

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

Anna E. Bartunek, M.D., Philippe R. Housmans, M.D., Ph.D.†*

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 multicompartment 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 $[Ca^{2+}]_i$ transient in a reversible, concentration-dependent manner. Increasing $[Ca^{2+}]_o$ 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 in 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 mm^2, 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

* Research Fellow, Department of Anesthesiology, Mayo Foundation; and Department of Cardiothoracic and Vascular Anesthesia and Intensive Care Medicine, University of Vienna, Vienna, Austria. † Associate Professor, Department of Anesthesiology, Mayo Foundation.

Received from the Department of Anesthesiology, Mayo Foundation, Rochester, Minnesota. Submitted for publication May 16, 2000. Accepted for publication August 10, 2000. Supported in part by Grant No. GM36365 from the National Institutes of Health, Bethesda, Maryland (Dr. Housmans); and by the Mayo Foundation, Rochester, Minnesota. Presented in part at the 44th Annual Meeting of the Biophysical Society, New Orleans, Louisiana, February 12–16, 2000.

Address reprint requests to Dr. Housmans: Department of Anesthesiology, 2-752 MB, Mayo Foundation, 200 First Street SW, Rochester, Minnesota. Address electronic mail to: housmans.philippe@mayo.edu.

stimuli. The muscles were stimulated and made to contract in alternating series of four isometric and four isotonic twitches at preload only during a 1–2-h period of stabilization.

At the end of the stabilization period, electrical stimulation was stopped, and multiple superficial cells were microinjected with the Ca^{2+}-regulated photoprotein aequorin (Friday Harbor Laboratories, University of Washington, Friday Harbor, WA) to allow for subsequent detection of the intracellular Ca^{2+} transient as previously described.[11] It was usually necessary to average luminescence and force signals of 64–256 twitches to obtain a satisfactory signal-to-noise ratio in aequorin luminescence signals. For all muscles (n = 32) combined, the peak aequorin signal was 10.18 ± 9.99 (mean ± SD) times the root mean square of the baseline "noise" value (range, 59.32–2.83).

We quantified peak systolic aequorin luminescence and time to peak aequorin luminescence. The decline of the aequorin signal was quantified by measuring the time from the stimulus to the time when aequorin luminescence had decreased to 25% of its peak value (t_{L25}), and the slope of the logarithm of aequorin luminescence from 50% peak to 10% peak ($k_{50/10}$). The measurement of $k_{50/10}$ is based on the fact that the decline of the aequorin signals in isometric (but not isotonic[12]) twitches follows an exponential decline, and that aequorin luminescence is approximately a 2.5 power function of $[Ca^{2+}]_i$.

The methods of delivery of sevoflurane were the same as for other volatile anesthetics.[13] In brief, oxygen flowed through the calibrated sevoflurane vaporizer and was allowed to mix in a 3-l reservoir bag. An occlusive roller pump (Masterflex; Cole-Parmer, Chicago, IL) delivered a continuous gas flow to the bubbler in the organ bath. The muscle chamber was covered with a tightly sealing Parafilm (American Can Company, Greenwich, CT) except for a narrow slit for the muscle clip and transducer hook. The concentration of sevoflurane was measured continuously between the reservoir bag and the roller pump with an anesthetic agent monitor (Ohmeda 5330, Madison, WI). Gas chromatography (Hewlett-Packard 5880A, Palo Alto, CA) measurements showed that 1% (vol/vol) sevoflurane corresponded to 0.18 mM in fluid at 30°C. The concentration of sevoflurane in fluid and the calculated partial pressure of sevoflurane in fluid followed closely imposed changes of anesthetic vapor concentration in the gas phase. After the sevoflurane administration was discontinued, its concentration in liquid declined rapidly and was always undetectable at 20 min.

Sevoflurane minimum alveolar concentration (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).[14] The ratio of isoflurane MAC to halothane MAC in the ferret is 1.5, similar to that found in humans, dogs, and horses.[15] Sevoflurane MAC values for humans, dogs, and horses are 2.05, 2.36, and 2.31, respectively.[16–18] The halothane MAC values for humans, dogs, and horses are reported to be 0.75, 0.86 and 0.88, respectively.[15] 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 humans (2.73), dogs (2.74), and horses (2.63) as well, which brings us to an estimated MAC of 2.7% (vol/vol) in the ferret used in this study.

All ferret papillary muscles were pretreated with bupranolol HCl 5×10^{-7} M before the onset of the experiment to abolish any β-adrenergic effects. All experiments were conducted with the initial muscle length set at L_{max}.

Experimental Design

Two experimental protocols were used to examine the mechanism of the inotropic effect of sevoflurane. Each muscle served as its own control. Muscles contracted isometrically throughout the experiments.

In group 1 muscles (n = 8), sevoflurane was applied in concentrations of 0.7%, 1.35%, 2.7%, and 4.05% (vol/vol). These concentrations correspond to 0.25, 0.5, 1.0, and 1.5 MAC in the ferret (see above). As soon as contractility had reached a steady state (which was usually the case after 10–12 min of equilibration with a particular sevoflurane concentration), signals of force and aequorin luminescence were averaged on a digital storage oscilloscope (Nicolet 4094C, Madison, WI). Averaged signals were stored on 5.25-inch floppy disks and transferred to a desktop computer by software programs written in WFBASIC (Blue Feather Software, New Glarus, WI), which also measures all variables of contraction, relaxation, and aequorin luminescence. One to three records with 64 averaged twitches each were taken at control, at each sevoflurane concentration, and after sevoflurane washout, and were averaged to further improve signal-to-noise ratio of the aequorin luminescence signals, if necessary, before quantification of variables. Variables of contraction and relaxation were determined from isometric twitches at the preload of L_{max}: peak developed force, time to peak force, and time from peak force to half-isometric relaxation.

In group 2 muscles (n = 24), we determined whether sevoflurane alters myofibrillar Ca^{2+} sensitivity. After measurement of control variables of the isometric twitch, group 2a, 2b, and 2c muscles were exposed to 1.35% (0.5 MAC), 2.7% (1.0 MAC), or 4.05% (1.5 MAC) sevoflurane, respectively. When aequorin luminescence and peak isometric force had reached steady state, extracellular Ca^{2+} was rapidly increased by adding small aliquots of a concentrated $CaCl_2$ solution (0.25 M) to the bathing solution, until the amplitude of peak developed force was equal to that in the control twitch. In three

Table 1. Muscle Characteristics during Control Conditions at L_{max} in Concentration–Response Experiments (Group 1) and Ca^{2+} Back-titration Experiments in Three Sevoflurane Concentrations (Groups 2a, 2b, 2c)

	L_{max} (mm)	CSA (mm^2)	R (mN/mm^2)	T (mN/mm^2)	R/T
Group 1 (n = 8)					
Mean ± SD	4.7 ± 1.3	0.47 ± 0.14	12.3 ± 2.0	56.0 ± 9.0	0.22 ± 0.02
Range	3.2–7.5	0.24–0.65	9.1–15.0	41.9–69.2	0.18–0.24
Group 2a (n = 8)					
Mean ± SD	5.5 ± 1.1	0.57 ± 0.22	10.5 ± 3.1	52.0 ± 13.5	0.20 ± 0.02
Range	3.6–7.1	0.20–0.89	6.2–15.8	34.6–70.5	0.17–0.22
Group 2b (n = 8)					
Mean ± SD	5.2 ± 1.2	0.39 ± 0.18	13.2 ± 4.8	66.3 ± 30.5	0.20 ± 0.03
Range	3.9–7.1	0.14–0.67	7.5–20.7	40.0–129.9	0.16–0.26
Group 2c (n = 8)					
Mean ± SD	5.0 ± 1.0	0.50 ± 0.15	12.4 ± 2.8	62.0 ± 8.6	0.20 ± 0.04
Range	3.6–6.4	0.32–0.74	7.4–17.3	50.0–76.6	0.15–0.27

L_{max} = length at which twitch active force is maximal; CSA = cross-sectional area; R = resting tension; T = total tension.

experiments, peak force slightly exceeded that of the control twitch after titration with $CaCl_2$. In these instances, $[Ca^{2+}]_o$ was decreased by the addition of 10–40 μl of EGTA 0.2 M, pH 7.0, to precisely match peak force to that in the control twitch. Free $[Ca^{2+}]_o$ in the Ca^{2+} back-titrated twitch was calculated with Fabiato's program.[19] Signals of force and aequorin luminescence were averaged and stored in the same way as in group 1. The protocol of Ca^{2+} back-titration allowed us to compare aequorin luminescence signals in the absence (control) and presence of sevoflurane at equal peak developed force. If the magnitude of the intracellular Ca^{2+} transient in the presence of sevoflurane (at equal peak force as in control) is different from that in the control twitch, there is likely to have been a change in myofibrillar Ca^{2+} sensitivity.

Theoretical Analysis

To assess the relative effects of sevoflurane on intracellular Ca^{2+} availability *versus* myofibrillar Ca^{2+} sensitivity, the isometric Ca^{2+} back-titration experiments were analyzed with the use of a multicompartment computer model[11] comprising the following compartments: free $[Ca^{2+}]_i$, Ca^{2+} bound to troponin C (TnC) and its force dependence, Ca^{2+} bound to calmodulin, and sarcoplasmic reticulum (SR) Ca^{2+} release and uptake. The model assumes that peak Ca^{2+} occupancy of TnC is the same at equal developed force with or without sevoflurane. For the sake of simplicity, all sources of myoplasmic Ca^{2+} delivery (transsarcolemmal Ca^{2+} current, Na^+–Ca^{2+} exchange, and SR Ca^{2+} release) were lumped into one myoplasmic Ca^{2+} delivery term (SR release) to gain one value, which is referred to as myoplasmic Ca^{2+} availability. Myoplasmic Ca^{2+} availability is expressed as percent of the value of the nonanesthetic control. Changes in myofibrillar Ca^{2+} sensitivity were expressed as changes in off-rate of Ca^{2+} from TnC at the Ca^{2+}-specific site II ($k_{off(TnC.Ca)}$; the on-rate is very fast and limited only by diffusion),[20] with the understanding that various mechanisms can generate changes in Ca^{2+} sensitivity (see Discussion). A detailed description of the analysis has been described elsewhere.[11] Myofibrillar Ca^{2+} sensitivity was defined as $1/k_{off(TnC.Ca)}$ and was expressed as percent of the value in the nonanesthetic control.

Statistical Analysis

Muscle characteristics between muscle groups were compared with one-way analysis of variance. Concentration–response relations between sevoflurane concentration and variables of contractility and aequorin luminescence were tested for differences with repeated-measures analysis of variance; pairwise comparisons *versus* control were conducted with Bonferroni-corrected paired *t* tests. In Ca^{2+} back-titration experiments, aequorin luminescence in sevoflurane and high $[Ca^{2+}]_o$ were compared with control by the Student paired *t* test. Because absolute values of aequorin luminescence varied from muscle to muscle, percentage values of aequorin luminescence and relative changes are reported in which each muscle serves as its own control.[11] Relative changes in Ca^{2+} sensitivity and availability derived at different sevoflurane concentrations were compared using one-way analysis of variance followed by pairwise comparison *versus* control with Bonferroni-corrected *t* test. Differences between relative values of Ca^{2+} sensitivity and availability in a particular sevoflurane concentration were compared with the Student paired *t* test. Values were reported as mean ± SD. Differences were considered significant at the *P* less than 0.05 level.

Results

Effects of Sevoflurane on Contractility and Intracellular Ca^{2+} Transient

Group 1 muscle characteristics in control conditions at L_{max} are shown in table 1. Figure 1 illustrates a representative example of a concentration–response experi-

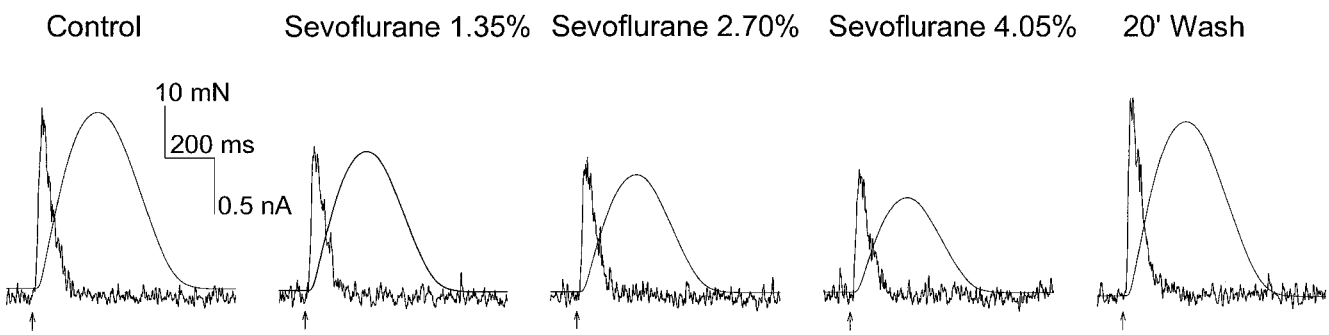

Fig. 1. Aequorin luminescence and force traces as a function of time during a cumulative concentration–effect experiment to sevoflurane in isometric twitches. One hundred twenty-eight twitches were averaged. The vertical arrow in each panel indicates the time of the electrical stimulus.

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 $[Ca^{2+}]$ 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.

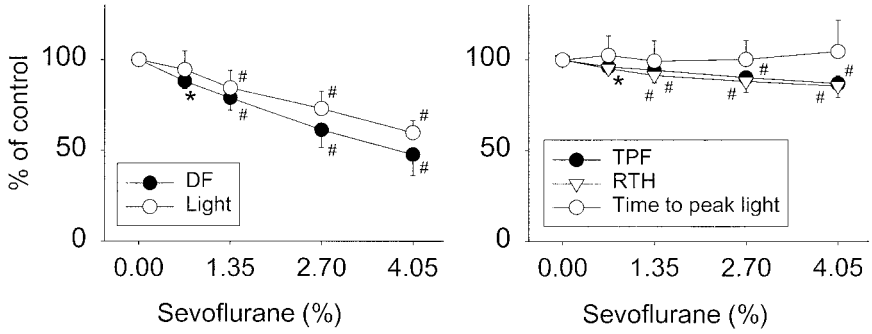

Fig. 2. Effects of sevoflurane on (*left*) peak force (DF) and peak aequorin luminescence (light), and on (*right*) time to peak aequorin luminescence, time to peak force (TPF), and time to half isometric relaxation (RTH) of isometric twitches. Data are mean ± SD. *$P < 0.05$, #$P < 0.01$ by repeated-measures 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 decreased 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 results 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]

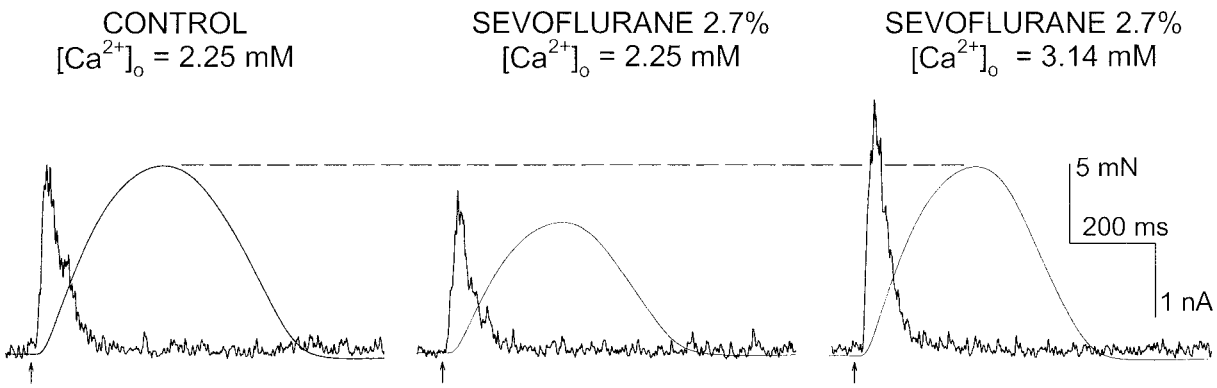

Fig. 3. Aequorin luminescence and force traces of isometric twitches during a typical Ca^{2+} back- titration experiment for sevoflurane 2.7%. After an initial control (*left*), muscles were exposed to sevoflurane 2.7% (*middle*), and $[Ca^{2+}]_o$ was rapidly increased (*right*) so that peak developed 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 TnC[25]; (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 chains[26]; 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 change[27] 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 changes 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+} transients 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

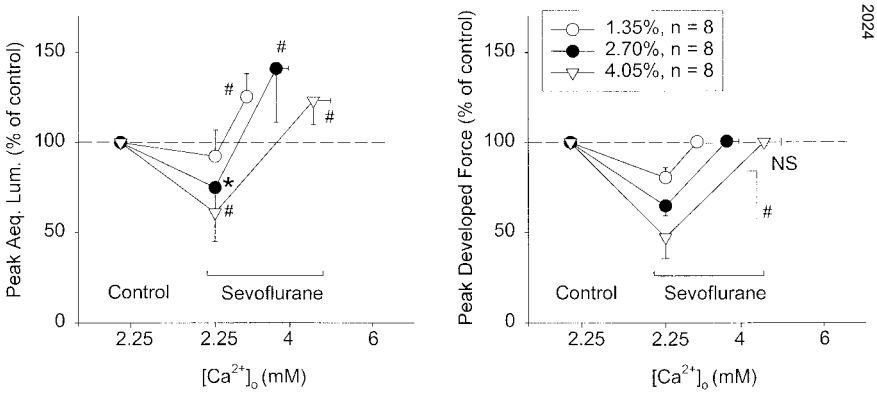

Fig. 4. Summary of isometric Ca^{2+} back-titration experiments for peak aequorin luminescence (*left*) and peak developed force (*right*) during control ($[Ca^{2+}]_o$ = 2.25 mM), sevoflurane exposure ($[Ca^{2+}]_o$ = 2.25 mM), and sevoflurane in increased $[Ca^{2+}]_o$ (> 2.25 mM) at equal peak force 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.

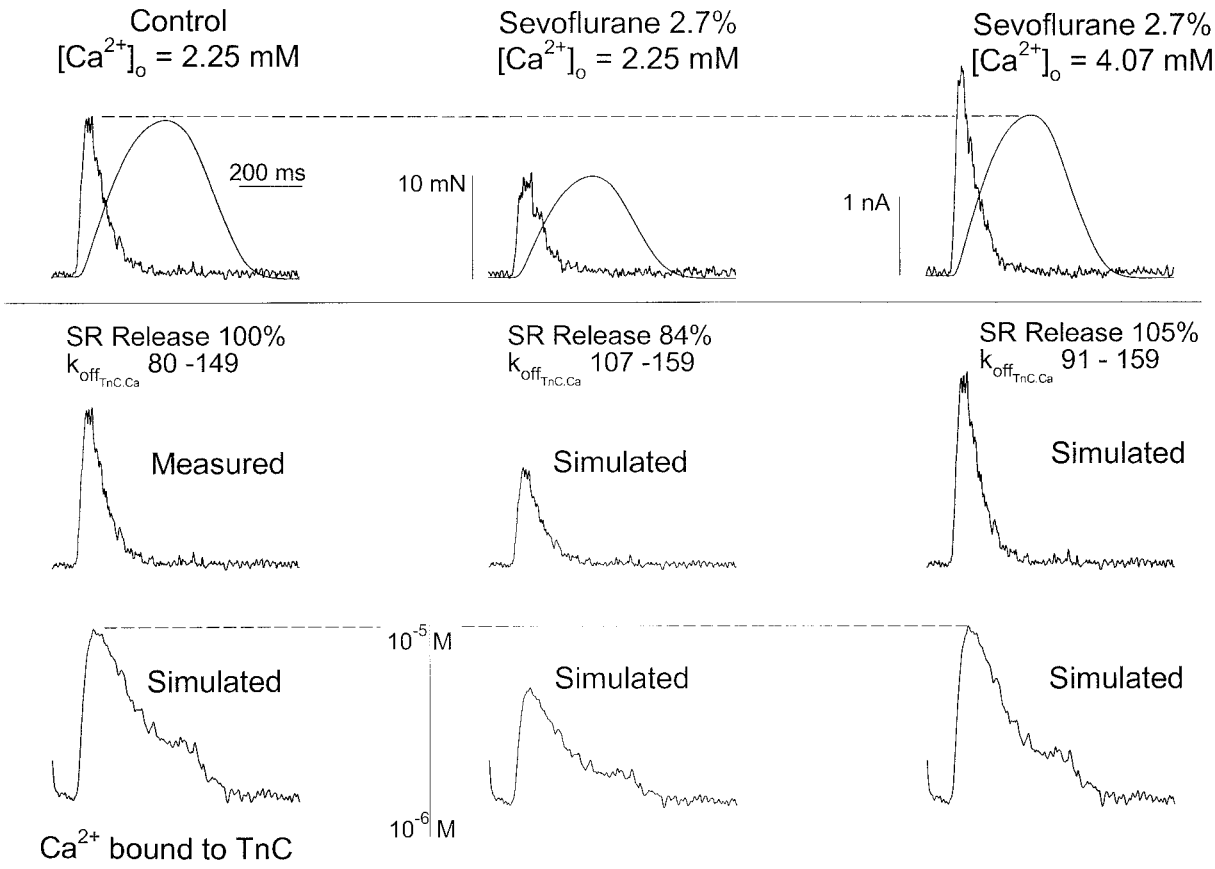

Fig. 5. Analysis of isometric Ca^{2+} back-titration experiment. (*Top*) Aequorin luminescence and force traces as a function of time in a typical Ca^{2+} back-titration experiment. Sixty-four twitch contractions were averaged. (*Middle*) Traces of aequorin luminescence obtained by computational simulation, except where marked "measured." (*Bottom*) Calculated traces of Ca^{2+} occupancy of troponin C. See text for details. SR = sarcoplasmic reticulum.

(similar to isotonic lengthening) are limited by the rate of decrease of the $[Ca^{2+}]_i$ transient.[30,31] The acceleration of isometric relaxation by sevoflurane in intact cardiac muscle might result from a decrease in Ca^{2+} sensitivity. This is in contrast to findings by Hanouz et al.,[22] who reported that sevoflurane and isoflurane did not induce a lusitropic effect under high load and concluded that these anesthetics might not modify myofilament Ca^{2+} sensitivity. The differences may result from species differences (rat *vs.* ferret).

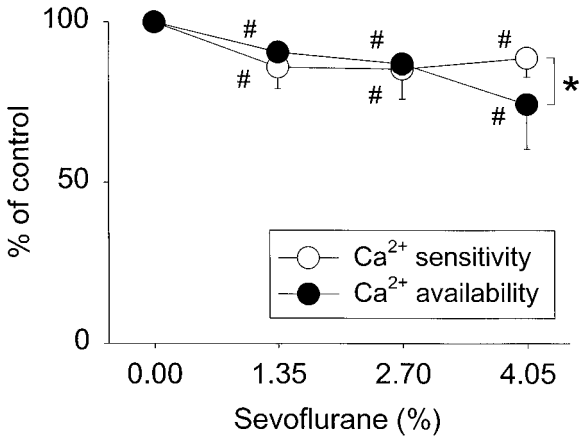

Fig. 6. Relative effects of sevoflurane on myoplasmic Ca^{2+} availability and myofibrillar Ca^{2+} sensitivity. The data points (mean ± SD) are derived from the same number of experiments as indicated in figure 4. *$P < 0.05$ by paired Student *t* test. #$P < 0.05$ by analysis of variance and Bonferroni-corrected *t* test for comparison with control.

To assess whether sevoflurane alters myofibrillar Ca^{2+} sensitivity in intact fibers, peak aequorin luminescence was compared with and without sevoflurane at equal peak force in Ca^{2+} back-titration experiments. The higher Ca^{2+} transient in sevoflurane and increased extracellular $[Ca^{2+}]$ than in control indicates a decreased Ca^{2+} sensitivity. To quantify the relative changes in myofibrillar Ca^{2+} sensitivity *versus* changes in myoplasmic Ca^{2+} availability, we analyzed the Ca^{2+} transients in the context of a multicompartment computer model that considered SR Ca^{2+} release, Ca^{2+} uptake, and binding to the principal intracellular Ca^{2+} buffers, TnC and calmodulin.[11] Published values for rate constants of association and dissociation of Ca^{2+} to the intracellular buffers TnC and calmodulin and a Michaelis-Menten kinetic scheme for SR Ca^{2+} uptake were used. The values of V_{max} (maximal rate of Ca^{2+} uptake by the SR) and of K_m ($[Ca^{2+}]_o$ at which Ca^{2+} 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 fibers[9] 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_{fs}) 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 conclude 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.

The authors thank Laurel Wanek, B.A., Mayo Foundation, Rochester, Minnesota, for outstanding support in this project.

References

1. Park WK, Pancrazio JJ, Suh CK, Lynch C 3rd: Myocardial depressant effects of sevoflurane: Mechanical and electrophysiologic actions in vitro. ANESTHESIOLOGY 1996; 84:1166–76

2. Azuma M, Matsumura C, Kemmotsu O: The effects of sevoflurane on contractile and electrophysiologic properties in isolated guinea pig papillary muscles. Anesth Analg 1996; 82:486–91

3. Hatakeyama N, Ito Y, Momose Y: Effects of sevoflurane, isoflurane, and halothane on mechanical and electrophysiologic properties of canine myocardium. Anesth Analg 1993; 76:1327–32

4. Hatakeyama N, Momose Y, Ito Y: Effects of sevoflurane on contractile responses and electrophysiologic properties in canine single cardiac myocytes. ANESTHESIOLOGY 1995; 82:559–65

5. Kanaya N, Kawana S, Tsuchida H, Miyamoto A, Ohshika H, Namiki A: Comparative myocardial depression of sevoflurane, isoflurane, and halothane in cultured neonatal rat ventricular myocytes. Anesth Analg 1998; 87:1041–7

6. Davies LA, Hamilton DL, Hopkins PM, Boyett MR, Harrison SM: Concentration-dependent inotropic effects of halothane, isoflurane and sevoflurane on rat ventricular myocytes. Br J Anaesth 1999; 82:723–30

7. Prakash YS, Cody MJ, Hannon JD, Housmans PR, Sieck GC: Comparison of volatile anesthetic effects on actin-myosin cross-bridge cycling in neonatal versus adult cardiac muscle. ANESTHESIOLOGY 2000; 92:1114–25

8. Bartunek AE, Housmans PR: Effects of sevoflurane on the contractility of ferret ventricular myocardium. J Appl Physiol 2000; 89:1778–86

9. Murat I, Ventura-Clapier R, Vassort G: Halothane, enflurane, and isoflurane decrease calcium sensitivity and maximal force in detergent-treated rat cardiac fibers. ANESTHESIOLOGY 1988; 69:892–9

10. Tavernier BM, Adnet PJ, Imbenotte M, Etchrivi TS, Reyford H, Haudecoeur G, Scherpereel P, Krivosic-Horber RM: Halothane and isoflurane decrease calcium sensitivity and maximal force in human skinned cardiac fibers. ANESTHESIOLOGY 1994; 80:625–33

11. Housmans PR, Wanek LA, Carton EG, Bartunek AE: Effects of halothane and isoflurane on the intracellular Ca^{2+} transient in ferret cardiac muscle. ANESTHESIOLOGY 2000; 93:189–201

12. Housmans PR, Lee NK, Blinks JR: Active shortening retards the decline of the intracellular calcium transient in mammalian heart muscle. Science 1983; 221:159–61

13. Housmans PR, Murat I: Comparative effects of halothane, enflurane, and isoflurane at equipotent anesthetic concentrations on isolated ventricular myocardium of the ferret. I. Contractility. ANESTHESIOLOGY 1988; 69:451–63

14. Murat I, Housmans PR: Minimum alveolar concentrations (MAC) of halothane, enflurane, and isoflurane in ferrets. ANESTHESIOLOGY 1988; 68:783–6

15. Quasha AL, Eger EId, Tinker JH: Determination and applications of MAC. ANESTHESIOLOGY 1980; 53:315–34

16. Aida H, Mizuno Y, Hobo S, Yoshida K, Fujinaga T: Determination of the minimum alveolar concentration (MAC) and physical response to sevoflurane inhalation in horses. J Vet Med Science 1994; 56:1161–5

17. Kazama T, Ikeda K: Comparison of MAC and the rate of rise of alveolar concentration of sevoflurane with halothane and isoflurane in the dog. ANESTHESIOLOGY 1988; 68:435–7

18. Scheller MS, Saidman LJ, Partridge BL: MAC of sevoflurane in humans and the New Zealand white rabbit. Can J Anaesth 1988; 35:153–6

19. Fabiato A: Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Methods Enzymolog 1988; 157:378–417

20. Johnson JD, Charlton SC, Potter JD: A fluorescence stopped flow analysis of Ca^{2+} exchange with troponin C. J Biol Chem 1979; 254:3497–502

21. Hanouz JL, Massetti M, Guesne G, Chanel S, Babatasi G, Rouet R, Ducouret P, Khayat A, Galateau F, Bricard H, Gerard JL: In vitro effects of desflurane, sevoflurane, isoflurane, and halothane in isolated human right atria. ANESTHESIOLOGY 2000; 92:116–24

22. Hanouz JL, Vivien B, Gueugniaud PY, Lecarpentier Y, Coriat P, Riou B: Comparison of the effects of sevoflurane, isoflurane and halothane on rat myocardium. Br J Anaesth 1998; 80:621–7

23. Graf BM, Vicenzi MN, Bosnjak ZJ, Stowe DF: The comparative effects of equimolar sevoflurane and isoflurane in isolated hearts. Anesth Analg 1995; 81:1026–32

24. Skeehan TM, Schuler HG, Riley JL: Comparison of the alteration of cardiac function by sevoflurane, isoflurane, and halothane in the isolated working rat heart. J Cardiothorac Vasc Anesth 1995; 9:706–12

25. Robertson SP, Johnson JD, Holroyde MJ, Kranias EG, Potter JD, Solaro RJ: The effect of troponin I phosphorylation on the Ca^{2+}-binding properties of the Ca^{2+}-regulatory site of bovine cardiac troponin. J Biol Chem 1982; 257:260–3

26. Hofmann PA, Metzger JM, Greaser ML, Moss RL: Effects of partial extraction of light chain 2 on the Ca^{2+} sensitivities of isometric tension, stiffness, and velocity of shortening in skinned skeletal muscle fibers. J Gen Physiol 990; 95:477–98

27. Blanck TJ, Chiancone E, Salviati G, Heitmiller ES, Verzili D, Luciani G, Colotti G: Halothane does not alter Ca^{2+} affinity of troponin C. ANESTHESIOLOGY 1992; 76:100–5

28. Vantrappen A, Wanek L, Sieck G, Potter J, Housmans P: Effect of halothane on Ca^{2+} binding to human recombinant cardiac troponin C [abstract]. ANESTHESIOLOGY 1998; 89:A622

29. Housmans P, Potter J: Effects of halothane on calcium binding kinetics of human recombinant cardiac troponin C [abstract]. Biophys J 2000; 78:107A

30. Backx PH, Gao WD, Azan-Backx MD, Marban E: The relationship between contractile force and intracellular [Ca^{2+}] in intact rat cardiac trabeculae. J Gen Physiol 1995; 105:1–19

31. Spurgeon HA, duBell WH, Stern MD, Sollott SJ, Ziman BD, Silverman HS, Capogrossi MC, Talo A, Lakatta EG: Cytosolic calcium and myofilaments in single rat cardiac myocytes achieve a dynamic equilibrium during twitch relaxation. J Physiol 1992; 447:83–102