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The Effects of Halothane, Enflurane, and Isoflurane on the Length–Tension Relation of the Isolated Ventricular Papillary Muscle of the Ferret

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The effects of halothane, enflurane, and isoflurane on the length–tension relation were investigated in papillary muscles of the right ventricle of adult male ferrets at 30° C. Isometric twitch contractions were obtained at lengths ranging from the shortest length yielding the greatest active force development under isometric conditions (L_{max}) to 86% of L_{max}, in two consecutive protocols: first in $[Ca^{2+}]_o$ ranging from 0.45 to 2.25 mM, and then in $[Ca^{2+}]_o$ 2.25 mM before, during, and after exposure to incremental concentrations of halothane (n = 9 muscles), enflurane (n = 9 muscles), and isoflurane (n = 9 muscles), each in steps of 0.25 MAC to total concentrations up to and including 1.5 MAC. Each of the three anesthetics caused a concentration-dependent decrease in developed force. The relative extent of the negative inotropic effect was not different at various muscle lengths. Because myofibrillar Ca^{2+} responsiveness (Ca^{2+}-affinity of troponin C) decreases at shorter muscle lengths, the results suggest that an alteration in myofibrillar Ca^{2+} responsiveness by volatile anesthetics is minor relative to the anesthetic-induced decreased intracellular Ca^{2+} availability in ventricular myocardium. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Cardiac muscle: length–tension relation. Heart: Starling's Law; contractility.)

THE DEVELOPMENT OF FORCE by mammalian cardiac muscle and ventricular performance depends on initial fiber length—a phenomenon that is well known as the Frank-Starling Law of the Heart.[1]

Current theories suggest that both mechanical factors, such as myofilament overlap, as well as activation factors, such as the amount of Ca^{2+} bound to troponin, are affected by muscle length and contribute to the length dependence of force generation. In fully activated frog skeletal muscle, changes in thick and thin filament overlap account for most of the changes in force along the descending limb of the sarcomere length–tension relation, at sarcomere lengths >2.25 μm,[2] whereas along the ascending limb (sarcomere length <2.0 μm), myofilament overlap, restoring forces, and effects of interference between overlapping ends of thin filaments play a dominant role.

In mammalian cardiac muscle, where the working range is restricted to sarcomere lengths between approximately 1.6 to 2.3 μm,[3] the slope of the ascending limb of the length–tension relation is much steeper than in skeletal muscle, so that mechanical factors alone cannot account for the length dependence of force generation in cardiac muscle. In maximally calcium-activated skinned cardiac cells[4] and trabeculae,[5] the slope of the ascending limb of the length–tension relation is less steep than in intact cardiac muscle, which is generally only partially activated, so that the reduction of force in intact cardiac muscle at short sarcomere lengths is much greater than can be accounted for by mechanical factors only. This finding was one of the earliest indications that changes in activation with muscle length may also contribute to the cardiac length–tension relation. Two independent mechanisms by which changes in length affect activation have so far been identified. Changes in affinity of troponin for Ca^{2+} seem to occur instantaneously with changes in length,[5,6] whereas slow changes in Ca^{2+} release by the sarcoplasmic reticulum (SR) seem to follow changes in muscle length.[7,8]

The volatile anesthetics halothane, enflurane, and isoflurane are well-known myocardial depressants. Negative inotropic effects of these drugs have been explained in terms of their actions in interfering with 1) the entry of Ca^{2+} through the sarcolemma; 2) the Ca^{2+} uptake and release from the SR; and 3) the Ca^{2+} responsiveness of the contractile apparatus.[9] Most previous studies on the cardiac contractile effects of anesthetics were conducted at a fixed initial fiber length. Since both volatile anesthetics and shorter muscles lengths decrease myofibrillar responsiveness, it is possible that the anesthetic negative inotropic effect is more pronounced at long than at short muscle lengths. The purpose of this study is 1) to document the effects of halothane, enflurane, and isoflurane on the length–tension relation of isolated ventricular myocardium; and 2) to test the hypothesis that these anesthetics have a more pronounced negative inotropic effect at long than at short muscle lengths.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee. Twenty-seven papillary muscles

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from the right ventricle of adult male ferrets (weighing 1,100–1,500 g, and 16–19 weeks of age) were used for this study. The animals were anesthetized with 100 mg/kg intraperitoneal pentobarbital. As soon as a surgical level of anesthesia was reached, the heart was excised quickly and placed in a beaker with freshly oxygenated (30° C) physiologic salt solution. After a few minutes of spontaneous beating, the aorta and coronary arteries were selectively perfused for one minute with oxygenated physiological salt solution at 30° C. The right ventricle was opened and oxygenated physiologic salt solution (30° C) dripped on the exposed right ventricle and papillary muscles, while the heart and papillary muscles were beating spontaneously. Papillary muscles were excised carefully and mounted vertically in a jacketed temperature-controlled (30° C) muscle chamber similar to Blinks'[10] bath. The chamber contained 80 ml physiologic salt solution of the following composition (mM): Na^+ 140; K^+ 5; Ca^{2+} 2.25; Mg^{2+} 1; Cl^- 103.5; HCO_3^- 24; HPO_4^{2-} 1; SO_4^{2-} 1; $acetate^{-1}$ 20; and glucose 10. This solution was made with deionized distilled water and was continuously bubbled with a gas mixture of 95% O_2–5% CO_2.

The tendinous end of each muscle was tied with a thin braided polyester thread (size 9.0 Deknatel® Surgical Tevdek) to the lever of a force–length servo transducer[11] with a moving mass equivalent to 250 mg and a static compliance of 0.28 μm/mN. The ventricular end of the muscle was held in a miniature Lucite clip with built-in platinum electrode. Two platinum wires ran longitudinally on either side of the muscle and served as anode during punctate stimulation.

All of the experiments of this study were conducted at a stimulus frequency of 0.25 Hz and at a temperature of 30° C to allow for comparison with other studies in ferret papillary muscle.[12,13] Previous experiments, though conducted in isotonic conditions, have shown that ferret isolated papillary muscles remain stable for many hours in those circumstances.[12]

A Grass S88D stimulator delivered rectangular pulses of 5-ms duration, and stimulus voltage was set 10% above threshold and ranged from 2 to 12 volts. After 10 min of preloaded isotonic twitch contractions, the muscles were made to contract in alternating series of four preloaded isotonic and four isometric twitches for at least 2 h, until twitch amplitude and time course reached steady state. During this stabilization period, the bathing solution was renewed at least twice with fresh physiologic salt solution. After the stabilization period, muscle length was set at the shortest muscle length yielding the greatest active force development under isometric conditions (L_{max}), and the basic characteristics were determined. Table 1 shows the basic muscle characteristics at L_{max} at the onset of the experiment. Waveforms of force, length, and rate of force development (dF/dt) were acquired each at 1 kHz and displayed as a function of time on a four-channel digital oscilloscope (Nicolet 4094A or 4094C) and on a four-channel pen recorder (Honeywell 1400). Waveforms of interest were permanently stored on floppy disks, transferred to a microcomputer (IBM PC-XT, Boca Raton, FL) and analyzed as previously described.[12] At this stage, ten muscles were excluded from the study for failure to meet previously described criteria,[12] which define suitable muscles as having, at L_{max}, a length ≥3.5 mm, a mean cross-sectional area ≤1.2 mm^2, and ratio of resting to total force (R/T) <0.25.

After a new stabilization period of 20 min under isometric conditions at L_{max}, each of a series of eight isometric contractions was recorded at a different length, in the following sequence: 100, 96, 92, 88, 98, 94, 90, and 86% of L_{max}. Changes in muscle length were applied 203.9 ± 3.0 ms (mean ± SD) before the stimulus by rapidly (<3 ms) switching electronically from the stop set at L_{max} to the stop at each shorter length. Each of these test contractions at a length shorter than L_{max} was separated by 15 isometric contractions at L_{max} to minimize the slow effects of length changes on force development.[8] This 8-min sequence of "test contractions" was repeated in the various conditions (*vide infra*) during the experiment. For each contraction the following variables were measured: resting force, total force, developed force (DF), maximal rate of force development ($+dF/dt_{max}$), maximal rate of decrease in force ($-dF/dt_{max}$), resting length, length at peak force, time to peak force (TPF), time to maximal rate of force development, time to maximal rate of decrease in force, and time from peak force to half-isometric relaxation ($RT\frac{1}{2}$).

TABLE 1. Muscle Characteristics at the Onset of the Experiments at L_{max}

	L_{max} (mm)	CSA (mm^2)	R ($mN \cdot mm^{-2}$)	T ($mN \cdot mm^{-2}$)	R/T
Halothane (n = 9)	5.59 ± 1.46 (3.50–7.50)	0.56 ± 0.19 (0.31–0.88)	8.76 ± 3.20 (3.07–14.09)	49.48 ± 21.32 (26.17–92.82)	0.186 ± 0.056 (0.100–0.263)
Enflurane (n = 9)	5.26 ± 1.13 (4.00–7.50)	0.66 ± 0.23 (0.36–0.98)	8.55 ± 3.29 (4.57–15.37)	44.64 ± 18.82 (25.61–78.07)	0.197 ± 0.041 (0.144–0.270)
Isoflurane (n = 9)	4.73 ± 0.69 (3.75–5.75)	0.61 ± 0.16 (0.33–0.83)	7.35 ± 3.67 (3.83–15.87)	44.29 ± 12.60 (27.37–65.00)	0.161 ± 0.041 (0.117–0.244)

Values are mean ± SD; range in parentheses. CSA = cross-sectional area; R = resting tension; T = total tension.

TABLE 2. Control Values of L_{max}

	DF ($mN \cdot mm^{-2}$)	$+dF/dt_{max}$ ($mN \cdot mm^{-2} \cdot s^{-1}$)	$-dF/dt_{max}$ ($mN \cdot mm^{-2} \cdot s^{-1}$)	TPF (ms)	RT½ (ms)
Halothane					
1	40.758 ± 20.002	332.049 ± 139.112	274.377 ± 76.090	214.750 ± 36.441	129.250 ± 49.552
2	33.825 ± 18.668**	286.411 ± 131.803**	238.770 ± 97.585*	203.875 ± 28.287	118.500 ± 42.440*
3	40.522 ± 19.791	342.436 ± 135.823	272.182 ± 94.562	205.222 ± 30.145	116.125 ± 30.187
4	36.205 ± 17.988†	302.808 ± 135.167†	242.895 ± 103.950	208.444 ± 35.739	119.875 ± 32.211
Enflurane					
1	36.772 ± 15.933	265.708 ± 105.825	194.575 ± 71.968	225.625 ± 25.939	136.000 ± 21.153
2	28.488 ± 12.612**	238.905 ± 96,995*	185.173 ± 71.706	216.500 ± 28.671*	123.875 ± 15.624**
3	36.132 ± 17.422	323.858 ± 177.517	236.449 ± 120.169	207.444 ± 29.134	121.222 ± 19.182
4	32.304 ± 15.992	281.957 ± 157.737	200.570 ± 97.790†	213.778 ± 26.541	123.444 ± 17.536
Isoflurane					
1	37.540 ± 9.448	301.152 ± 63.996	240.524 ± 72.719	218.444 ± 17.565	131.000 ± 23.281
2	31.590 ± 9.879**	269.781 ± 67.977*	226.123 ± 73.511	208.222 ± 17.845**	120.111 ± 13.495**
3	36.287 ± 9.536	312.117 ± 74.004	263.691 ± 77.197	205.333 ± 15.572	115.778 ± 16.200
4	32.016 ± 10.108†	268.936 ± 83.389††	216.363 ± 70.802††	211.778 ± 20.987†	124.889 ± 14.632††

Values are mean ± SD.

DF = developed force; $+dF/dt_{max}$ = maximal rate of rise of force; $-dF/dt_{max}$ = maximal rate of fall of force; TPF = time to peak force; RT½ = time from peak force to half isometric relaxation.

* $P < 0.05$, ** $P < 0.01$ for comparison with control 1 by Student's paired t test. † $P < 0.05$, †† $P < 0.01$ for comparison with control 3 by Student's paired t test.

Once the initial series of test contractions had been recorded (control 1, table 2, fig. 1) the solution was changed to a low-$[Ca^{2+}]_o$ (0.45 mM) solution, and muscles were allowed to reach steady state. Series of test contractions were recorded after a 15-min equilibration, at each of the following Ca^{2+} concentrations (mM): 0.45, 0.90, 1.35, 1.80, and 2.25 (control 2, table 2), achieved by addition of small aliquots of a $CaCl_2$ (112.5 mM) solution to the bath. The solution then was replaced with fresh physiologic salt solution ($[Ca^{2+}]_o$ 2.25 mM), and muscles were allowed to equilibrate. A third series of test contractions then was recorded (control 3, table 2) immediately before exposure to anesthetic. Each muscle was exposed to one anesthetic only, and nine muscles were tested for each anesthetic. Following MAC values previously determined for the ferret at 37° C,[14] we studied the effects of equipotent concentrations of halothane, enflurane, and isoflurane from 0–1.50 MAC in 0.25 MAC increments. Since the experiments were carried out at 30° C, however, the MAC fractions and multiples used (derived at 37° C) were in fact greater than the 0–1.5 MAC values, by approximately 16%. Sixty minutes after anesthetic delivery to the bathing solution was terminated, a final set of control contractions was recorded (control 4, table 2).

The concentration of anesthetic was measured continuously between the reservoir bag and the roller pump with a calibrated fast-response acoustic gas analyzer.[12] This device was calibrated with 100% O_2 and 100% N_2O immediately before the first concentration of anesthetic was tested, and the calibration was corrected for the presence of 5% CO_2 in the carrier gas.

At each anesthetic concentration, stable values of DF were reached usually after 20–35 min. Test contractions then were recorded, such that total exposure at each anesthetic concentration was 30–45 min. After test contractions had been recorded at 1.50 MAC, the vaporizer was turned off, the reservoir bag emptied, and the muscles allowed to recover for 1 h. A final set of test contractions (control 4, fig. 1) then was recorded; at the conclusion of the experiment the muscles were weighed after very light blotting. The duration of the experiments, measured from the commencement of the measurements, was 456 ± 84 min (mean ± SD) with a range of 300–624 min.

STATISTICAL ANALYSIS

For each muscle and at each experimental step, the relationship between muscle length and the following

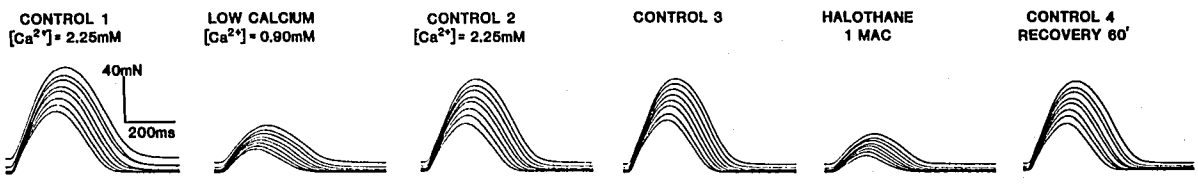

FIG. 1. Each of the six panels represents the time course of force of isometric twitches at lengths from 100 to 86% of L_{max} at 2% L_{max} intervals in different conditions (see text). The superimposed force traces in each panel represent twitches recorded at different lengths.

variables were studied: DF, maximum positive dF/dt ($+dF/dt_{max}$), maximum negative dF/dt ($-dF/dt_{max}$), TPF, and RT½. Least-squares linear regression analysis was carried out between each variable and muscle length, expressed as percent of L_{max}. Values of slopes were tested for differences between anesthetic agent groups with one-way analysis of variance (ANOVA), at corresponding anesthetic concentrations.

Least-squares linear regression analysis also was carried out between each variable and anesthetic concentration expressed as fraction or multiple of MAC. Values of slope were tested for differences between anesthetic agent groups with one-way ANOVA at each resting length. Student's *t* tests were used to compare two groups when appropriate.

At each length, the value of each variable at a given anesthetic concentration was computed as a percent of its value in control conditions at that length. Linear regression analysis was carried out between these percent values and length. Values of slope were tested for difference from zero for each anesthetic concentration. The same steps were followed for the low-$[Ca^{2+}]_o$ study, with $[Ca^{2+}]_o$ expressed as log $[Ca^{2+}]_o$.

The time variables of the isometric twitch—TPF and RT½—were displayed as a function of peak DF for each group of muscles at each length. Within each group, least-squares linear regression through individual (time and amplitude) values yielded sets of (slope and intercept) values for each muscle for change in $[Ca^{2+}]_o$ and in anesthetic concentration. Within each group of muscles, we tested for statistically significant differences in (slope and intercept) values between the $[Ca^{2+}]_o$ and anesthetic concentration data with Hotelling's T^2 test.[15]

Results

Anesthetic Experiments

When muscle length was decreased from L_{max}, there consistently was a decrease in DF in control conditions, in low $[Ca^{2+}]_o$, and in the presence of anesthetic (fig. 2). The slope of the DF–length relationship was computed for each muscle in each tested condition. When computed from absolute values of DF, the slopes of the DF–length relationship already were significantly different from control at 0.25 MAC (Student's paired *t* test, $P < 0.01$) for each of the three tested anesthetics (fig. 3, top). The difference between control and anesthetic became more pronounced at higher concentrations ($P < 0.001$ at ≥0.50 MAC). Values of slopes before and after the anesthetic concentration–effect experiment were not statistically significantly different ($P > 0.05$).

There were no differences between DF–length slopes among anesthetic groups ($P = 0.16$, one-way ANOVA).

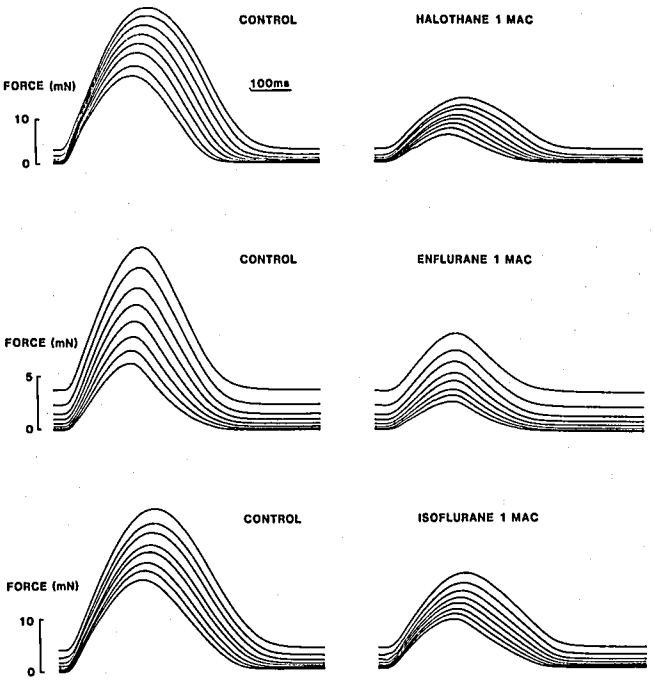

FIG. 2. Time course of force development in isometric twitches at lengths from 100 to 86% in each of three muscles: control and 1 MAC halothane (*top*); control and 1 MAC enflurane (*middle*); control and 1 MAC isoflurane (*bottom*). The superimposed force tracings in each panel represent twitches recorded at different lengths.

DF then was plotted as a percent value of DF at L_{max} for each anesthetic concentration (fig. 3, middle). This plot showed that at high anesthetic concentrations (1.50 MAC), muscles often performed optimally at lengths shorter than L_{max}, typically 98% of L_{max}. This effect was reversible upon discontinuing the anesthetic and happened more often and in a more pronounced way with enflurane and halothane than with isoflurane. Substituting the new L_{max} for the initial one when appropriate did not affect the overall results of the study. The bottom part of figure 3 displays, for each length, how DF compared with its length-matched control before the anesthetic was applied. The slopes of the relationship between relative values $DF_{anes}/DF_{control}$ and length did not differ significantly from zero. In other words, if a given anesthetic concentration decreased DF in a certain proportion at one length, it did so in the same proportion at any other length.

Also in every muscle, DF decreased in a dose-dependent fashion during anesthetic exposure at every tested length (fig. 2 and fig. 4, top). At L_{max}, 1.50 MAC isoflurane reduced DF to (mean ± SD) 30.90 ± 7.52% of its original value; 1.50 MAC enflurane to 17.41 ± 9.73%; and 1.50 MAC halothane to 18.77 ± 8.59%. The effect of the anesthetic may be characterized by the relationship between the slope of DF and anesthetic concentration (MAC),

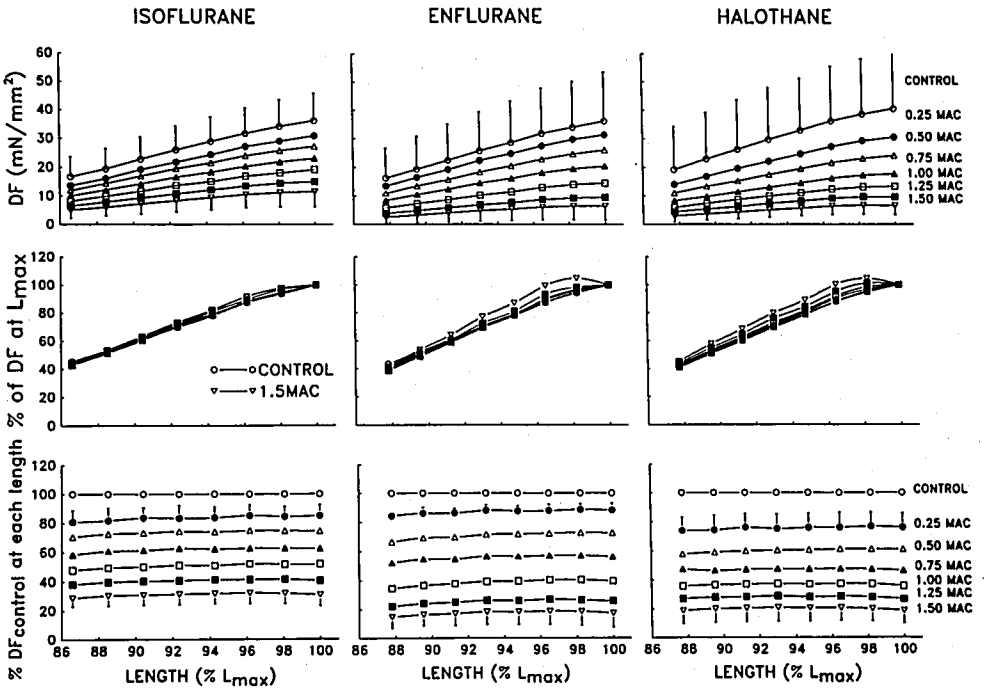

FIG. 3. (*Top*) Relationships of developed force (DF) of isometric twitches at different lengths (force–length relation) before and during exposure to incremental concentrations of isoflurane (n = 9), enflurane (n = 9), or halothane (n = 9). Data are mean ± SD. For clarity, error bars are shown only for control and 1.5 MAC anesthetic. (*Middle*) Same as *top*, except that DF values are shown as percent of DF at L_{max}. (*Bottom*) Same as *top*, except that DF values are shown as percent of DF at each corresponding length.

which was plotted for L_{max} and 86% L_{max} (fig. 4). These slopes vary with length according to the effect of length change on the absolute value of DF (fig. 5, top). There were no differences among anesthetics in their actions on the DF–length relationship when studied by comparing the slopes of DF ≠ MAC at varying lengths.

The same steps were followed to analyze the changes of other variables during the experiments. We will now consider separately the positive and negative rates of force change ($+dF/dt_{max}$ and $-dF/dt_{max}$, respectively) and time variables for force development and relaxation (TPF and RT½).

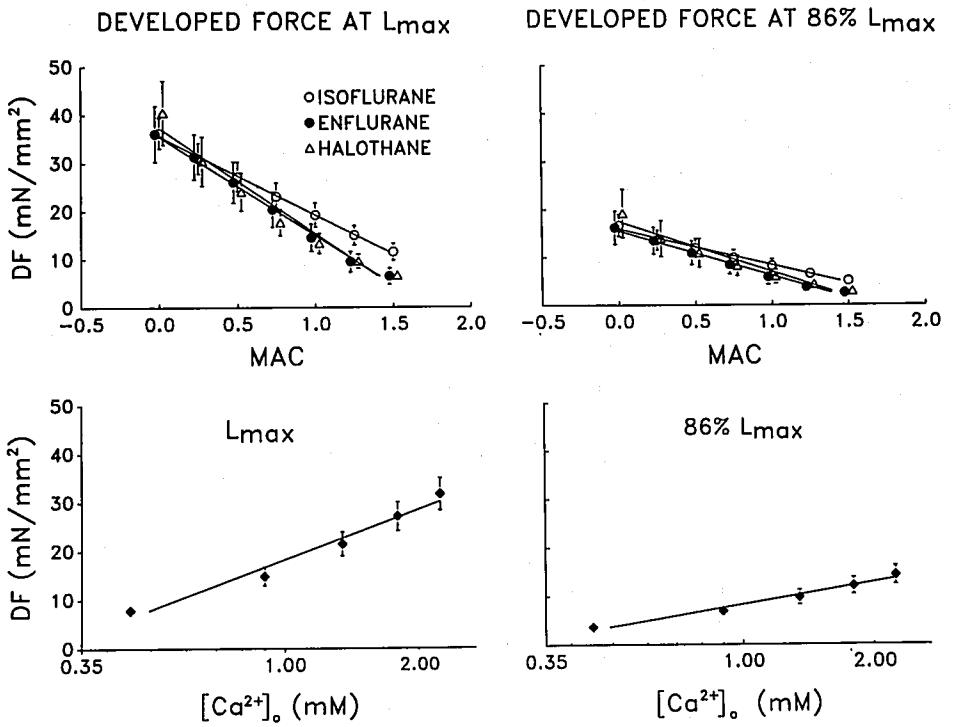

FIG. 4. (*Top*) Cumulative dose–response relationships of developed force (DF; mean ± SEM) in isometric twitches to isoflurane, enflurane, and halothane at L_{max} (*left*) and 86% L_{max} (*right*), with corresponding least-squares linear regression lines. (*Bottom*) Dose–response relationship of DF to $[Ca^{2+}]_0$ at L_{max} (*left*) and 86% of L_{max} (*right*), with superimposed least-squares linear regression lines.

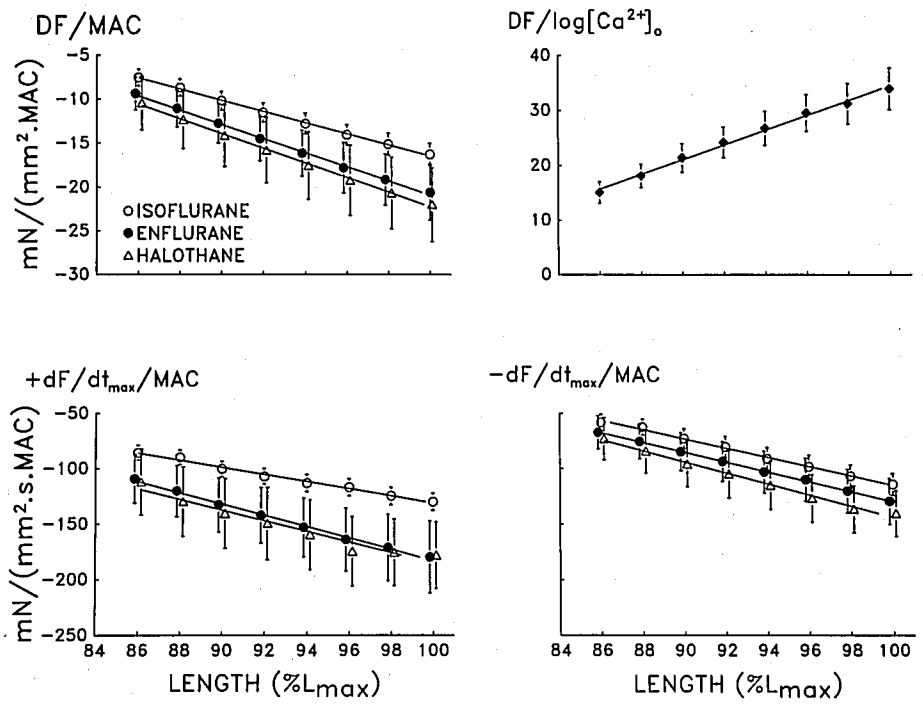

FIG. 5. Length dependence of DF/MAC, $+dF/dt_{max}$/MAC, and $-dF/dt_{max}$/MAC for isoflurane, enflurane and halothane, and of DF/log $[Ca^{2+}]_0$. Data are mean ± SEM (n = 9 per group), with superimposed least-squares linear regression lines.

Before anesthetics were applied, $+dF/dt_{max}$ and $-dF/dt_{max}$ decreased to approximately 55% of the value at L_{max} when length was decreased to 86% of L_{max}, similar to a decrease in DF to 44% of L_{max} values. The slopes of the $+dF/dt_{max}$–length and $-dF/dt_{max}$–length relationships became significantly different from control at 0.25 MAC halothane ($P < 0.01$) for $+dF/dt_{max}$ and $-dF/dt_{max}$; at 0.25 MAC enflurane for $-dF \neq dt_{max}$ ($P < 0.05$) and at 0.5 MAC for $+dF \neq dt_{max}$ ($P < 0.05$); at 1.0 MAC isoflurane ($P < 0.05$) for $-dF/dt_{max}$ and at 0.50 MAC for $+dF/dt_{max}$ ($P < 0.01$). There were significant differences between anesthetic groups for $+dF/dt_{max}$, but not for $-dF/dt_{max}$. Halothane decreased $+dF/dt_{max}$ more at any length than did isoflurane ($P = 0.0009$) or enflurane ($P = 0.0022$). The slopes of the relationship of percent values of $+dF/dt_{max.anes}/+dF/dt_{max.control}$ and $-dF/dt_{max.anes}/-dF/dt_{max.control}$ *versus* length did not differ significantly from zero. The slopes of the relationship between $+dF/dt_{max}$/MAC and length were different between anesthetic groups ($P = 0.0002$, one-way ANOVA; halothane *vs.* enflurane, $P = 0.002$; halothane *vs.* isoflurane, $P = 0.0009$; enflurane *vs.* isoflurane, $P = 0.02$; fig. 5, bottom left). At 1.5 MAC halothane and enflurane, maximal $+dF/dt_{max}$ and $-dF/dt_{max}$ values were found at 98% of L_{max}.

RT½ decreased to approximately 75–80% of control when length was decreased from L_{max} to 86% of L_{max}. At L_{max}, isoflurane, enflurane, and halothane at 1.5 MAC reduced RT½ to 85.48 ± 8.98, 79.24 ± 11.08, and 80.64 ± 11.75% of preanesthetic values, respectively.

The slopes of the relationship of percent values of $RT½_{anes}/RT½_{control}$ *versus* length did not differ from zero. The slopes of the relationship between RT½/MAC and length differed between halothane and isoflurane ($P = 0.0068$, one-way ANOVA; halothane *vs.* isoflurane, $P = 0.01$; fig. 6, top right). Finally, at 1.5 MAC halothane and enflurane, maximal values of RT½ were found at 98% of L_{max}.

TPF decreased little (typically to 80–85% of control) when length decreased from L_{max} to 86% of L_{max}. The slopes of TPF–length relationships became significantly different from their corresponding control at 0.25 MAC halothane, 0.75 MAC enflurane, and 1.0 MAC isoflurane. The differences between groups were highly significant ($P < 0.0001$, one-way ANOVA; halothane *vs.* enflurane $P = 0.0003$; halothane *vs.* isoflurane $P = 0.0001$; enflurane *vs.* isoflurane $P = 0.01$). The slopes of the relationship of percent values of $TPF_{anes}/TPF_{control}$ *versus* length changed with increasing anesthetic concentrations. They became significantly different (from the slope before anesthetic) at 0.75 MAC for isoflurane ($P < 0.05$), at 1.25 MAC for enflurane ($P < 0.01$), and at 0.5 MAC for halothane ($P < 0.01$).

The slopes of the TPF ≠ MAC *versus* length relationship were different between the halothane and isoflurane groups between 96% and 86% of L_{max}. Differences between enflurane and halothane groups reached statistical significance only at 92% of L_{max} and at L_{max} (fig. 6, top left).

CALCIUM EXPERIMENTS

The effects of low extracellular Ca^{2+} concentrations were tested in eight muscles of the halothane group, eight

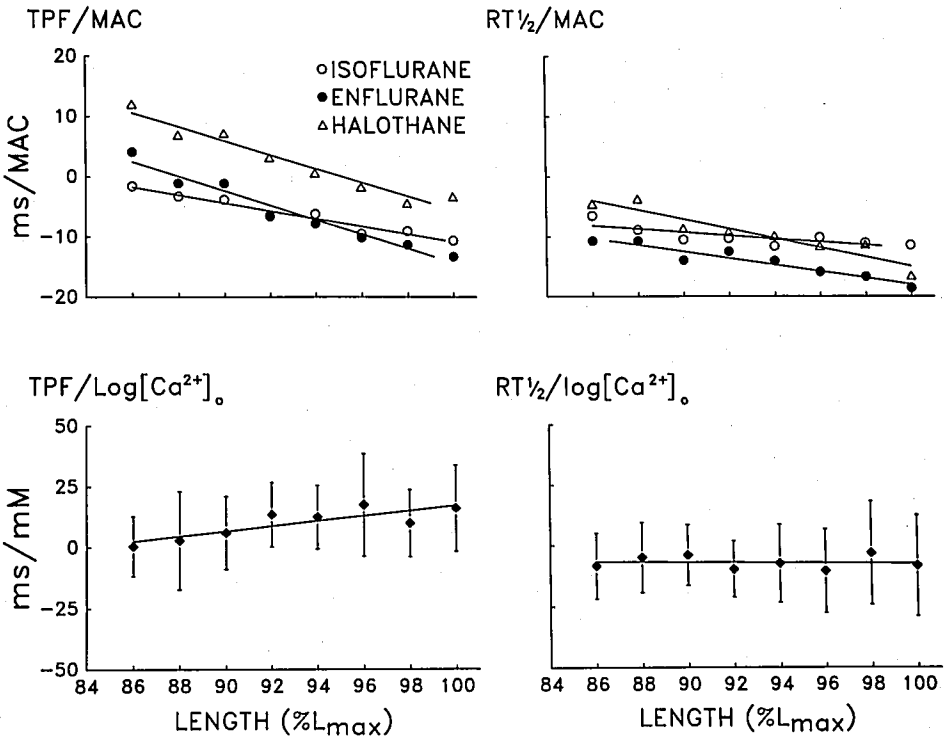

FIG. 6. Length dependence of slopes of linear regression lines relating TPF and RT½ to MAC (*top*) and to log $[Ca^{2+}]_o$ (*bottom*) in each of the experimental groups. For clarity, SD values are not shown in the top panels; values in lower panels are mean ± SD. Least-squares linear regression lines are superimposed.

of the enflurane group, and nine of the isoflurane group. Data from the latter group are shown in figures 4–7. For each muscle, the low $[Ca^{2+}]_o$ experiment was carried out before the anesthetic experiment.

Decreasing $[Ca^{2+}]_o$ reduced DF at all lengths (fig. 1 and 4, bottom), whereas decreasing resting length reduced DF at any $[Ca^{2+}]_o$ (fig. 7). DF at a $[Ca^{2+}]_o$ of 0.45 mM was 25.14 ± 6.67% of control for the isoflurane group (n = 9), 31.21 ± 12.27% for the enflurane group (n = 8), and 32.41 ± 8.80% for the halothane group (n = 8). $+dF/dt_{max}$ and $-dF/dt_{max}$ also were reduced at low Ca^{2+} concentrations in the same proportions. TPF and RT½ did *not* decrease at low $[Ca^{2+}]_o$, and therefore the slopes of the relationship of these variables to log $[Ca^{2+}]_o$ was not significantly altered with length (fig. 6, bottom), in contrast to the result with anesthetics (fig. 6, top). The slopes of relative values of variables (*i.e.*, variables at a tested $[Ca^{2+}] \div$ variables at a $[Ca^{2+}]$ of 2.25 mM) *versus* length never were different from zero, for any variable or at any tested $[Ca^{2+}]_o$. In these three series of experiments, decreasing Ca^{2+} concentration in the bath did not change these variable–length relationships significantly. The three series of experiments also were tested for consistency. The effect of log $[Ca^{2+}]_o$ on the different variables was not statistically different between groups, at all lengths.

However, when absolute values of time course variables (TPF and RT½) were plotted as a function of peak twitch amplitude (DF) both for changes in $[Ca^{2+}]_o$ and for anesthetic concentration in isometric twitches at L_{max} (Fig. 8, left), RT½ was lower. In other words, isometric relaxation was abbreviated by anesthetic when compared with that in low $[Ca^{2+}]_o$ at equal twitch amplitude, whereas at shorter muscle lengths (86% L_{max}) there was no difference between low $[Ca^{2+}]_o$ and anesthetic in the time course of isometric relaxation (Fig. 8, right). There were no statistically significant differences in TPF between anesthetic and low $[Ca^{2+}]_o$ at either long (L_{max}) (except for enflurane) or short (86% L_{max}) lengths. RT½ was significantly smaller at L_{max} in the halothane group, at L_{max}, 98% and 96% of L_{max} in the enflurane group, and at L_{max} down to and including 90% L_{max} in the isoflurane group.

Finally, all changes reported in the anesthetic and calcium experiments were reversible after return to control conditions. There was a significant decrease in DF between controls 1 and 2 ($P < 0.05$, paired t test) and between controls 3 and 4 ($P < 0.05$, paired t test). Yet the decreases in DF from control 1 to control 2 and from control 3 to control 4 respectively were not statistically different among the three muscle groups ($P > 0.05$, one-way ANOVA).

Discussion

This study demonstrates that the increase of force with increasing myocardial fiber lengths persists under clinically relevant concentrations of halothane, enflurane, and

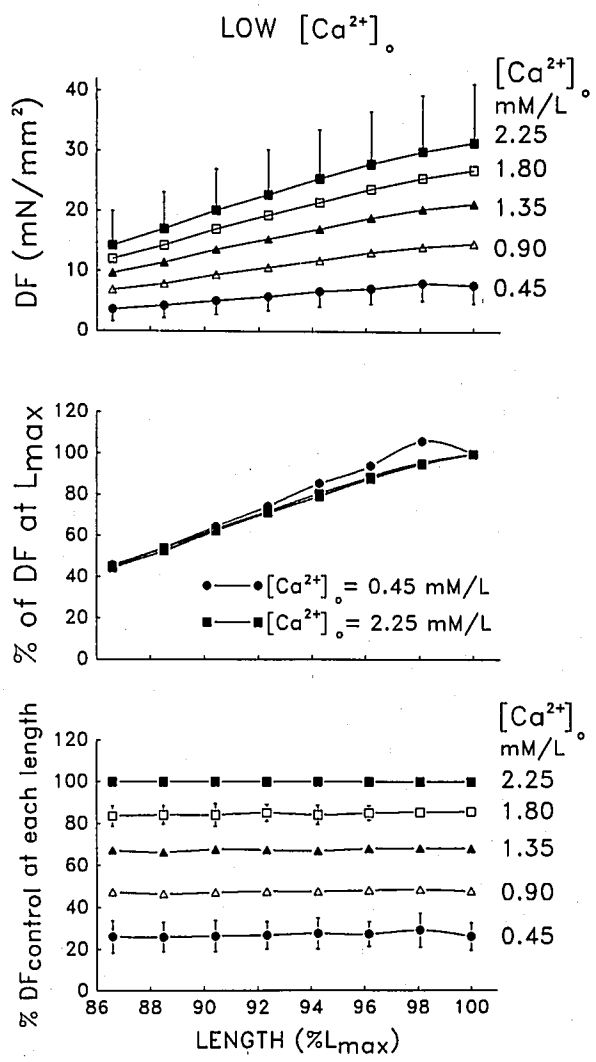

FIG. 7. Force–length relations during isometric twitches during cumulative dose–response experiments to changes in $[Ca^{2+}]_0$ (mean ± SD; n = 9; isoflurane group of muscles). Format is the same as in figure 3.

isoflurane. Despite the severe reduction in force developed under isometric conditions when the muscles were exposed to high concentrations of volatile anesthetics, the effect was consistently demonstrated at every concentration of anesthetic and in every muscle tested.

A fundamental property of cardiac muscle is the dependence of force development on muscle length, a property classically known as Starling's Law of the Heart (for a recent review, see ref. 1). Our understanding of the cellular mechanisms that account for the length dependence of force generation is still incomplete, but current theories suggest that both mechanical factors—such as myofilament overlap, intracellular and extracellular restoring forces—and activation factors are affected by muscle length and contribute to the length dependence of force generation.

In fully activated frog skeletal muscle, changes in thick and thin filament overlap account for most of the changes in force along the descending limb of the sarcomere length–tension relationship, at sarcomere lengths >2.25 μm,[2] whereas along the ascending limb (sarcomere length <2.0 μm), myofilament overlap, restoring forces, and effects of interference between overlapping ends of thin filaments play a dominant role. In mammalian cardiac muscle, where the working range is restricted to sarcomere lengths between 1.6 to 2.3 μm,[3] mechanical factors alone cannot account for the length dependence of force generation along the ascending limb of the length–tension relationship, the slope of which is much steeper than in skeletal muscle. In maximally calcium-activated skinned cardiac cells[4] and trabeculae,[5] the slope of the ascending limb of the length–tension relation is less steep than in intact cardiac muscle, which is generally only partially activated, so that the decrease in force at short sarcomere lengths in intact cardiac muscle is much greater than can be accounted for by mechanical factors alone. At least two independent mechanisms by which changes in length affect activation have so far been identified: these are changes in affinity of troponin for Ca^{2+} and slow changes in Ca^{2+} release by the SR, as discussed in detail below.

After a decrease in length during diastole in cardiac muscle, force is decreased in the next isometric twitch. In subsequent contractions, the twitch amplitude decreases slowly over 5–20 min. The immediate decrease in force after a decrease in length is accompanied by minimal changes[7] or even a slight increase in amplitude[16] of the intracellular Ca^{2+} transient. The declining phase of the calcium transient is prolonged, whereas the contraction is abbreviated. These observations have led to suggestion that the affinity of troponin for Ca^{2+} is decreased at short lengths. During the slow decline in force after a shortening step, force and $[Ca^{2+}]_i$ decreased in parallel, suggesting that the slow component may be due to SR Ca^{2+} release or Ca^{2+} entry.[7] Nichols[17] and Allen *et al.*[18] showed that the slow effects are a consequence of muscle length during diastole rather than during systole. They proposed that resting $[Ca^{2+}]_i$ decreases at short lengths, which results in reduced SR Ca^{2+} loading and hence reduced Ca^{2+} release, smaller Ca^{2+} transients, and reduced force development.

In support of the hypothesis of a decrease in the affinity of troponin C for Ca^{2+} at short lengths are the observations that in skinned rat cardiac fibers,[6] the steady-state force–*p*Ca curve was shifted toward higher $[Ca^{2+}]$ at shorter lengths. Furthermore, when native cardiac troponin C was substituted with skeletal troponin C in skinned hamster ventricular trabeculae, most of the length-dependent force generation was lost.[19] Although the exact mechanism by which troponin C senses length changes is unknown, there is evidence that length *per se*

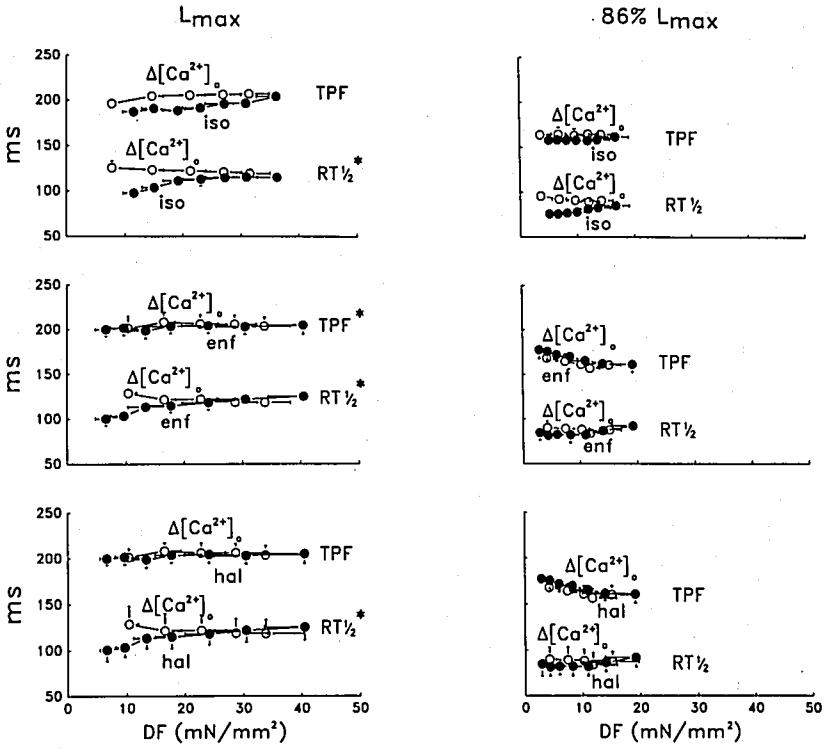

FIG. 8. Dependence of time to peak force (TPF) and of time to half-isometric relaxation (RT½) on peak developed force in isometric twitches at L_{max} (*left*) and at 86% L_{max} (*right*) in two different conditions: changes in $[Ca^{2+}]_o$ from 0.45 to 2.25 mM in 0.45 mM increments (open circles) and of anesthetic concentration from 0 to 1.5 MAC in 0.25 MAC increments (filled circles) in $[Ca^{2+}]_o$ 2.25 mM. Values (mean ± SEM) in a given graph are from the same group of muscles (n = 9); for each of the two conditions, the three graphs represent experiments on three different groups of muscles. Levels of significance by Hotelling's T^2 test for differences between (slope, intercept) values of least-squares linear regressions among $[Ca^{2+}]_o$ and [anesthetic] experiments are indicated for TPF and RT½ in each panel.

may not be the primary determinant. Force development increases the Ca^{2+} affinity of troponin C,[1,20,21] and the influence of fiber length on the intracellular Ca^{2+} transient is attenuated when force development is decreased by application of hypertonic solutions, by substitution of D_2O for H_2O, or by 2,3-butanedione 2-monoxime, all of which interfere with cross-bridge attachment.[22] These observations have raised the possibility that fiber length *per se* has no influence on the Ca^{2+} affinity of troponin C in resting muscle, but that the changes observed are secondary to cross-bridge attachment.[22]

Still uncertain is the extent to which a change in length alters Ca^{2+} release from the SR and Ca^{2+} entry across the sarcolemma. On the basis of results from studies in skinned cardiac cells,[4,23] Fabiato and Fabiato suggested that Ca^{2+} loading of the SR was not affected by length, but that the effectiveness of Ca^{2+} as a trigger for Ca^{2+}-induced Ca^{2+} release was altered by stretch. Yet, from further studies, Fabiato[24] concluded that there is no evidence that calcium-induced release of calcium from the cardiac SR is length-dependent.

Halothane, enflurane, and isoflurane are well-known myocardial depressants. Negative inotropic effects of these drugs have been explained in terms of their actions in interfering with 1) the entry of Ca^{2+} through the sarcolemma; 2) the Ca^{2+} uptake and release from the SR; and 3) the Ca^{2+} responsiveness of the contractile apparatus.[9] In previous studies[12,13] we postulated that halothane, enflurane, and isoflurane, in addition to their effects to reduce intracellular calcium availability, decreased myofibrillar Ca^{2+} responsiveness, by decreasing the affinity of troponin C for Ca^{2+} and/or by affecting cross-bridge kinetics. More recently, we proposed that the anesthetic-induced changes in myofibrillar Ca^{2+} responsiveness are small relative to decreases in intracellular Ca^{2+} availability.[25] In rabbit papillary muscle in Ba^{2+} contracture, halothane, enflurane, and isoflurane did not alter the frequency of sinusoidal length and force oscillations at which dynamic stiffness was minimal[26]; this observation was taken as evidence that these anesthetics did not alter actin–myosin adenosine triphosphatase (ATPase) kinetics in those conditions. The anesthetic-induced decrease of the high-frequency stiffness in Ba^{2+} contracture was proposed to reflect a decrease in the number of cross-bridge interactions. In skinned rat cardiac fibers, Murat *et al.*[27] found that clinically relevant concentrations of halothane, enflurane, and isoflurane decreased myofibrillar calcium sensitivity and decreased maximal activated force. The mechanism for these changes was proposed to involve an anesthetic-induced decrease in the number of force-generating cross-bridges, a decrease in force developed by each cross-bridge, and a decrease in the rate of ATP hydrolysis.[28] Yet, since the changes observed were small at relatively high anesthetic concentrations (2 MAC), they probably contribute only to a minor extent to the overall negative inotropic effect of these anesthetics.

In view of the evidence for a length- or force-dependent change in Ca^{2+} responsiveness and for a decrease in myo-

fibrillar Ca^{2+} responsiveness by volatile anesthetics, one would predict that the relative negative inotropic effect of volatile anesthetics would be different at long *versus* short lengths. Examination of the length dependence of maximal DF, however, does not disclose such an influence. As anesthetics depressed DF at L_{max}, so did they at all other lengths also, in proportion to the preanesthetic control initial force. In other words, if a muscle developed 75% of control force at L_{max} when exposed to anesthetic, it would also develop the same fraction of the preanesthetic control force at any other length at the same anesthetic concentration. The same findings apply for the other variables analyzed ($+dF/dt_{max}$, $-dF/dt_{max}$, TPF, and RT½).

Comparison between the effects of exposure to low extracellular $[Ca^{2+}]$ and to volatile anesthetics may provide insights into mechanisms of action of anesthetics and length–tension relationships. Therefore, each muscle was exposed first to a stepwise increase in $[Ca^{2+}]_o$ and then to increasing concentrations of anesthetic at normal $[Ca^{2+}]_o$. This sequence may have introduced a time effect and reduced the value of any conclusion that could be drawn from comparing the two dose–response curves. However, the unavoidable decline in muscle performance in either protocol was not statistically significantly different. The responses of every muscle were similar under both protocols. Except for TPF, reducing $[Ca^{2+}]_o$ produced for each variable the same pattern of effects as did increasing volatile anesthetic concentration. However, upon comparison of the time course of isometric relaxation at equal twitch amplitude, values of RT½ were lower (*i.e.*, isometric relaxation was shorter) in anesthetic than in low $[Ca^{2+}]_o$ at L_{max}, although they did not differ between the protocols with varied $[Ca^{2+}]_o$ and anesthetic at the much shorter lengths. These results are the only indication from this study to suggest that volatile anesthetics decrease myofibrillar Ca^{2+} responsiveness at long muscle lengths but not at short lengths. This effect is detectable most likely because comparison of time course variables at equal twitch amplitude with different inotropic interventions is more sensitive than when contractions of different amplitudes are compared.[25]

In some muscles, L_{max} shifted slightly toward lower muscle lengths (98% L_{max}) at anesthetic concentrations greater than 1 MAC or at low $[Ca^{2+}]_o$. The latter phenomenon has been reported previously.[29] To compensate for this, the data were analyzed also after normalizing measured forces to the force measured at 98% of L_{max} instead of L_{max}. This modification did not affect the results.

Our current findings are in agreement with those of Komai and Rusy,[30] who reported that halothane reduced absolute changes in DF that accompany an increase in resting tension from 0.5 to 1.5 g/mm^2 in rabbit right ventricular papillary muscle stimulated at 0.1–1.0 Hz. In this range of stimulation frequencies, halothane's effect could be compensated for by increasing the frequency of stimulation, and thus increasing calcium availability. This led to the conclusion that the extent of Ca^{2+} binding to troponin C in the presence and absence of halothane remained the same provided that Ca^{2+} availability was the same.[30] Our results are also in agreement with those obtained in chronically instrumented dogs by Van Trigt *et al.*[31] When the data from their figure 8 are recalculated and expressed relative to the maximal left ventricular (LV) end-systolic transmural pressure, one finds that this variable is affected in a constant proportion at equal LV end-systolic minor axis diameter, with or without halothane.

This study was conducted under muscle length control, without information on sarcomere length. Consequently, force development at short lengths may well have been overestimated relative to that at L_{max} because of greater internal shortening at long than at short muscle lengths.[32,33] The above consideration is of little relevance to this study, however, since we compared amplitude and time courses of isometric twitches at a given length before and during drug interventions, and subsequently compared time course variables at a given muscle length at equal twitch amplitude. Furthermore, in order to preserve our preparation from early degradation, the experiments were carried out at nonphysiologic temperature and stimulation frequencies. The effect of anesthetics may be different under other, untested, conditions.

In summary, the data from this study show that in isolated intact ventricular muscle the length dependence of force generation is not significantly altered by halothane, isoflurane, and enflurane up to 1.5 MAC. These anesthetics act at any length as though less Ca^{2+} acted on the contractile apparatus, *i.e.*, as though there were a reduction of intracellular Ca^{2+} availability, possibly coupled to a decreased myofibrillar calcium responsiveness, detected only when the time course of equal amplitude twitches were compared in low $[Ca^{2+}]_o$ *versus* in anesthetic. Such a minor effect on myofibrillar Ca^{2+} sensitivity is not surprising in view of recent studies on the intracellular Ca^{2+} transient detected in aequorin-injected ferret papillary muscles.[34,35]

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