were averaged. The vertical arrow in each panel indicates the time of the electrical stimulus.

ment to incremental concentrations of sevoflurane on aequorin luminescence and force in isometric twitches. Table 2 and figure 2 summarize the values of variables of contractility and of aequorin luminescence during control and cumulative concentration-response experiments to sevoflurane. Sevoflurane caused a concentration-dependent decrease of force and aequorin luminescence over the concentration range studied (figs. 1 and 2, left). Sevoflurane shortened the duration of the isometric twitch (time to peak force) and isometric relaxation half time in a concentration-dependent manner (fig. 2, right). Sevoflurane did not change time to peak light (fig. 2, right) and did not affect the decline of aequorin luminescence measured by t_{L25} and the slope $k_{50/10}$ of the logarithm of aequorin luminescence during decline from 50% to 10% of peak.

Effects of Sevoflurane on Myofibrillar Ca^{2+} Sensitivity

There were no statistically significant differences for L_{max} , mean cross-sectional area, resting force or preload at L_{max} , total force, and the ratio of resting to total force

among the three muscle groups 2a, 2b, and 2c used in this experimental protocol (table 1). A typical Ca^{2+} back-titration experiment is shown in figure 3. Aequorin luminescence and isometric force were measured in control (fig. 3, left) and during exposure to sevoflurane 2.7% (1 MAC; fig. 3, middle), both in $[Ca^{2+}]_o$ 2.25 mM. Extracellular $[Ca^{2+}]_o$ was then rapidly increased with small aliquots of a concentrated $CaCl_2$ solution (0.25 M) until peak developed force in sevoflurane and high $[Ca^{2+}]_o$ was equal to that in the control twitch. At equal peak developed force, aequorin luminescence was higher in the presence of sevoflurane and elevated $[Ca^{2+}]_o$ (fig. 3, right) than in its absence. The resulting $[Ca^{2+}]_o$ values at the end of the procedure were 3.00 ± 0.09 , 3.70 ± 0.29 , and 4.59 ± 0.42 mM in 0.5, 1.0, and 1.5 MAC sevoflurane, respectively. Figure 4 summarizes the results of the Ca^{2+} back-titration experiments in each of three sevoflurane concentrations.

Figure 5 shows a typical Ca^{2+} back-titration experiment (fig. 5, top) and part of the analysis procedure (fig. 5, middle and bottom). First, aequorin luminescence signals that would match the amplitude and time course

Table 2. Aequorin Luminescence and Variables of Contractility and Relaxation during Cumulative Concentration-Response Experiments to Sevoflurane (n = 8) in Group 1 Muscles in Isometric Twitches at the Preload of L_{max}

	Control	0.7%	1.35%	2.7%	4.05%	20-min Washout
Peak systolic aequorin luminescence (%)	100	94 ± 10	84 ± 10*	73 ± 10*	60 ± 7*	105 ± 10
Time to peak aequorin luminescence (ms)	44 ± 6	45 ± 5	43 ± 6	44 ± 4	45 ± 4	44 ± 6
Peak developed force (mN/mm ²)	43.8 ± 7.5	38.6 ± 6.9†	34.5 ± 6.2*	26.8 ± 6.1*	20.8 ± 6.1*	44.0 ± 8.9
Time to peak force (ms)	265 ± 36	255 ± 31†	250 ± 27*	239 ± 24*	230 ± 21*	260 ± 27
Time to half isometric relaxation (ms)	171 ± 28	162 ± 22	155 ± 19*	149 ± 17*	145 ± 16*	165 ± 20
Time to 25% aequorin luminescence (ms)	123 ± 12	119 ± 11	119 ± 7	118 ± 11	122 ± 9	118 ± 13
$k_{50/10}$	11.3 ± 1.9	11.7 ± 2.1	11.4 ± 1.4	11.3 ± 1.8	10.1 ± 1.3	11.9 ± 1.6

Values are mean ± SD.

* $P < 0.01$, † $P < 0.05$, one-way repeated-measures analysis of variance followed by comparisons versus control by Bonferroni-corrected paired t tests.

L_{max} = length at which twitch active force is maximal; $k_{50/10}$ = slope of logarithm of aequorin luminescence from 50% peak to 10% peak by least-squares linear regression.

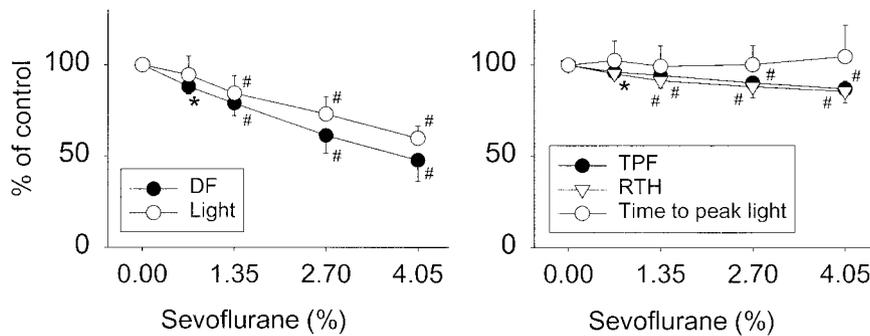


Fig. 2. Effects of sevoflurane on (left) peak force (DF) and peak aequorin luminescence (light), and on (right) time to peak force (TPF), and time to half isometric relaxation (RTH) of isometric twitches. Data are mean \pm SD. * $P < 0.05$, # $P < 0.01$ by repeated-measures analysis of variance and comparison versus control by Bonferroni-corrected paired t test.

of experimental gained aequorin luminescence in sevoflurane in high $[Ca^{2+}]_o$ (fig. 5, top right) were simulated by varying SR release and $k_{off(TnC,Ca)}$ in small steps. Ca^{2+} availability and Ca^{2+} sensitivity of the Ca^{2+} back-titrated twitch were derived by the SR release and $k_{off(TnC,Ca)}$ values of the simulated aequorin luminescence signals that produced (1) the best fit to the measured Ca^{2+} transient in high $[Ca^{2+}]_o$ plus sevoflurane by least squares differences, and (2) equal peak Ca^{2+} occupancies of TnC in control and in sevoflurane plus high $[Ca^{2+}]_o$ at equal peak force (fig. 5, bottom). This procedure yielded a value of myofibrillar Ca^{2+} sensitivity in sevoflurane. The effect of sevoflurane in control $[Ca^{2+}]_o$ on myoplasmic delivery was found by searching the least squares fit to the experimentally measured aequorin luminescence signal (fig. 5, top, middle) by varying SR release only and using the $k_{off(TnC,Ca)}$ value found earlier for the aequorin luminescence signal in the back-titrated twitch.

Figure 6 summarizes the analyses for all Ca^{2+} back-titration experiments and shows the relative effects of sevoflurane on myoplasmic Ca^{2+} availability and on myofibrillar Ca^{2+} sensitivity. The results of the analysis are as follows: (1) sevoflurane decreases myoplasmic Ca^{2+} availability in a concentration-dependent manner; (2) sevoflurane decreases myofibrillar sensitivity; (3) the decrease of myofibrillar Ca^{2+} sensitivity is already fully present at 2.7% (vol/vol; 1 MAC) sevoflurane; and (4) sevoflurane decreases myoplasmic Ca^{2+} availability and myofibrillar Ca^{2+} sensitivity to the same relative extent, except at 4.05% (vol/vol; 1.5 MAC), where sevoflurane decreases Ca^{2+} availability more than Ca^{2+} sensitivity.

Discussion

Studies in isolated heart, intact cardiac muscle tissue preparations, and single ventricular myocytes demonstrated a concentration-dependent decrease of indices of contractility by sevoflurane in various species such as rat, dog, guinea pig, and humans.^{1-6,21-24} As for other volatile anesthetics, the negative inotropy of sevoflurane might be associated with (1) effects on transsarcolemmal Ca^{2+} flux; (2) alteration of SR function; (3) a decrease of free intracellular $[Ca^{2+}]$ level during systole; and (4)

modification of the responsiveness of the contractile proteins to activation by Ca^{2+} . The current study is the first to show the effects of sevoflurane on the intracellular Ca^{2+} transient and to assess whether sevoflurane alters myofibrillar Ca^{2+} sensitivity in intact ventricular myocardium. Measurements of free intracellular Ca^{2+} using the bioluminescent protein aequorin, and of contractile force show a concomitant concentration-dependent decrease in these variables, indicating that the negative inotropy of sevoflurane is related to a decrease in intracellular Ca^{2+} availability.

Indeed, the negative inotropic effect of sevoflurane has been mainly attributed to depression of transsarcolemmal Ca^{2+} influx,¹⁻⁵ whereas the effects of sevoflurane on the SR seem to be modest.^{1,2,22} Sevoflurane interacts with the L-type Ca^{2+} channel in cultured neonatal rat ventricular myocytes since the L-type Ca^{2+} channel agonist Bay K 8644 significantly prevented the sevoflurane-depressed contractile amplitude.⁵ The sevoflurane-induced depression of myocardial contractility was accompanied by a shortening of duration of action potential in canine ventricular muscle strips and resulted from significant blockade of transsarcolemmal Ca^{2+} current.^{3,4} In guinea pig papillary muscle, sevoflurane decreased contractile force and duration of action potential in a concentration-dependent fashion.² Sevoflurane depressed contractile force at rested state and at low stimulation frequencies, whereas it did not suppress potentiated state contractions and also depressed contractile force after ryanodine.² A comprehensive study on the mechanical and electrophysiologic effects of sevoflurane was conducted by Park *et al.*¹ In guinea pig myocardium, sevoflurane decreased maximum rate of force development at low stimulation rates but not at high stimulation rates. Sevoflurane decreased the initial rate of force development less than the rate of "late" force development, and selectively decreased late peak force in high K^+ Tyrode solution without changing early peak force. Taken together, these observations led to the conclusion that sevoflurane has almost no effect on SR Ca^{2+} release. Sevoflurane increased duration of action potential, decreased peak L-type Ca^{2+} current, and suppressed the delayed K^+ current, which appears to underlie the increased duration of action potential.¹

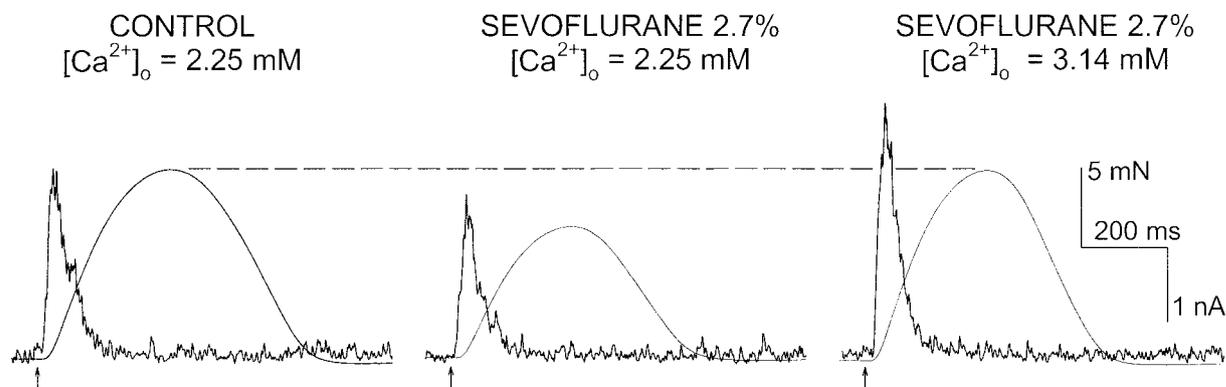


Fig. 3. Aequorin luminescence and force traces of isometric twitches during a typical Ca^{2+} back-titration experiment for sevoflurane 2.7%. After an initial control (*left*), muscles were exposed to sevoflurane 2.7% (*middle*), and $[Ca^{2+}]_o$ was rapidly increased (*right*) so that peak developed force equaled that in control. At equal peak force, aequorin luminescence was higher in the presence of sevoflurane. Sixty-four twitches were averaged in each panel. The vertical arrow in each panel indicates the time of the electrical stimulus.

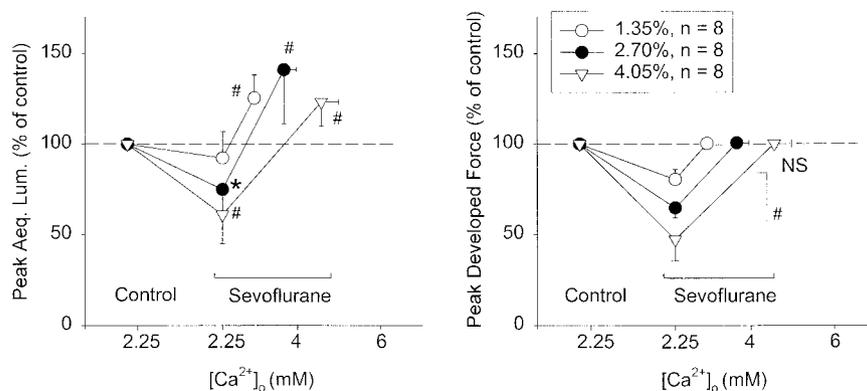
A decrease in myofibrillar Ca^{2+} sensitivity by sevoflurane may contribute to the overall negative inotropic effect of sevoflurane in cardiac ventricular muscle. Ca^{2+} sensitivity is defined as the contractile response (usually measured as force) of the myofibrils to a given myoplasmic Ca^{2+} concentration. Changes in any of the following processes can lead to a change of myofibrillar Ca^{2+} sensitivity: (1) the affinity of TnC for Ca^{2+} (Ca^{2+} -specific binding site II); (2) interactions of TnC with TnI and TnT; (3) the state of phosphorylation of TnI that changes the Ca^{2+} affinity for TnC²⁵; (4) signal transduction *via* tropomyosin to actin; (5) the interaction of actin with myosin heads *via* the formation of cross-bridges; (6) regulation of contraction *via* myosin light chains²⁶; and/or (7) possibly other mechanisms. The affinity of TnC for Ca^{2+} is determined by the off-rate of TnC's Ca^{2+} -specific binding site II, as the on-rate is very fast and only limited by diffusion.²⁰ Halothane did not change²⁷ or slightly increased the Ca^{2+} affinity of isolated cardiac TnC *in vitro*²⁸ and decreased $k_{off}(TnC,Ca)$ in human recombinant cardiac TnC.²⁹ These effects cannot account for the decrease in myofibrillar Ca^{2+} sensitivity by halothane. The effects of sevoflurane on Ca^{2+} binding to TnC have not yet been investigated.

In rat skinned cardiac fibers, sevoflurane decreased the

rate of force redevelopment after a release-stretch cycle (k_{tr}).⁷ When interpreted in a two-state cross-bridge model, this finding suggests a decrease in the cross-bridge apparent attachment rate (f_{app}) with no change in the cross-bridge detachment rate (g_{app}). This would keep fewer cross-bridges in the force-generating state, so that less force is generated, even if the Ca^{2+} transient were not changed. This would manifest itself as a decreased myofibrillar Ca^{2+} sensitivity in intact fibers as observed in this study.

In intact cardiac muscle, there is indirect evidence for a decrease in myofibrillar Ca^{2+} sensitivity by sevoflurane.⁸ The negative inotropic effect of sevoflurane was more pronounced in isometric conditions, where the native myofibrillar Ca^{2+} sensitivity is high, than in unloaded contractions, where the native myofibrillar Ca^{2+} sensitivity is low.⁸ Sevoflurane abbreviated both time to peak force and isometric relaxation half-time. The acceleration of isometric relaxation was not a consequence of the concomitant decrease in peak force, as isometric relaxation half-time was unchanged in control conditions over the range of extracellular Ca^{2+} concentrations of 0.45–2.25 mM.⁸ Isometric relaxation in cardiac muscle is controlled by the contractile proteins themselves, whereas cell relengthening rates in isolated myocytes

Fig. 4. Summary of isometric Ca^{2+} back-titration experiments for peak aequorin luminescence (*left*) and peak developed force (*right*) during control ($[Ca^{2+}]_o = 2.25$ mM), sevoflurane exposure ($[Ca^{2+}]_o = 2.25$ mM), and sevoflurane in increased $[Ca^{2+}]_o$ (> 2.25 mM) at equal peak force as in control as a function of $[Ca^{2+}]_o$. * $P < 0.05$, # $P < 0.01$ by repeated-measures analysis of variance and comparison *versus* control by Bonferroni-corrected paired *t* test.



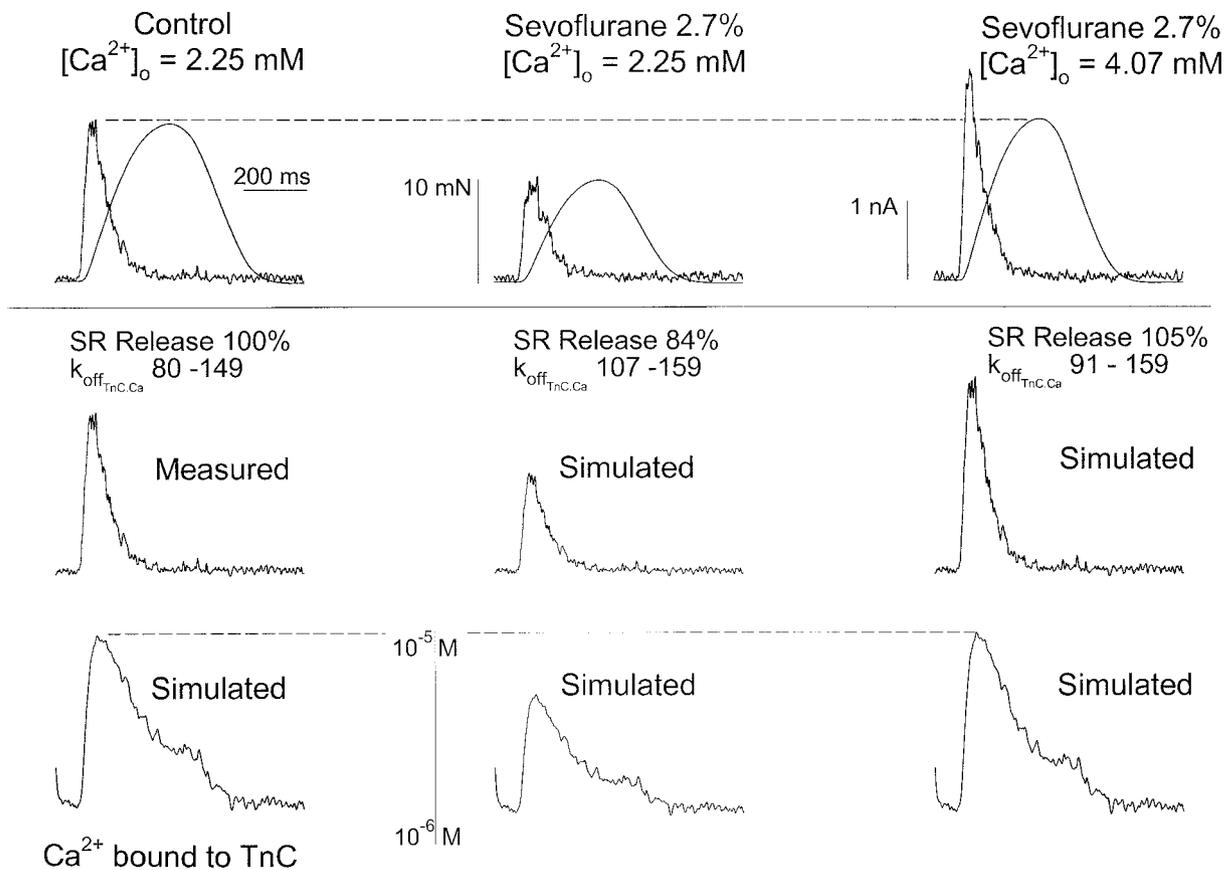


Fig. 5. Analysis of isometric Ca²⁺ back-titration experiment. (Top) Aequorin luminescence and force traces as a function of time in a typical Ca²⁺ back-titration experiment. Sixty-four twitch contractions were averaged. (Middle) Traces of aequorin luminescence obtained by computational simulation, except where marked "measured." (Bottom) Calculated traces of Ca²⁺ occupancy of troponin C. See text for details. SR = sarcoplasmic reticulum.

(similar to isotonic lengthening) are limited by the rate of decrease of the [Ca²⁺]_i transient.^{30,31} The acceleration of isometric relaxation by sevoflurane in intact cardiac muscle might result from a decrease in Ca²⁺ sensitivity. This is in contrast to findings by Hanouz et al.,²² who

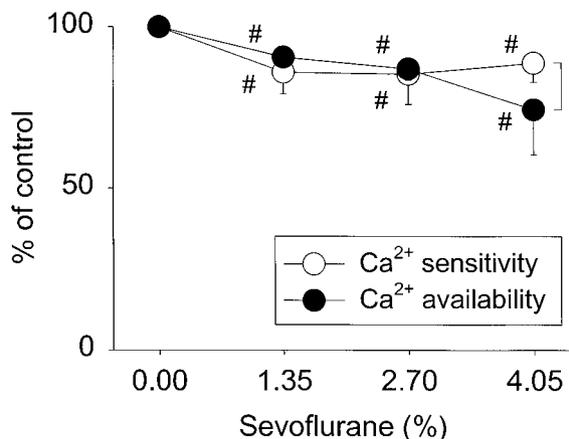


Fig. 6. Relative effects of sevoflurane on myoplasmic Ca²⁺ availability and myofibrillar Ca²⁺ sensitivity. The data points (mean \pm SD) are derived from the same number of experiments as indicated in figure 4. * $P < 0.05$ by paired Student t test. # $P < 0.05$ by analysis of variance and Bonferroni-corrected t test for comparison with control.

reported that sevoflurane and isoflurane did not induce a lusitropic effect under high load and concluded that these anesthetics might not modify myofilament Ca²⁺ sensitivity. The differences may result from species differences (rat *vs.* ferret).

To assess whether sevoflurane alters myofibrillar Ca²⁺ sensitivity in intact fibers, peak aequorin luminescence was compared with and without sevoflurane at equal peak force in Ca²⁺ back-titration experiments. The higher Ca²⁺ transient in sevoflurane and increased extracellular [Ca²⁺]_o than in control indicates a decreased Ca²⁺ sensitivity. To quantify the relative changes in myofibrillar Ca²⁺ sensitivity *versus* changes in myoplasmic Ca²⁺ availability, we analyzed the Ca²⁺ transients in the context of a multicompartiment computer model that considered SR Ca²⁺ release, Ca²⁺ uptake, and binding to the principal intracellular Ca²⁺ buffers, TnC and calmodulin.¹¹ Published values for rate constants of association and dissociation of Ca²⁺ to the intracellular buffers TnC and calmodulin and a Michaelis-Menten kinetic scheme for SR Ca²⁺ uptake were used. The values of V_{max} (maximal rate of Ca²⁺ uptake by the SR) and of K_m ([Ca²⁺]_o at which Ca²⁺ uptake rate equals V_{max}/2) were not allowed to vary.¹¹ However, sevoflurane might slightly

increase the rate of Ca^{2+} removal from the cytoplasm, as reflected by a faster and earlier isotonic relaxation when compared with amplitude-matched twitches in low $[Ca^{2+}]_o$.⁸ This would cause a faster decline of the Ca^{2+} transient. If this were the case, the effect would be very small, a 6-ms shorter signal (measured at the midpoint of the decline) for a 20% increase in SR Ca^{2+} uptake rate (V_{max}) in experimental conditions of this study. Measurements of t_{L25} and $k_{50/10}$ were not affected by sevoflurane, yet these variables may not be able to resolve small changes in the decline of the Ca^{2+} transient.

Sevoflurane up to a concentration of 2.7% (vol/vol; 1 MAC) changes Ca^{2+} sensitivity and Ca^{2+} availability to the relative same extent. At 4.05% (vol/vol; 1.5 MAC), Ca^{2+} availability was significantly more decreased than Ca^{2+} sensitivity. This pattern of relation between decrease in Ca^{2+} sensitivity and Ca^{2+} availability is similar to that observed for isoflurane.¹¹ By contrast, halothane decreased myofibrillar Ca^{2+} sensitivity less than Ca^{2+} availability over the entire concentration range (0–1.5 MAC).¹¹ Sevoflurane decreased myoplasmic Ca^{2+} availability to the same extent as isoflurane yet less than halothane ($P < 0.01$, one-way analysis of variance and Bonferroni-corrected t tests). Yet the decrease in myofibrillar Ca^{2+} sensitivity was not concentration-dependent. Anesthetic-induced decreases in myofibrillar Ca^{2+} sensitivity were already maximal at 0.5 MAC halothane and 1 MAC isoflurane and sevoflurane. This suggests a saturable process, the nature of which remains to be defined. In skinned rat cardiac fibers, 2 MAC sevoflurane decreased k_{tr} and f_{app} more than 1 MAC sevoflurane, observations that suggest a concentration-dependent effect.⁷ Yet, because of the loss of certain natively present constituents and of membrane regulation of contractility, results obtained in skinned muscle fiber preparations are difficult to extrapolate to intact myocardium.

Halothane, isoflurane,¹¹ and sevoflurane (current study) at equipotent concentrations decreased Ca^{2+} sensitivity in intact muscle to the relative same extent (one-way analysis of variance, $P > 0.1$). This is consistent with observations that there were no differences between effects of volatile anesthetics on pCa-force relations and maximal activated force in skinned rat cardiac fibers⁹ and in human skinned cardiac fibers.¹⁰ However, sevoflurane and halothane exerted differential effects on cross-bridge cycling parameters. Sevoflurane did not decrease the fraction of attached cross-bridges (α_{fg}) and g_{app} , whereas halothane did. Sevoflurane 1 MAC decreased k_{tr} and f_{app} less than 1 MAC halothane, and 2 MAC sevoflurane decreased k_{tr} and f_{app} more than 2 MAC halothane.⁷ Studies of cross-bridge kinetics have shown differences between anesthetics that were not resolved from examination of pCa-force relations of skinned fibers and from analysis of myofibrillar Ca^{2+} sensitivity in intact fibers. Therefore, one might con-

clude that various sites of contractile proteins are affected by volatile anesthetics.

The results of this study must be interpreted in the context of the experimental conditions in which they were obtained. Results obtained here at 30°C and a stimulus interval of 4 s may differ from those that one could obtain at the more physiologic conditions of the animal, 37–38°C and 200 beats/min.

In summary, in intact ferret papillary muscle, the negative inotropic effect of sevoflurane is caused by a decrease of myoplasmic Ca^{2+} availability and of myofibrillar Ca^{2+} sensitivity in equal proportions, except at 1.5 MAC, where myoplasmic Ca^{2+} availability decreases more. These changes are at the basis of the negative inotropic effect of sevoflurane in mammalian ventricular myocardium.

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