Effects of volatile anesthetics on elastic stiffness in isometrically contracting ferret ventricular myocardium

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Bartunek, Anna E, Victor A. Claes, and Philippe R. Housmans. Effects of volatile anesthetics on elastic stiffness in isometrically contracting ferret ventricular myocardium. *J Appl Physiol* 92: 2491–2500, 2002. First published February 15, 2002; 10.1152/japplphysiol.00841.2001.—The effects of halothane, isoflurane, and sevoflurane on elastic stiffness, which reflects the degree of cross-bridge attachment, were studied in intact cardiac muscle. Electrically stimulated (0.25 Hz, 25°C), isometrically twitching right ventricular ferret papillary muscles ($n = 15$) at optimal length (L_{max}) were subjected to sinusoidal length oscillations (40 Hz, 0.25–0.50% of L_{max} peak to peak). The amplitude and phase relationship with the resulting force oscillations was decomposed into elastic and viscous components of total stiffness in real time. Increasing extracellular Ca^{2+} concentration in the presence of anesthetics to produce peak force equal to control increased elastic stiffness during relaxation, which suggests a direct effect of halothane and sevoflurane on cross bridges.

sinusoidal length oscillations; cross bridges; calcium

THERE IS EVIDENCE THAT the volatile anesthetics halothane, isoflurane, and sevoflurane inhibit actomyosin cross-bridge function at a level "downstream" from Ca^{2+} binding to thin filament regulatory proteins (3, 17, 26, 27, 29, 34). The contribution of this direct effect on cross-bridge function to the overall negative inotropic effect of volatile anesthetics appears to be small, but its relevance in physiological conditions is actually not known. Information about anesthetics' effects on cross-bridge performance was obtained mostly in skinned fibers (26, 27, 29, 34), yet results from intact cardiac muscle with intact cell membranes might reflect more accurately the in vivo physiological conditions. Instantaneous changes in stiffness on activation arise from an elastic component associated with each individual attached cross bridge; stiffness therefore expresses a measure of the number of attached cross bridges at any one moment (14, 15). Stiffness measurements were commonly utilized to explore the influence of different interventions on cross-bridge performance (3, 11, 26, 29, 31, 33, 39). The effects of volatile anes-

thetics on active cross bridges were recently studied by detecting changes in elastic stiffness that occur throughout shortening and lengthening of isotonic twitches of intact ferret papillary muscle. Halothane and isoflurane increased elastic stiffness at equal peak shortening and higher extracellular Ca^{2+} concentration ($[Ca^{2+}]$; $[Ca^{2+}]_o$) when compared with control, an observation that strongly suggests a direct effect of anesthetics on cross-bridge function (3).

In the present study, stiffness was measured throughout isometric contraction by imposing sinusoidal length oscillations of small amplitude and analyzing the amplitude and phase relations of the resultant force oscillations. Stiffness was instantaneously separated into its viscous and elastic components, the latter of which is associated with the elastic elements in the cross bridges. Instantaneous elastic stiffness measurement in Ca^{2+} back-titration experiments indicates that halothane and sevoflurane most likely affect cross-bridge performance during isometric relaxation in intact cardiac muscle tissue.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the Mayo Foundation, with protocols completed in accordance with the 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; 16–19 wk of age) were anesthetized with pentobarbital sodium (100 mg/kg ip), and the heart was quickly removed through a thoracotomy. During generous superfusion with oxygenated physiological 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 muscle chamber containing a physiological salt solution made up in ultrapure water (Nanopure Infinity, Barnstead, Dubuque, IA) and with the following composition (in mM): 137.5 Na^+, 5.0 K^+, 2.25 Ca^{2+}, 1.0 Mg^{2+}, 127.0 Cl^-, 1.0 SO_4^{2-}, 20.0 acetate^{-1}, 10.0 glucose, and 5.0 MOPS, pH 7.40, bubbled with 100% O_2. Suitable prepa-

rations were selected on the basis of the following criteria: length at which twitch active force is maximal (L_{max}) ≥ 3.5 mm, a mean cross-sectional area ≤ 1.2 mm^2, and a ratio of resting to total force in an isometric twitch at L_{max} ≤ 0.30. The tendinous end of each muscle was tied with a thin braided polyester thread (size 9.0 Deknatel Surgical Suture, Fall River, ME) to the end of a stiff glass rod (7.5 cm, 40 mg), which was attached to the lever of a force-length servotransducer. The attachment was sealed with paraffin. This transducer system (Innovi, Belgium) allows one simultaneously to *1*) measure shortening up to 3 mm (resolution 0.25 µm), *2*) impose loads up to 299 mN, *3*) measure force by feedback sensing (resolution <0.1 mN), and *4*) impose abrupt changes of load (load clamp) or of initial length (length clamps: quick release and quick stretch). The equivalent moving mass was 250 mg, and the static compliance was 0.28 µm/mN. The resonant frequency of the transducer was 250 Hz. The ventricular end of each muscle was held in a miniature Lucite clip (Dupont, Wilmington, DE) 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. A Grass S88D stimulator (Astro-Med, West Warwick, RI) delivered rectangular pulses of 5-ms duration. Stimuli at 10–20% above threshold, range 5–10 V, were used to minimize the release of endogenous norepinephrine by the driving stimuli. Throughout the stabilization period, the muscles were stimulated and made to contract in alternating series of four isometric and four isotonic twitches for at least 1 h at 0.25 Hz at the temperature of the bathing solution of 30°C. The solution was changed at least once during this stabilization period. When muscles had reached steady state, initial muscle length was set at L_{max}. Thereafter the temperature was decreased to 25°C, the definite temperature in which the experiments were carried out. The muscles were then allowed to stabilize for another hour first in the same manner as above and then in isometric twitches only for muscles contracted isometrically at the preload of L_{max} throughout the experiment. To minimize effects of release of endogenous catecholamines in ferret cardiac muscle, β-adrenoceptor blockade was achieved by the administration of (±)-bupranolol hydrochloride (10^{-7} M).

On-line measurement of elastic stiffness. When load or length of a muscle is forced to vary in a sinusoidal manner, the resulting periodic changes are also sinusoidal, but they will not generally speaking be in phase with the imposed oscillations. This phase shift is quantified by resolving the sinusoid of the received signal into two components, one in phase with the imposed sinusoid and one in quadrature to the imposed sinusoid (i.e., leading or lagging by 90°). We imposed continuous small rapid length perturbations on the muscle attached to the electromagnetic lever system, all of which behaves as a linear second-order mechanical system (24, 35, 36, 38). The relation of the sinusoidal responses to the imposed sinusoidal changes can be described mathematically

$$m \times dv/dt + B \times v + 1/C_m \times \int v \cdot dt = f(t)$$

where m is moving mass, B is viscosity, C_m is mechanical compliance, v is velocity, f is force, and t is time. Imposing periodic length oscillations of certain frequencies induces force oscillations that are slightly out of phase with length by a phase angle Φ (Fig. 1*A*). The analysis uses force and velocity vectors that are separated by a phase angle difference of (90° − Φ). The force vector is decomposed into a force
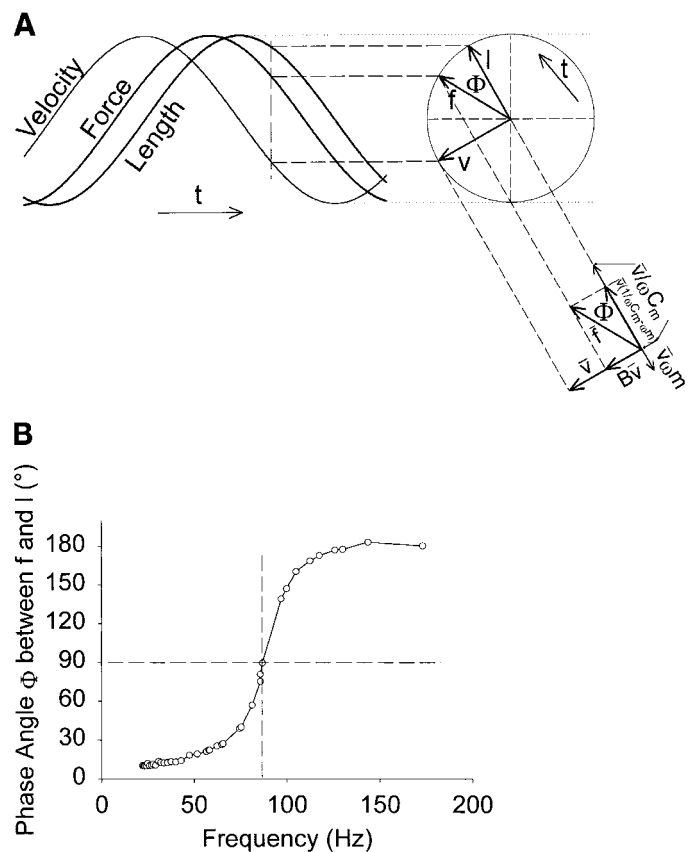

Fig. 1. *A*: schematic representation of analysis methods of the amplitude and phase relationships of length and force sinusoidal oscillations (*left*). Force, length, and velocity vectors are projected on a rotor diagram (*right*) in which the vectors rotate counterclockwise, 360° per cycle. The velocity and force vectors are projected (*right*) into a *x-y* plane in which the force vector f̄ is decomposed into a resistive component (Bv̄, where B is viscosity and v̄ is velocity) and, orthogonal to that, an [elastic (v̄/ωC$_m$) − inertial (v̄ωm)] component. At frequencies well below resonant frequency, the inertial component is negligibly small, and this component represents pure elastic stiffness. Analog correlators yielded both in-phase and out-of-phase components of total stiffness from the amplitude and phase relationship between force and velocity oscillations. *B*: Bode plot for a representative muscle at rest at the preload of length at maximal twitch force (L_{max}), illustrating the dependence of the phase angle Φ on oscillation frequency. The resonance frequency in this example was 87 Hz. The phase angle Φ between force and length is small (<15°) and independent of oscillation frequency at frequencies < 50% of resonance frequency. At resonance, the phase angle Φ is 90° and force and velocity are in phase with each other. At frequencies above resonance frequency, force and length tend to go out of phase. B, viscosity; C$_m$, mechanical compliance; f, force; l, length; m, equivalent moving mass; *t*, time; *v*, velocity; Φ, phase angle; ω, angular frequency.

component resisting the imposed velocity oscillations (Bv̄: viscosity) and the vectorial sum of the forces across moving mass (v̄ωm: inertia) and elastic components (v̄/ωC$_m$: mechanical compliance). At resonance, force and velocity oscillations are in phase, and inertial and elastic forces are equal in magnitude but of opposite signs and therefore they cancel each other out. Below resonance frequency, as shown in Fig. 1*A*, the elastic force is much larger than the inertial force, yet the opposite is true above resonance frequency. Figure 1*B* shows a Bode plot of a muscle at the preload of L_{max} at rest (not stimulated) displaying the phase angle Φ between force

and length oscillations at each of a large range of frequencies. Resonance frequency ($\Phi = 90°$) in this example was 87 Hz. In previous studies, we found that the resonance frequency of ferret papillary muscle at preload of L_{max} was in the range of 83–154 Hz (3). During isometric contraction, the resonance frequency would be higher as muscle stiffness increases with force. We used an oscillation frequency of 40 Hz in all experiments to minimize the contribution of inertial forces to the measurement of elastic stiffness. At frequencies <50% of resonance frequency, the phase angle Φ was indeed smaller than 15–20° (Fig. 1B), and the small error introduced by the inertial component can be ignored. The vectors $B\bar{v}$ and $\bar{v}/\omega C_m - \bar{v}\omega m$, viscous and elastic components of total stiffness, respectively, were obtained in real time by correlating the sine and cosine functions used with amplitude and phase relations of force and velocity oscillations by means of an electronic circuit of analog correlators. Henceforth we refer to elastic stiffness as "stiffness" unless otherwise stated. Viscosity changes were minimal during contraction and were not pursued in this investigation. We have previously shown in cat papillary muscle that viscous stiffness is one order of magnitude smaller than elastic stiffness (24). The imposed length perturbations, which could be switched on and off with a toggle switch, were of total amplitude of 0.25 and 0.50% L_{max} peak to peak in Ca^{2+} back-titration experiments and concentration response experiments, respectively. Continuous length oscillations resulted in depression of active force development by $5.1 \pm 1.1\%$ (3.5–6.6%) when oscillation amplitude was 0.50% L_{max} and by $1.7 \pm 0.5\%$ (0.8–2.4%) when muscles were oscillated with an amplitude of 0.25% L_{max}.

Methods of delivery of volatile anesthetics. The methods of delivery of anesthetics were the same as previously described (4, 19). In brief, oxygen flowed through the calibrated vaporizer for halothane, isoflurane, or sevoflurane and was allowed to mix with the respective anesthetic 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, Greenwich, CT) except for a narrow slit for the muscle clip and the glass rod. The concentration of the anesthetic 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 the concentrations of halothane, isoflurane, and sevoflurane and their calculated partial pressure in fluid followed closely imposed changes of anesthetic vapor concentration in the gas phase. After the administration of anesthetic was discontinued, anesthetic concentration was always undetectable at 30 min.

Experimental design. Two experimental protocols were used to examine the effects of anesthetics on stiffness in cardiac muscle in which each muscle served as its own

control. Anesthetics were studied in random order. Table 1 summarizes the characteristics of the muscles used in each of the two experimental series. Muscles twitched isometrically at the preload of L_{max} throughout the experiments. In the first series of experiments, muscles ($n = 7$) were exposed to halothane, isoflurane, and sevoflurane in random order and in concentrations of 0.0, 0.5, 1.0, and 1.5 minimum alveolar concentration (MAC) each. MAC is an anesthetic half-maximal effective dose 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. One MAC halothane, isoflurane, and sevoflurane in the ferret corresponds to 1% (vol/vol), 1.5% (vol/vol), and 2.7% (vol/vol) of the respective anesthetic (4, 25). Effects of each anesthetic on isometric contraction at the preload of L_{max} were monitored until a steady state was reached, and this was usually the case after 10–18 min of equilibration in each concentration. Subsequently, the concentration of the anesthetic was increased. In control conditions, in each anesthetic concentration, in anesthetic washout, and in Ca^{2+} back-titration, two records were taken: one with no oscillations and one when muscle was exposed to sinusoidal velocity oscillations; the latter was used for quantification of variables of interest. After the highest concentration of anesthetic, the anesthetic was turned off, the reservoir bag was emptied, and the gas-delivering system was flushed with oxygen. The muscle was allowed to recover for 1 h before new control measurements were taken and the administration of the next anesthetic was commenced.

In the second series of experiments ($n = 8$ muscles), we determined whether anesthetics change cross-bridge function by a mechanism different from that exerted by anesthetic-induced reduced intracellular $[Ca^{2+}]$. Muscles were stabilized in the same manner as above. Records, one with no oscillation and one when muscle was exposed to sinusoidal velocity oscillations, were taken in control conditions and in one MAC anesthetic, when peak force had reached steady state. Extracellular $[Ca^{2+}]$ was then rapidly increased by adding small aliquots of a concentrated $CaCl_2$ solution (0.25 M) to the bathing solution, until the amplitude of peak force was equal to that in control twitch. In 10 of 24 experiments, peak force slightly exceeded that of the control twitch after titration with $CaCl_2$. In these instances, extracellular $[Ca^{2+}]$ was decreased by addition of 20–400 µl of 0.1 M EGTA, pH 7.0, to precisely match peak force to that in the control twitch. Free Ca^{2+} in the Ca^{2+} back-titrated twitch was calculated by Fabiato's program (13). After recording the back-titrated twitch, the anesthetic was turned off, the reservoir bag was emptied, and the gas-delivering system was flushed with oxygen. The solution was changed to control physiological salt solution (extracellular $[Ca^{2+}] = 2.25$ mM) and replaced at least four times over a period of 15 min. The

Table 1. *Muscle characteristics during control conditions at L_{max}*

	L_{max}, mm	CSA, mm^2	R, mN/mm^2	T, mN/mm^2	R/T	RS, mN/mm^3
Dose-response ($n = 7$)						
Mean ± SD	4.94 ± 0.49	0.53 ± 0.16	9.98 ± 2.40	68.37 ± 12.57	0.15 ± 0.02	138.10 ± 64.00
Range	4.29–5.70	0.36–0.76	6.97–14.79	49.66–86.53	0.12–0.17	70.03 ± 252.67
Ca^{2+} Back-titration ($n = 8$)						
Mean ± SD	6.39 ± 0.68	0.50 ± 0.18	13.12 ± 4.88	81.68 ± 24.46	0.17 ± 0.05	169.86 ± 64.55
Range	5.00–7.14	0.29–0.86	7.06–19.96	43.86–114.86	0.08–0.24	98.99–283.10

Values are means ± SD. L_{max}, length at which twitch active force is maximal; CSA, cross-sectional area; R, resting tension; T, total tension; RS, resting stiffness.

muscle was allowed to recover for 1 h before the same protocol with a different anesthetic was repeated. This protocol of Ca^{2+} back-titration allowed us to compare elastic stiffness in the absence (control) and presence of anesthetic at equal peak force.

All waveforms of force and elastic stiffness were displayed as a function of time on a four-channel digital oscilloscope (Nicolet 4094C, Madison, WI). Waveforms were stored on 5.25-in. 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 and stiffness. Stiffness trace was generated by the correlator with a 17-ms delay. Original force trace with oscillations and filtered force trace with 17-ms delay were stored (Fig. 1). Maximal amount of developed force (DF), time to DF, relaxation half time, and the time from DF to half isometric relaxation were analyzed from filtered and delayed force traces. To obtain stiffness in the active muscle, resting stiffness was subtracted from total stiffness. Peak active stiffness (PS), active stiffness at DF, active stiffness at half isometric relaxation, time to PS, and time from PS to half active stiffness during relaxation were analyzed from stiffness traces. Time to DF and time to PS were measured from the stimulus and corrected for 17-ms delay. Stiffness and force were normalized for cross-sectional area.

Statistics. In the concentration-response experiments, measurements during exposure to different anesthetic concentrations were compared with control measurements obtained just before exposure to the respective anesthetic by means of repeated-measures ANOVA, followed by comparisons vs. control by Bonferroni-corrected paired t-test. Comparisons between anesthetics of the same clinically effective concentration were performed by repeated-measures ANOVA, followed by pairwise comparisons with Bonferroni-corrected paired t-test. In Ca^{2+} back-titration experiments, comparisons between measurements in control and in exposure to 1 MAC, anesthetic and elevated extracellular $[Ca^{2+}]$ were compared by Student's paired t-test. Differences were considered significant at the $P < 0.05$ level. Data are reported as means $\pm$ SD, except in Figs. 3 and 4 where SE are plotted for reasons of clarity.

RESULTS

Table 2 summarizes variables of isometric contraction and elastic stiffness at the onset of the experiment. Figure 2, *B* and *C*, illustrates force and length traces of

Table 2. *Variables of contraction and elastic stiffness at the onset of the experiment*

	Dose Response (n = 7)	Ca^{2+} Back-titration (n = 8)
DF, mN/mm^2	58.39 ± 10.73	68.57 ± 22.04
PS, mN/mm^3	366.48 ± 68.99	169.86 ± 64.55
SDF, mN/mm^3	339.23 ± 66.54	291.62 ± 82.77
SHF-relax, mN/mm^3	265.70 ± 54.40	204.93 ± 56.21
TPF, ms	444.71 ± 30.85	466.25 ± 76.45
RTH, ms	369.57 ± 32.35	426.25 ± 79.42
TPS, ms	569.00 ± 42.49	578.00 ± 88.92
SHT, ms	307.86 ± 44.39	372.50 ± 71.31

Values are mean $\pm$ SD. DF, developed force at maximal force amplitude; PS, peak active stiffness; SDF, active stiffness at DF; SHF-relax, active stiffness at half isometric relaxation; TPF, time to peak force; RTH, time from peak developed force to half isometric relaxation; TPS, time to peak stiffness; SHT, time from peak active stiffness to half active stiffness during relaxation.
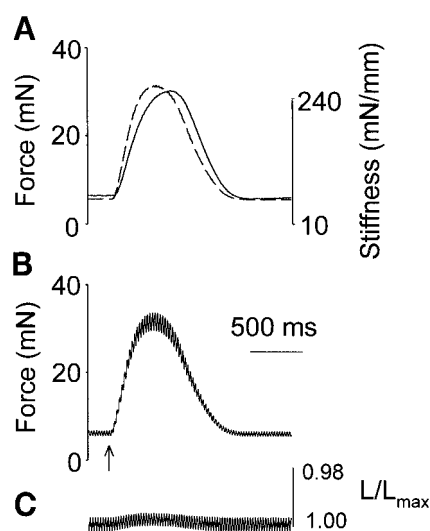

Fig. 2. Elastic stiffness (solid trace, *A*) obtained from sinusoidal length oscillations at 40 Hz (*C*) and resulting force oscillations (*B*) during isometric contraction in a representative muscle of the concentration-response experiment series in control conditions. Stiffness and filtered force trace (dashed trace in *A*) are delayed by 17 ms compared with unfiltered force and length trace. Vertical arrow in *B* indicates the electric stimulus. Muscle characteristics: L_{max}, 4.29 mm, mean cross-sectional area, 0.42 mm^2, ratio of resting to total force (R/T) = 0.14.

an isometrically twitching muscle in control conditions in a concentration-response experiment. Length oscillations with amplitude of 0.47% L_{max} (peak to peak) at a frequency of 40 Hz are visible on the length trace, and the resulting force oscillations are apparent on the force waveform. Figure 2*A* shows the stiffness trace, which is derived from the correlator with a 17-ms delay, and the force trace (dashed line), which is identical to that in the Fig. 2*B*, but filtered and delayed by 17 ms. The length trace shows a very small amount of shortening during the isometric contraction (Fig. 2*C*), which is consistent with a static compliance of 0.28 $\mu m/mN$ in the transducer system. In control conditions of concentration-response experiments, peak stiffness occurs 135 ± 17 ms (114–164 ms) after peak developed force. The time difference between peak force and peak stiffness decreased with increasing anesthetic concentrations (not shown) and recovered to control values when extracellular $[Ca^{2+}]$ was raised until peak force was the same as in control conditions (not shown). Volatile anesthetics did not alter resting elastic stiffness measured immediately before the stimulus (not shown).

In the first series of experiments, the effects of halothane, isoflurane, and sevoflurane on force and active stiffness in isometric twitches were examined in random order in seven muscles in concentrations of 0.0, 0.5, 1.0, and 1.5 MAC and after 30 min of anesthetic washout. Figure 3 shows typical force and stiffness waveforms in control conditions and 1 MAC anesthetic from a muscle of the first series of experiments. One MAC sevoflurane, isoflurane, and halothane decreased developed force, active stiffness, time to peak force, and time to peak active stiffness. Figure 3*C*, depicts stiff-
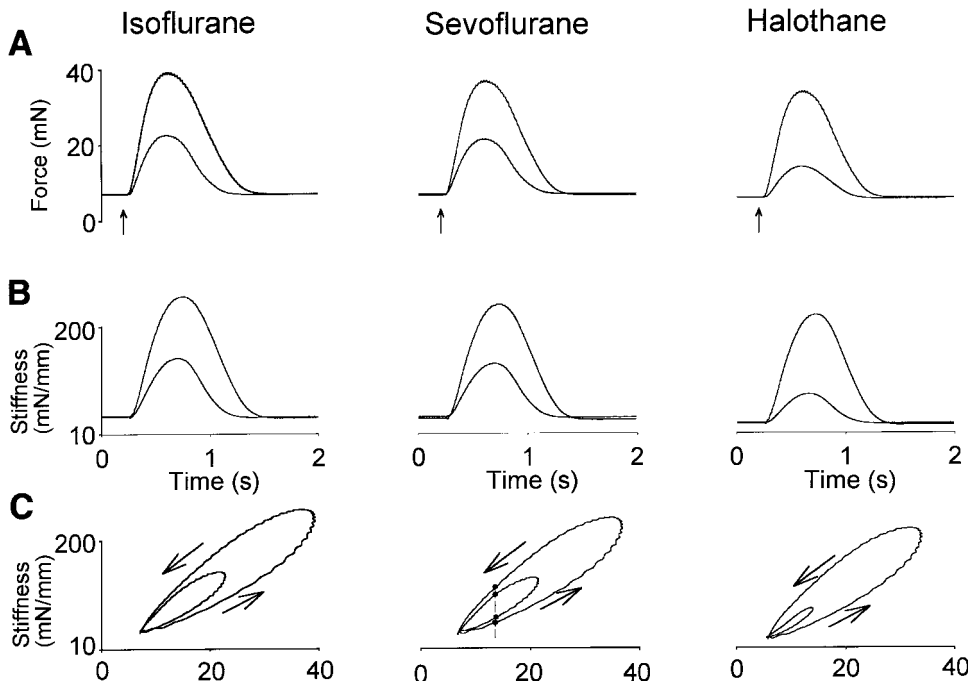
Fig. 3. Force (*A*) and stiffness (*B*) as a function of time in control conditions and during exposure to 1 minimum alveolar concentration (MAC) isoflurane, sevoflurane, and halothane in a representative muscle. Stiffness is plotted against force in *C*. Vertical arrow in *A* indicates the electric stimulus. Muscle characteristics: L_{max}, 5.7 mm, mean cross-sectional area, 0.56 mm^2, R/T = 0.13.

ness plotted against force. It shows that stiffness at any given force is higher during relaxation than during contraction. At any given force, anesthetics increased stiffness during contraction and decreased stiffness during relaxation resulting in a narrower hysteresis (Fig. 3*C*).

Concentration-dependent and washout effects of volatile anesthetics on developed force and active stiffness during isometric contraction are displayed in Figs. 4 and 5. Halothane, isoflurane, and sevoflurane decreased peak force and peak active stiffness

in a concentration-dependent reversible manner (Fig. 4, *A* and *B*). Each of the three anesthetics shortened time to peak force and, to a greater extent, time to peak stiffness (Fig. 4, *C* and *D*). They decreased active stiffness at peak force (Fig. 5*A*) and active stiffness at half isometric relaxation (Fig. 5*C*). They caused a shortening of time from peak force to half isometric relaxation (Fig. 5*B*) and time from peak stiffness to half active stiffness during relaxation (Fig. 5*D*). Relaxation half time did not recover to normal values after 30-min anesthetic washout, and time
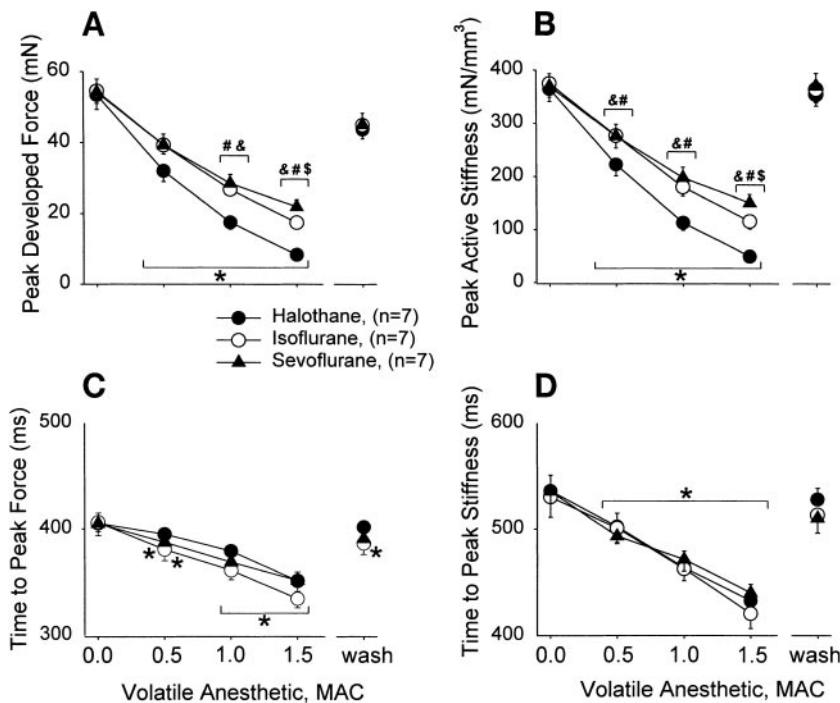

Fig. 4. Concentration-response effects of halothane, isoflurane, and sevoflurane on peak developed force (*A*), peak active stiffness (*B*), time to peak force (*C*), and time to peak active stiffness (*D*) in isometric twitches. *$P < 0.05$, by repeated-measures ANOVA and comparisons vs. corresponding control by Bonferroni-corrected paired *t*-test. Significant differences, $P < 0.05$, by repeated-measures ANOVA and pairwise comparisons between equipotent concentrations of anesthetics by Bonferroni-corrected paired *t*-test: #halothane vs. sevoflurane, $isoflurane vs. sevoflurane, &halothane vs. isoflurane. Values (means ± SE) in each graph are from the same muscles.

Fig. 5. Concentration-response effects of halothane, isoflurane, and sevoflurane on active stiffness at peak force (*A*), relaxation half time, i.e., time from peak developed force to half isometric relaxation (*B*), active stiffness at half isometric relaxation (*C*), and stiffness half time, i.e., time from peak active stiffness to half active stiffness during relaxation (*D*). *$P < 0.05$, by repeated-measures ANOVA and comparisons vs. corresponding control by Bonferroni-corrected paired *t*-test. Significant differences, $P < 0.05$, by repeated-measures ANOVA and pairwise comparisons between equipotent concentrations of anesthetics by Bonferroni-corrected paired *t*-test: #halothane vs. sevoflurane; $isoflurane vs. sevoflurane; &halothane vs. isoflurane. Values (means ± SE) in each graph are from the same muscles.
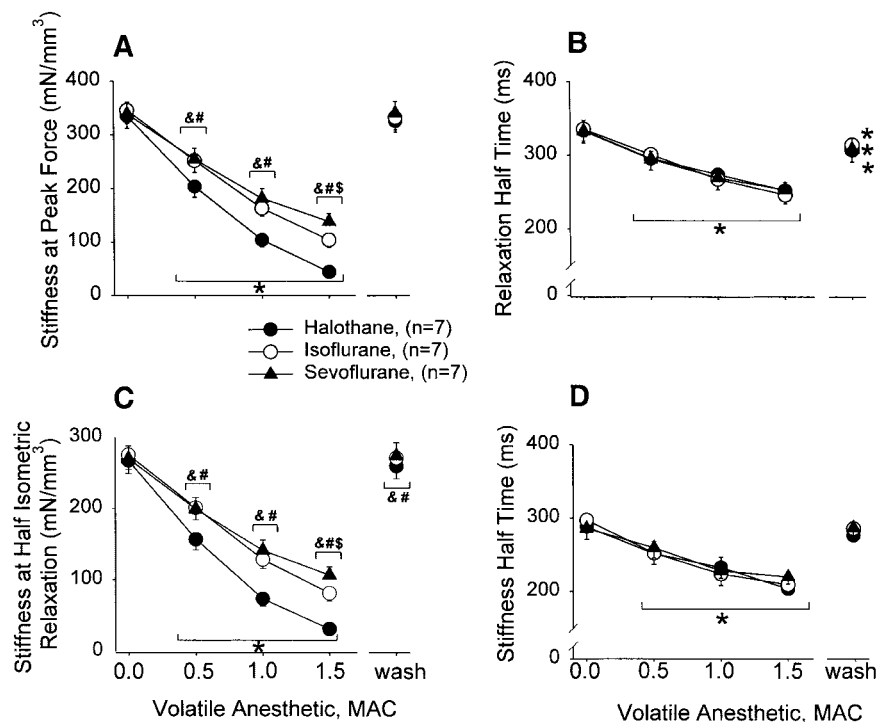
to peak force did not recover completely in halothane washout (Figs. 5*B* and 4*C*).

Ca^{2+} back-titration experiment. To determine whether anesthetics change active stiffness by a mechanism different from that exerted by anesthetic-induced decrease in intracellular [Ca^{2+}], Ca^{2+} back-titration experiments were performed (Fig. 6). Force and active stiffness were measured in control and during exposure to anesthetic 1 MAC, both in extracellular [Ca^{2+}] = 2.25 mM. Extracellular [Ca^{2+}] was then rapidly increased until peak force in anesthetic was the same as in the control twitch. In two muscles exposed to 1 MAC halothane, force amplitude could not be raised to control values by increasing extracellular [Ca^{2+}]. These muscles were excluded from further analysis regarding the halothane Ca^{2+} back-titration but were used for analysis of Ca^{2+} back-titration in isoflurane and sevoflurane. In Ca^{2+} back-titration experiments in the presence of isoflurane, peak force was minimally higher in each muscle (2.4–0.6 mN/mm^2). This resulted in a significant different mean peak force (0.64 ± 0.76 mN/mm^2). Therefore, limitations exist in drawing conclusions regarding the effects of isoflurane. Table 3 summarizes results of the Ca^{2+} back-titration experiments to 1 MAC halothane, isoflurane, and sevoflurane. In Ca^{2+} back-titration, active stiffness at peak force was slightly higher in sevoflurane. Halothane and sevoflurane significantly increased active stiffness at half isometric relaxation. In the presence of anesthetics and elevated Ca^{2+}, time from peak force to half isometric relaxation and time from peak active stiffness to half active stiffness during relaxation were significantly abbreviated.

DISCUSSION

We used continuous small-amplitude sinusoidal length perturbations to determine the elastic component of stiffness throughout isometric twitch contractions in the presence of halothane, isoflurane, and sevoflurane. The three volatile anesthetics decreased peak developed force and concomitantly active stiffness in a concentration-dependent manner, with halothane being most and sevoflurane least effective, an observation that is caused mainly by decreased intracellular availability of activating Ca^{2+} and decreased Ca^{2+} sensitivity of the contractile system. When in Ca^{2+} back-titration experiments peak force was equal to that in control conditions, peak active stiffness was increased by sevoflurane and active stiffness during relaxation was increased by halothane and sevoflurane, indicating that cross-bridge performance is affected by a mechanism downstream of Ca^{2+} binding to troponin C.

When heart muscle length or load is disturbed sinusoidally, it responds with sinusoidal force or length alterations like a linear second-order mechanical system (24, 35, 36, 38). Inertia, viscosity, and elasticity present in muscle preparations are affected by sinusoidal forcing functions, and measurement of stiffness therefore reflects the response of all three components together. Elastic stiffness is considered to be a static or time-independent property. Consequently, an elastic component responds to a stretch with a change in tension that is dependent only on the magnitude of the stretch and not on the rapidity of the stretch. In contrast, viscous and inertial stiffness both reflect dynamic or time-dependent properties (36, 39). In the
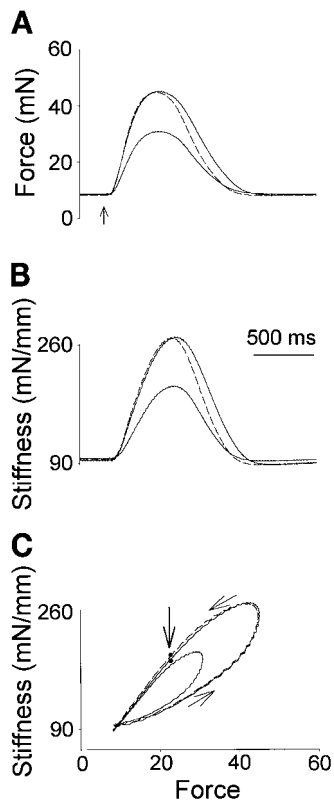
Fig. 6. Ca^{2+} back-titration experiment. Traces of force and elastic stiffness as a function of time in control conditions, during exposure to sevoflurane 1 MAC and during sevoflurane 1 MAC and elevated extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$; 4.07 mM) (dashed trace) are superimposed in A and B. C: stiffness plotted vs. force in control conditions, in 1 MAC sevoflurane, and in 1 MAC sevoflurane and elevated $[Ca^{2+}]_o$. In 1 MAC sevoflurane and elevated $[Ca^{2+}]_o$, stiffness for a given force during the relaxation phase is increased as indicated by the small vertical arrow at half peak force. Small vertical arrow in A indicates the electric stimulus. Muscle characteristics: L_{max}, 6.43 mm; mean cross-sectional area, 0.39 mm²; R/T = 0.18.

present study, the elastic component of stiffness was separated and recorded instantaneously throughout isometric contraction. In single activated frog skeletal fibers, elastic stiffness was thought to reside mainly in attached cross bridges, and the compliance attributable to cross bridges was found to be 80–90% of the measured instantaneous compliance of the fiber (14). Thus changes in stiffness reflect changes in the number of attached cross bridges. It is also generally accepted that the level of force generated by a muscle fiber is proportional to the number of cross bridges in the force-generating state (22). Therefore, if an intervention affects recruitment of cross bridges during Ca^{2+} activation, developed force and active stiffness will change in parallel. Disparate changes in stiffness and force, as we encountered in our experiments, however, can be explained by a change in the proportion of force generating to non-force-generating cross bridges. This might be induced by an influence on the kinetics of cross-bridge cycle with a modulation in rate of transition between passive and force-generating cross-bridge states during activation.

Interpretation of stiffness in isometric twitch contraction. During isometric contraction in papillary muscle, elastic stiffness lags behind tension during the tension rise and lags behind during relaxation (Fig. 2). This results in hysteresis when plotted as stiffness-tension relation during the isometric contraction cycle, with stiffness for a given force being higher during relaxation than during contraction (Fig. 3). This is in agreement with earlier investigations in intact cat papillary muscle, in which hysteresis of total (viscoelastic) stiffness-force relation was shown (33, 36) but not with findings in frog skeletal muscle fiber, in which stiffness rises earlier than tension during the onset of a tetanus (9, 16) and the plots of force vs. stiffness during development of force and during relaxation of tetanus were approximately the same (2). The reason for the discrepancy might be that in intact papillary muscle a complex composition of intracellular and extracellular elements forming the entirety of series elasticity induces a nonlinearity of the elastic-stiffness tension relation. Sarcomere compliance residing in the myofilament itself (18, 23, 41), damaged ends of intact muscle preparation (28), and shearing through Z-lines and intercalated discs (1) probably contribute to series elasticity. This might limit to some extent the value of conclusions drawn from elastic stiffness measurement in intact cardiac muscle on cross-bridge performance. Yet a change of elastic stiffness attributable to an intervention's influence on cross-bridge performance can still be detected and compared, by assuming that the intervention does not affect passive components in muscle during activation.

The effects of volatile anesthetics on active elastic stiffness. Evidence exists that contractile proteins undergo changes in the presence of volatile anesthetics, which result in the modulation of contractile force (17, 26, 29). Volatile anesthetics' effect on cross-bridge performance became evident in skinned (26, 29) and tetanized (17) cardiac muscle tissue but not in papillary muscle activated with Ba^{2+} (31). In intact isotonically twitching papillary muscle, halothane and isoflurane seemed to exert a direct effect on cross-bridge performance, as indicated by changes in elastic stiffness (3). Intact isotonically or isometrically twitching cardiac muscle tissue might better represent in vivo physiological conditions than skinned, tetanized, or Ba^{2+}-stimulated muscle. In isometric Ba^{2+}-stimulated contracture, the Ca^{2+}-free conditions might impair Ca^{2+}-dependent protein kinase involved in modulation of myofilament action. The skinning procedure is likely to change the microenvironment surrounding the myofilaments and allows the diffusion out of the cell of low-molecular-weight proteins, which play an important role in the modulation of muscle contractile function (43). To explore whether volatile anesthetics' interference with cross-bridge function is evident in intact isometrically contracting muscle, extracellular $[Ca^{2+}]$ was increased during exposure to 1 MAC anesthetic until peak force was the same as in control conditions. The underlying assumption is that at equal peak developed force, the Ca^{2+} binding sites responsi-

Table 3. *Summary of isometric Ca^{2+} back-titration experiments*

	Control	Ca^{2+} Back-titration in 1 MAC Anesthetic	P
Peak developed force, mN/mm^2			
Halothane	61.08 ± 16.22	61.09 ± 16.14	0.987
Isoflurane	66.22 ± 17.68	$66.86 \pm 18.09*$	0.049
Sevoflurane	66.61 ± 22.40	67.09 ± 22.50	0.064
Peak active stiffness, mN/mm^3			
Halothane	313.99 ± 84.69	326.99 ± 95.06	0.062
Isoflurane	324.88 ± 79.45	333.55 ± 82.56	0.051
Sevoflurane	322.26 ± 96.51	$329.15 \pm 95.91*$	0.036
Active stiffness at peak force, mN/mm^3			
Halothane	287.29 ± 80.81	295.65 ± 93.55	0.202
Isoflurane	297.21 ± 71.84	301.38 ± 76.73	0.392
Sevoflurane	293.28 ± 88.57	297.02 ± 86.13	0.269
Active stiffness at half isometric relaxation, mN/mm^3			
Halothane	194.57 ± 61.91	$207.43 \pm 68.23*$	0.026
Isoflurane	218.10 ± 53.19	$234.94 \pm 58.02*$	<0.001
Sevoflurane	211.79 ± 65.96	$225.31 \pm 65.26*$	0.002
Time to peak force, ms			
Halothane	435.33 ± 58.38	415.17 ± 48.63	0.094
Isoflurane	440.13 ± 59.73	$397.00 \pm 35.87*$	0.004
Sevoflurane	428.50 ± 59.92	400.00 ± 32.78	0.105
Time to peak stiffness, ms			
Halothane	551.33 ± 78.05	541.17 ± 59.65	0.475
Isoflurane	562.00 ± 74.16	$505.25 \pm 61.45*$	0.001
Sevoflurane	547.50 ± 76.11	520.63 ± 48.33	0.103
Relaxation half time, ms			
Halothane	380.00 ± 82.78	$324.83 \pm 58.62*$	0.022
Isoflurane	368.00 ± 48.07	$306.88 \pm 49.57*$	<0.001
Sevoflurane	389.75 ± 73.75	$335.50 \pm 67.43*$	<0.001
Stiffness half time, ms			
Halothane	330.00 ± 75.91	$264.33 \pm 43.30*$	0.016
Isoflurane	314.63 ± 25.18	$267.25 \pm 35.88*$	<0.001
Sevoflurane	337.13 ± 72.32	$282.88 \pm 52.19*$	0.001
$[Ca^{2+}]_o$, mmol/l			
Halothane	2.25	8.77 ± 2.25	
Isoflurane	2.25	4.77 ± 0.67	
Sevoflurane	2.25	4.15 ± 0.62	

Values are means $\pm$ SD. Variables of developed force and active stiffness in control and exposure to 1 MAC halothane ($n=6$), isoflurane ($n=8$), or sevoflurane ($n=8$) in increased extracellular Ca^{2+} concentration ($[Ca^{2+}]_o > 2.25$ mM) at equal peak force as in control. Peak force in Ca^{2+} back-titration in isoflurane was increased significantly above control value, therefore no conclusions will be drawn out of isoflurane data, which are presented here for the sake of completeness. $*P < 0.05$, by Student's paired t-test (no statistics in $[Ca^{2+}]_o$).

ble for Ca^{2+} regulation of the contractile system are occupied to an equal extent. The effects observed therefore are assumed to be caused by effects on contractile proteins downstream to Ca^{2+} binding to troponin C. Because peak force in Ca^{2+} back-titration in isoflurane experiments was raised significantly over that in control, we had to exclude isoflurane from discussion, although data analysis was performed and data are shown for completeness. In Ca^{2+} back-titration experiments, halothane and sevoflurane accelerated isometric relaxation as indicated by a decreased time from peak force to half isometric relaxation and increased active stiffness during relaxation as indicated by an increased stiffness at half isometric relaxation. The rate-limiting factor in tension relaxation of mammalian muscle is most likely the rate with which cross bridges detach and not Ca^{2+} uptake by the sarcoplasmic reticulum, because the decline of intracellular free $[Ca^{2+}]$ far precedes the decline of tension (44). When interpreted in a two-state cross-bridge model, an increase in the rate constant of cross-bridge detachment (g_{app}), an effect which has been shown in skinned rat

cardiac muscle preparations under exposure of 2 MAC halothane but not sevoflurane (29), could explain an acceleration of isometric relaxation. However, at submaximal levels of activation as in our experiments, cross-bridge kinetics are primarily controlled by the kinetics of Ca^{2+} binding to troponin C and the related transitions of the regulatory proteins. It is conceivable, therefore, that volatile anesthetics induce a faster Ca^{2+} off-rate from troponin C. Halothane did not alter the Ca^{2+} affinity of isolated troponin C (7), but this could not be shown for intracellular troponin C hitherto. The elevated active stiffness during relaxation indicates a higher stiffness-to-force ratio in the presence of halothane and sevoflurane. This is consistent with findings in skinned rat cardiac fibers, in which halothane, enflurane, and isoflurane increase the stiffness-to-force ratio (26). We interpret the disparate change in stiffness and force during exposure to halothane and sevoflurane as an effect on cross-bridge cycling kinetics. A modulation in rate of transition between passive and force-generating cross-bridge states could influence the proportion of force-generat-

ing to non-force-generating cross bridges. The molecular levels at which volatile anesthetics modulate cross-bridge performance cannot be detected by the technique used in this study. Anesthetics might modify rate constants of transitions between various states in the cross-bridge cycle by affecting contractile proteins themselves or by influencing second messenger cascades. There is growing evidence that volatile anesthetics activate protein kinase C, a mechanism that seems to play a role in myocardial protection exerted by volatile anesthetics (10, 40). Combined with the observation that protein kinase C inhibits cross-bridge cycling rate by phosphorylation of troponin I (32), this second messenger pathway might be considered to be involved in the effects observed.

The present findings must be interpreted within the constraints of several possible limitations. Although ferret papillary muscle is stable for many hours (3–5, 17, 19–21), we experienced a significant decrease of developed force from 55.5 mN/mm^2 in the first control measurement to 50.0 mN/mm^2 in the washout measurement of the last anesthetic $\sim$5 h later. Because anesthetics were studied in random order, this should not markedly confound the results.

Second, as mentioned earlier, in the presence of a high amount of series and parallel elastic elements and nonuniformities in intact muscle preparations, limitations might exist to deduct cross-bridge mechanical properties from whole muscle measurements (8). An alternative to the intact papillary muscle is the use of skinned fiber preparations (26, 29, 42), in which effects on myofilaments, practically anatomically isolated, can be tested. The studies by Prakash et al. (29) and Murat et al. (26) support our findings that volatile anesthetics affect cross-bridge kinetics.

A third limitation of our study is that we did not determine frequency dependence of stiffness. Dynamic transfer function of stiffness (42) and frequency at which stiffness is minimal (6, 30, 31) would give valuable information about dynamics and cycling rates of cross bridges. But those methods can only be used in muscle preparations in which steady-state activation of the contractile system is carried out, either in skinned fiber preparations or in intact tetanized or Ba^{2+}-activated muscle.

In conclusion, volatile anesthetics exert a direct effect on cross-bridge performance, which becomes apparent during isometric relaxation (halothane and sevoflurane) and as shown recently during isotonic contraction (halothane and isoflurane) in intact cardiac muscle tissue, an effect that might contribute significantly to their depressant action on the heart.

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