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Comparative Effects of Halothane, Enflurane, and Isoflurane at Equipotent Anesthetic Concentrations on Isolated Ventricular Myocardium of the Ferret. I. Contractility

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The effects of halothane, enflurane, and isoflurane on myocardial contractility were compared in papillary muscles of the right ventricle of adult male ferrets at 30° C. Isotonic and isometric variables of contractility were measured before, during, and after exposure to incremental concentrations of halothane (n = 9 muscles), enflurane (n = 9 muscles), and isoflurane (n = 9 muscles), in steps of 0.25 MAC up to 1.5 MAC of halothane and of enflurane, and up to 2.0 MAC of isoflurane. Each of the three anesthetics caused a dose-dependent reversible decrease in contractility. The onset of maximal myofibrillar activation was delayed in a dose-dependent manner, and time to peak shortening of the isotonic preloaded twitch was unchanged, except for a slight decrease at >1 MAC of enflurane. Isoflurane's negative inotropic effects were clearly less than those of either halothane or enflurane. Comparison of the time course of contraction and relaxation in both isometric and isotonic twitches suggests that, in addition to effects on intracellular calcium availability, these anesthetics decrease the myofibrillar responsiveness to calcium and/or the calcium sensitivity of the contractile proteins. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Cardiac muscle. Heart: contractility; relaxation.)

THE VOLATILE ANESTHETICS, halothane, enflurane, and isoflurane are potent myocardial depressants. Their negative inotropic effect has been demonstrated in humans,[1-5] in experimental animals,[6-11] and in isolated atria and ventricles of various mammalian species.[12-25]

In isolated right ventricular cat papillary muscle, the negative inotropic effect of enflurane was found by Brown and Crout[18] to be greater than that of halothane at equipotent anesthetic concentrations. By contrast, in the same preparation in similar experimental conditions, Shimosato *et al.*[22] and Kemmotsu *et al.*[24] found that 1 MAC of halothane was more cardiodepressant than 1 MAC of enflurane. The myocardial effects of halothane and enflurane were difficult to compare because of differences in experimental protocols and variations in methods to assess myocardial contractility *in vitro*. Recent experimental evidence suggests that isoflurane depresses myocardial contractility in human[3-5] and dog[9,10] to a lesser extent than do halothane or enflurane, but the observed changes could in part be related to differential effects on the systemic circulation.[10] The results from these *in vivo* studies contrast sharply with the reported myocardial depressant effect of isoflurane in isolated cat papillary muscle,[24] which was found to be comparable to that of halothane. However, in a recent study on guinea pig papillary muscle, Lynch[25] demonstrated that isoflurane is a less potent myocardial depressant than either halothane or enflurane at stimulation rates greater than 1 Hz.

In view of these contradictory results, the present study was undertaken to critically re-evaluate comparative effects of halothane, enflurane, and isoflurane at equipotent anesthetic concentrations on isolated ventricular myocardium of the ferret. A complete analysis of cardiac muscle contractility was undertaken during exposure to cumulative concentrations of these anesthetics over a range of clinically useful anesthetic concentrations and during recovery after prolonged exposure. Furthermore, changes in the time course of contraction and relaxation were examined, as these are valuable in identifying mechanisms of drug action. Effects of these anesthetics on myocardial relaxation are the subject of an associated study.[26]

This article is accompanied by an editorial. Please see: Rusy BF: Anesthetic action in heart muscle: Further insights through the study of myocardial mechanics. ANESTHESIOLOGY 69:445-447, 1988.

Materials and Methods

Twenty-seven papillary muscles from the right ventricle of adult male ferrets (weighing 1100-1500 g, and 16-19 weeks of age) were used for this study. Ferrets have the advantage over cats and rats of providing for a more homogeneous population of long, thin right ventricular papillary muscles of cylindrical geometry with a yield of one to three papillary muscles per animal. The animals were anesthetized with sodium pentobarbital (100 mg/kg i.p.). As soon as a surgical level of anesthesia was reached, the heart was quickly excised, the right ventricle was opened, and suitable papillary muscles were carefully excised and mounted horizontally in a temperature-con-

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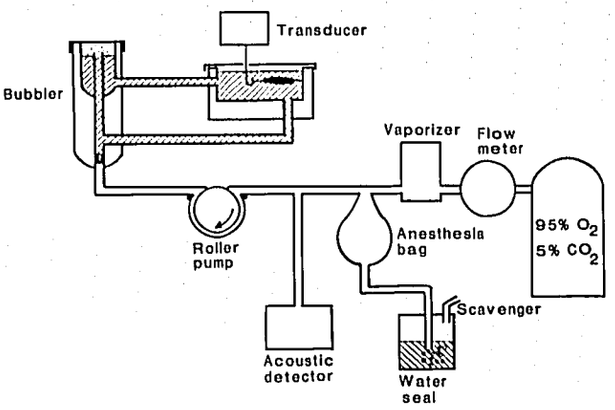

FIG. 1. Schematic diagram of the experimental setup. See text for details.

trolled (30° C) muscle chamber that contained a physiological salt solution made up in glass distilled water and with the following composition (mM): Na^+ 135; 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; glucose 10. The bathing solution was equilibrated with 95% O_2-5% CO_2 and the *p*H was 7.4. The design of the jacketed muscle chamber and oxygenating vessel is a modification of Blinks' organ bath.[27] The experimental setup is schematically illustrated in figure 1. Even though there may be a difference in drug effects on ferret ventricular myocardium at different temperatures, and intrinsic depressant effects of these anesthetics differ at physiologic frequencies (1 Hertz or higher),[25] we elected to conduct the experiments for this study at 30° C and at a stimulus frequency of 0.25 Hertz, since 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 L_{max} (*i.e.*, the length at which twitch active force was maximal) $\geq$3.5 mm, a mean cross-sectional area (CSA) $\leq$ 1.2 mm^2, and a ratio of resting (R) to total force (T), (R/T) $\leq$ 0.25. Table 1 summarizes the muscle characteristics during control conditions at L_{max}. 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. 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 milliNewton (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 ventricular end of each muscle was held in a subminiature lucite 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 stimulator at a stimulus interval of 4 s. Stimuli only barely above threshold voltage (10% above threshold, range 2–12 V) were used to minimize the release of endogenous norepinephrine by the driving stimuli.[28] The muscles were stimulated and made to contract in alternating series of four isometric and four isotonic twitches for 2–3 h. At the end of this stabilization period, muscles had reached steady-state, and initial muscle length was set at L_{max}, *i.e.*, the length at which twitch active force was maximal. During stabilization, the bathing solution was continuously renewed with fresh physiological salt solution at the temperature of the bath at a rate of 200 ml/h. Throughout the stabilization period and the experimental procedure, the temperature of the bathing solution was kept at 30° C.

A gas mixture of 95% O_2-5% CO_2 (2–3 l/min) flowed through a calibrated vaporizer for either halothane (Fluotec Mark 3), enflurane (Enfluratec), or isoflurane (Airco, Ohio Medical Products), 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 at a rate of 500 ml/min. The concentration of anesthetic vapor was measured continuously between the reservoir bag and the roller pump by means of an acoustic gas analyzer (Section of Engineering, Mayo

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
Halothane (n = 9)	Mean	5.03	0.68	6.69	39.91	0.167
	SD	0.95	0.21	3.08	10.96	0.053
	Range	3.80–6.50	0.43–0.94	2.70–12.73	26.31–56.44	0.077–0.230
Enflurane (n = 9)	Mean	4.89	0.49	8.50	44.35	0.196
	SD	0.99	0.14	2.00	6.77	0.053
	Range	3.50–6.00	0.23–0.70	5.54–11.02	40.21–57.71	0.108–0.250
Isoflurane (n = 9)	Mean	4.44	0.61	6.80	42.38	0.163
	SD	0.34	0.27	2.48	9.82	0.049
	Range	4.20–5.00	0.24–1.12	4.22–11.60	21.71–56.17	0.100–0.245

CSA = cross-sectional area; R = resting tension; T = total tension.

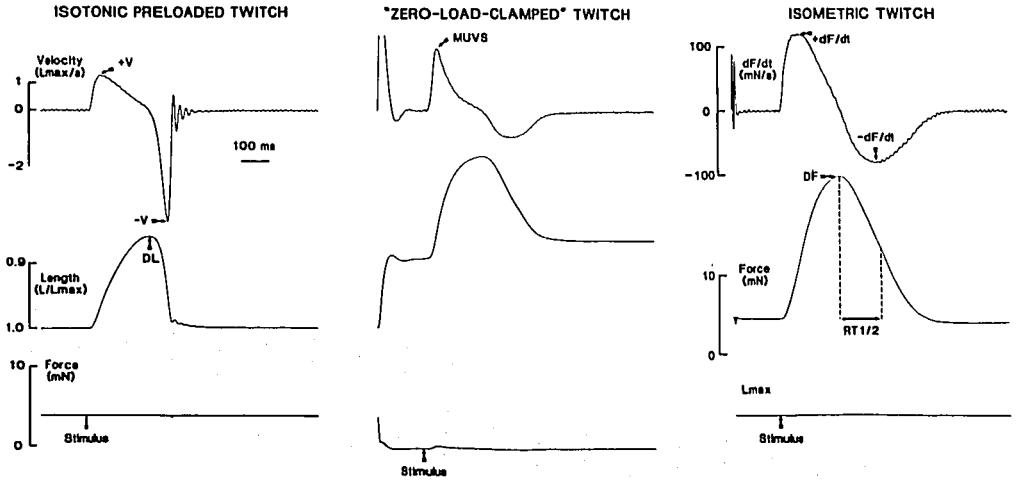

FIG. 2. Variables of contraction and relaxation determined in this study. *Left panel.* Velocity (top), length (middle), and force (lower) traces of a preloaded isotonic twitch where muscle shortening proceeds against a constant load (preload). Peak shortening (DL) and time to peak shortening (TDL), and maximal velocity of shortening (+V) and of lengthening (−V) were obtained in isotonic preloaded twitches. *Middle panel.* Velocity (top), length (middle), and force (lower) traces of a zero-load-clamped twitch, where load was rapidly decreased from preload to zero at the onset of the sweep. At the stimulus, muscle shortening proceeds against zero load and the maximal rate of shortening (maximal unloaded velocity of shortening, MUVS) was determined, as well as TMUVS, the time to MUVS measured from the stimulus. *Right panel.* dF/dt (top), force (middle), and length (lower) traces of an isometric twitch at the muscle length L_{max}. Peak developed force (DF), time to peak force (TPF), maximal rate of rise (+dF/dt), and of fall of force (−dF/dt), and the time from peak force to when developed force falls to half its peak value (RT½) were determined from isometric twitches. Muscle characteristics L_{max}, 4.5 mm; mean cross-sectional, area 0.51 mm^2; ratio of resting to total tension at L_{max}, 0.163.

Foundation), which operates on the physical principle that the velocity of sound in a gas is inversely proportional to the square root of the gas density (nominally) at a given barometric pressure and temperature.‡ This acoustic detector was calibrated with known gas mixtures of halothane, enflurane, and isoflurane (all from 0–4% in steps of 1%) verified with a mass spectrometer (Perkin Elmer 1100), and provided for measurements with an accuracy of at least 0.03% vapor concentration. Daily calibrations were carried out with 100% O_2 (in which sound velocity is 317.2 m/s) and 100% N_2O (in which sound velocity is 261.8 m/s) immediately before the experimental protocol was started, and the calibration was corrected for the presence of 95% O_2 and 5% CO_2 in the carrier gas. The concentration of the delivered anesthetic was followed throughout the experiment and set to predetermined levels. The acoustic detector withdrew 600 ml/min of gas for this purpose. The distal end of the reservoir bag was also connected to a 1-cm underwater seal. Continuous bubbling of excess gas at this site confirmed the fact that at no time did negative pressures develop in the anesthetic delivery system due to insufficient gas flow. The jacketed bubbler was covered with tightly sealing Parafilm® (American Can Company, Greenwhich, CT). The muscle chamber was covered with glass cover slides throughout the experiment, except for a 1 × 0.5 mm slit for the transducer hook. The continuous superperfusion of Krebs-Ringer solution throughout the experiment was set at 40 ml/min.

‡ Isaacson J: Personal communication.

In four control experiments for each anesthetic (12 total), the concentration of anesthetic in fluid was measured. In each experiment, two samples per anesthetic concentration were taken and measured by gas chromatography (Hewlett Packard 5880A). Using anesthetic densities corrected to 30° C,[29] 1% halothane, enflurane, and isoflurane (all in gas phase) corresponded to, respectively, 0.36 mM, 0.42–0.46 mM, and 0.24–0.29 mM in fluid at 30° C. The concentration of halothane, enflurane, and isoflurane in fluid and the calculated partial pressure of anesthetic in fluid followed closely imposed changes of anesthetic vapor concentration in the gas phase. After the administration of anesthetic was discontinued, anesthetic concentration in liquid declined rapidly and was always undetectable at 30 min.

Muscles contracted isotonically at the preload of L_{max} throughout the experiment. After 15 min in each anesthetic concentration, a series of variables of contraction and relaxation were determined from three types of contraction (fig. 2). The first contraction was an isotonic twitch at the preload of L_{max} from which were measured the maximal amount of shortening (DL), peak velocity of shortening (+V) and of lengthening (−V), and corresponding times to peak values measured from the stimulus. The second contraction was a "zero-load-clamp" contraction; that is, an isotonic twitch at the preload of L_{max} where load was rapidly (<3 ms) decreased electronically to zero during the latent period.[30] This contraction gave a measurement of maximal unloaded velocity of shortening (MUVS) and time to MUVS (TMUVS). Theoretical and technical considerations of the zero-load-clamp technique to obtain maximal shortening velocity

TABLE 2. Variables of Contraction and Relaxation at the Onset of the Experiment; Values are Mean ± SD; *P* Value Obtained from One-way ANOVA

	Halothane (n = 9)	Enflurane (n = 9)	Isoflurane (n = 9)	*P*
DF	33.22	35.85	35.57	0.77
($mN \cdot mm^{-2}$)	± 9.08	± 7.19	± 8.92	
+dF/dt	241.8	271.9	229.8	0.56
($mN \cdot mm^{-2} \cdot s^{-1}$)	± 92.6	±47.0	± 63.1	
−dF/dt	157.7	182.1	147.4	0.34
($mN \cdot mm^{-2} \cdot s^{-1}$)	± 63.1	± 34.0	±50.1	
TPF	229.8	217.7	226.4	0.69
(ms)	± 31.5	± 24.8	± 32.0	
$RT_{\frac{1}{2}}$	161.6	154.0	180.7	0.51
(ms)	± 63.2	± 21.9	± 49.5	
DL	0.145	0.148	0.145	0.97
($L \cdot L_{max}^{-1}$)	± 0.033	± 0.033	± 0.026	
TDL	232.6	226.8	228.1	0.86
(ms)	± 23.5	± 20.8	± 23.6	
MUVS	2.19	2.30	2.21	0.82
($L \cdot L_{max}^{-1} \cdot s^{-1}$)	± 0.33	± 0.43	± 0.43	
TMUVS	57.6	56.1	53.3	0.33
(ms)	± 5.7	± 6.8	± 5.4	
−V	4.20	4.03	3.62	0.66
($L \cdot L_{max}^{-1} \cdot s^{-1}$)	± 1.19	± 1.33	± 1.49	

mN = millinewton; DF = developed force; +dF/dt = maximal rate of rise of force; −dF/dt = maximal rate of fall of force; TPF = time to peak force; $RT_{\frac{1}{2}}$ = time from peak force to half isometric relaxation; DL = peak shortening; TDL = time to peak shortening; MUVS = maximal unloaded velocity of shortening; TMUVS = time to maximal unloaded velocity of shortening; −V = maximal velocity of lengthening.

at zero load (MUVS) have been discussed earlier.[30] The third contraction was an isometric twitch, which gave measurements of peak developed force (DF), maximal rate of rise (+dF/dt), and fall of force (−dF/dt), corresponding time to peak values, and time to half isometric relaxation ($RT_{\frac{1}{2}}$) measured from the time to peak force (TPF). Each of these three contractions was separated by seven isotonic twitches at the preload of L_{max} to eliminate effects of loading history of preceding contractions.[31–33] Right ventricular ferret papillary muscles often have a spontaneous tendency to exhibit a slightly varying strength of contraction (5–10% over several beats), which is presumably caused by effects upon the β-adrenoceptor of norepinephrine released from post-synaptic adrenergic nerve endings. To minimize effects of release of endogenous catecholamines in ferret cardiac muscle, β-adrenoceptor blockade was achieved by the administration of (±)-bupranolol hydrochloride (10^{-6} M), a competitive β-blocking agent that is more potent than propranolol and apparently devoid of agonistic effects in heart muscle.[34,35]

All waveforms of force, length, velocity, and dF/dt were displayed as a function of time on a four-channel digital oscilloscope (Nicolet® 4094A), stored permanently on 5¼″ floppy disks, and recorded at slow speed on a four-channel pen recorder (Honeywell® 1400). All waveforms of interest recorded on the digital oscilloscope were transferred to a host laboratory computer (IBM PC-XT, Boca Raton, FL) under the command of a dedicated program written in WFBASIC (Blue Feather Software, New Glarus, WI), which also measured all parameters of contraction and relaxation and corresponding time values. This program was extensively tested and was error-free and accurate; this approach has also eliminated inter-observer variations in waveform analysis and errors commonly encountered during manual calculations.

A cumulative dose-response curve for halothane, enflurane, and isoflurane, respectively, was established in nine muscles for each agent. Effects of each anesthetic concentration on contraction and relaxation were followed until a steady state was reached, and this was usually the case after 10–15 min of equilibration at each concentration. After 15 min at each concentration, the test contractions required for analysis of contractility and of relaxation were then recorded, after which the concentration of the anesthetic was increased. The procedures required to record test contractions for analysis of contraction and relaxation lasted about 8 min, such that total exposure to a particular anesthetic concentration was about 22–23 min. The following incremental anesthetic concentrations were used: for halothane, from 0 to 1.5% in steps of 0.25%; for enflurane, from 0 to 3% in steps of 0.50%; for isoflurane, from 0 to 3% in steps of 0.375%. Each step corresponded to an increment of 0.25 MAC of the respective anesthetic in the ferret at 37° C.[36] This experimental design allowed us to compare inotropic effects at exactly equipotent anesthetic concentrations. Yet, as the experiments were carried out at 30° C, the MAC multiples used in this study (derived at 37° C) were in fact greater by some 16%[36] than values in the range (0–1.5 MAC) stated. It was not possible to examine effects of halothane and enflurane at >1.5 MAC, because most muscles became inexcitable. Even at 1.5 MAC, the intensity of the stimulus had to be increased to elicit contractions, because the threshold for stimulation had increased. After the highest concentration, the vaporizer was turned off and the reservoir bag emptied. Muscle recovery was followed under exactly similar conditions for 60 min and variables of contraction and relaxation were recorded at 15, 30, and 60 min of recovery. In an additional series of six muscles, an identical protocol was executed except that the administration of anesthetic was omitted. Values of variables of contractility and relaxation during these non-anesthetic controls were obtained at times equivalent to those in the anesthetic experiments.

To test the hypothesis of whether there is a dose-response relationship for a particular variable to a given anesthetic, and whether there are differences between anesthetics and/or between each anesthetic and the non-anesthetic controls, the relationship between contractile variable and anesthetic concentration was subjected to

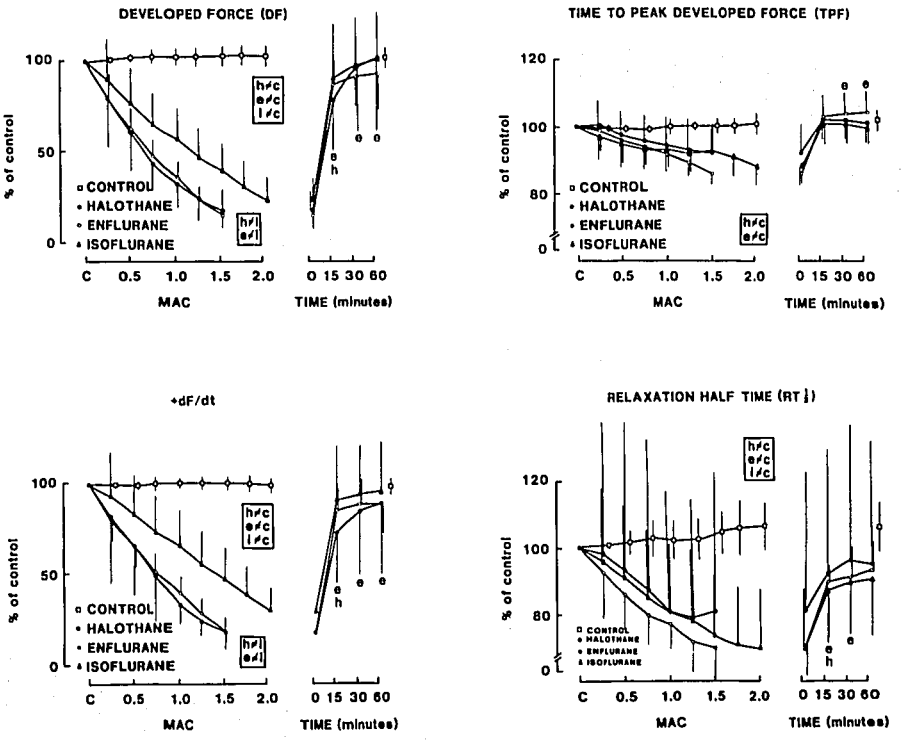

FIG. 3. Cumulative dose-response relationships to and time course of recovery after exposure to halothane, enflurane, and isoflurane for isometric twitches: values of peak developed force (DF), of time to peak developed force (TPF), of maximal rate of rise of force (+dF/dt), and of time to half isometric relaxation (RT½) are plotted as percent of control at time zero ± SD. Statistically significant differences between absolute values during recovery *versus* values at time zero within each group (two-sided paired Student's *t* test) are indicated in each panel (e = enflurane; h = halothane) at $P < 0.05$. Values (mean ± SD) obtained during non-anesthetic control experiments (n = 6) were superimposed for comparison. In the non-anesthetic control group, differences between absolute values at time zero and at times equivalent to 60 min of recovery were not statistically significant (two-sided paired Student's *t* test; $P > 0.05$, n = 6).

response curve analysis,[37] specifically by least squares linear regression analysis, whenever possible. Linear regression analysis was deemed appropriate when the correlation coefficient was greater than the value that was significant at the 0.01 level. For every individual muscle, linear regression analysis was carried out on each variable. Values of individual slopes were tested for differences between groups (halothane, enflurane, isoflurane, non-anesthetic control) with one-way analysis of variance (one-way ANOVA). Pairwise comparisons between means of two groups were made with the least significant difference method at the 95% confidence level.

Results

There were no statistically significant differences for L_{max}, mean cross-sectional area (CSA), resting force or preload at L_{max} (R), total force (T), and ratio of resting to total force (R/T) among the three groups of muscles used in this study (table 1) (one-way ANOVA, $P > 0.2$). The absolute values of variables of contraction and relaxation at the onset of each dose-response experiment for each of the three muscle groups are reported in table 2. There were no statistically significant differences between the three muscle groups for all variables (one-way ANOVA). The coefficients of variation of the variables shown in table 2 were less than 30% for DF, TPF, DL, TDL, MUVS, and TMUVS, and less than 40% for +dF/dt, −dF/dt, RT½, and −V. Accordingly, we considered it justified to normalize all results as percent of individual control values of each muscle.

Figure 3 illustrates dose-response relationships to halothane, enflurane, and isoflurane for DF, TPF, +dF/dt, and RT½, all derived from isometric contractions. The three anesthetics caused a reversible dose-dependent decrease in DF and +dF/dt, and abbreviated both contraction (shortened TPF) and isometric relaxation (shortened RT½). Figure 4 shows that the three anesthetics also caused a reversible dose-dependent decrease in DL and MUVS measured from isotonic contractions. Halothane slightly increased, whereas enflurane (>1 MAC) shortened the duration of the isotonic twitch (TDL). Isoflurane had no effect on TDL. All three agents increased TMUVS. Variables of relaxation, (−dF/dt) and (−V), shown in figure 5 exhibit a similar dose-dependent change. Values (mean ± SD) of variables of contraction and relaxation obtained in six non-anesthetic controls were superimposed in figures 3–5. For all variables of contraction and relaxation, the relationship between concentration and effect was linear. Therefore, for each individual muscle, a least squares linear regression was performed relating the absolute value of each variable to MAC. For the non-anesthetic controls, MAC values were used equivalent to those at which contraction and relaxation variables were measured in the anesthetic groups at equivalent times. The mean (±SD) values of individual slopes and median values of coefficients of determination (r^2) are shown for each variable and muscle group (halothane, enflurane, isoflu-

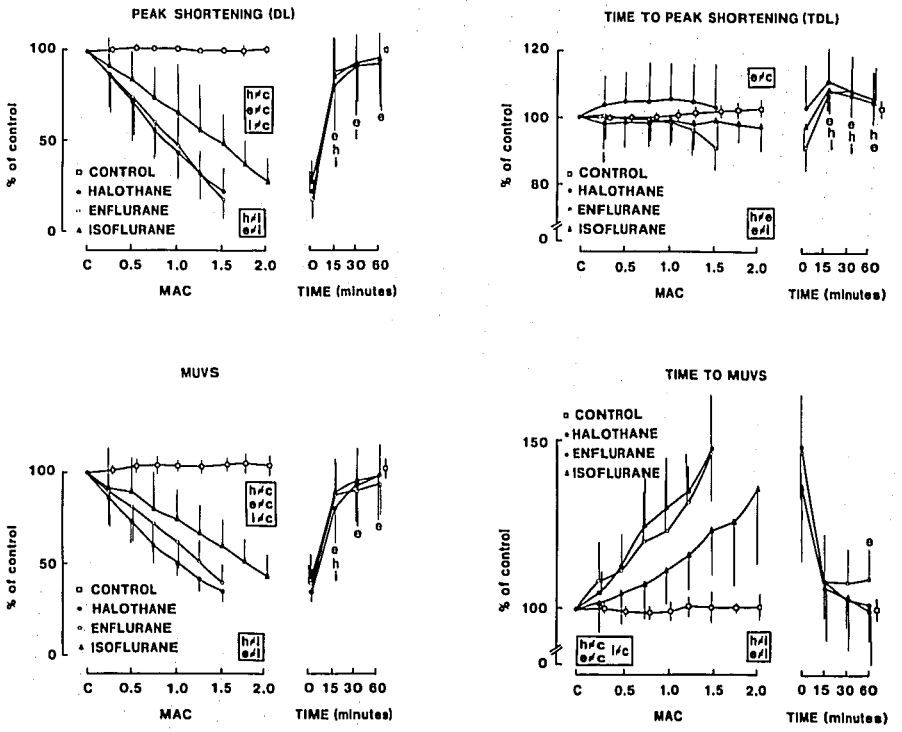

FIG. 4. Cumulative dose-response to and time course of recovery after exposure to halothane, enflurane, and isoflurane for isotonic twitches: values of peak shortening (DL), of time to peak shortening (TDL) of the isotonic preloaded twitch, of amplitude (MUVS) and time to maximal unloaded velocity of shortening (TMUVS) of the zero-load-clamped twitch are plotted as percent of control at time zero ± SD. Statistically significant differences between absolute values during recovery *versus* values at time zero within each group (two-sided paired Student's *t* test) are shown in each panel (e = enflurane, h = halothane, i = isoflurane) at $P < 0.05$. Values (mean ± SD) obtained during non-anesthetic control experiments (n = 6) were superimposed for comparison. In the non-anesthetic control group, differences between absolute values at time zero and at times equivalent to 60 min of recovery were not statistically significant (two-sided paired Student's *t* test; $P > 0.05$, n = 6).

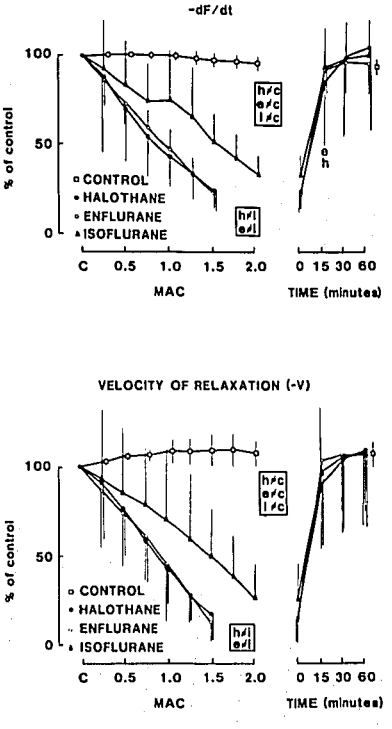

FIG. 5. Cumulative dose-response to and time course of recovery after exposure to halothane, enflurane, and isoflurane for variables of relaxation: maximal rate of decrease in force of isometric twitches (−dF/dt), and maximal lengthening velocity in isotonic twitches (−V). Values are plotted as percent of control at time zero ± SD. Statistically significant differences between absolute values during recovery *versus* values at time zero within each group (two-sided paired Student's *t* test) are shown at $P < 0.05$ (e = enflurane, h = halothane). Values (mean ± SD) obtained during non-anesthetic control experiments (n = 6) were superimposed for comparison. In the non-anesthetic control group, differences between absolute values at time zero and at times equivalent to 60 min of recovery were not statistically significant (two-sided paired Student's *t* test; $P > 0.05$, n = 6).

rane, non-anesthetic control) in table 3. For each variable, the four groups of slopes were tested for significant differences with one-way ANOVA. Pairwise comparisons between groups (see Materials and Methods) revealed the following: 1) there were no differences between the three anesthetics with respect to their effect in decreasing the duration of isometric contraction (TPF) and relaxation (RT½); 2) significant differences were found between halothane and isoflurane, and between enflurane and isoflurane for the other variables listed in table 3; 3) each of the three anesthetic groups differed from the non-anesthetic control for all variables, except for TPF (isoflurane not significantly different from control) and TDL (halothane and isoflurane not significantly different from control); and 4) at no time did we detect any statistically significant differences of effect between halothane and enflurane.

In a further step to compare inotropic effects of halothane, enflurane, and isoflurane, we calculated from the linear regression lines the anesthetic concentration (in MAC units) necessary to reduce the amplitude of contractile variables by 50%. Table 4 shows that halothane and enflurane were equipotent myocardial depressants. In contrast, one would need a concentration of isoflurane approximately 50% (range 41–61%) greater to obtain a similar negative inotropic effect. However, genuine anesthetic equipotency exists with certainty only at 1 MAC,

TABLE 3. Mean (±SD) Absolute Values of Slopes and Median Values of Coefficients of Determination (r^2) of Linear Regression Lines Relating Each Variable to MAC

	Halothane (n = 9)		Enflurane (n = 9)		Isoflurane (n = 9)		Control (n = 6)		
	Slope (Mean ± SD)	r^2 (Median)	Slope (Mean ± SD)	r^2 (Median)	Slope (Mean ± SD)	r^2 (Median)	Slope (Mean ± SD)	S.E.E. (Median)	P (Between Groups)
DF/MAC ($mN \cdot mm^{-2} \cdot MAC^{-1}$)	18.34* ± 5.40**	0.94	20.13† ± 5.84**	0.98	13.62 ± 4.21**	0.97	−0.0127 ± 0.0195	0.3665	<0.001
+dF/dt/MAC ($mN \cdot mm^{-2} \cdot s^{-1} \cdot MAC^{-1}$)	132.7† ± 61.4**	0.96	143.5† ± 42.1**	0.99	80.5 ± 23.2**	0.99	−0.0061 ± 0.1297	4.0326	<0.001
−dF/dt/MAC ($mN \cdot mm^{-2} \cdot s^{-1} \cdot MAC^{-1}$)	80.95* ± 40.67**	0.96	93.27† ± 29.77**	0.99	47.30 ± 22.28¶	0.94	0.0696 ± 0.0726	2.9674	<0.001
TPF/MAC ($ms \cdot MAC^{-1}$)	13.65 ± 11.35§	0.80	20.05 ± 11.53¶	0.89	10.68 ± 15.25	0.71	0.0254 ± 0.0350	1.5756	<0.01
$RT\frac{1}{2}$/MAC ($ms \cdot MAC^{-1}$)	24.62 ± 12.11**	0.86	30.62 ± 9.01**	0.89	28.41 ± 19.53**	0.94	−0.0706 ± 0.0828	2.3318	<0.0001
DL/MAC ($L \cdot L_{max}^{-1} \cdot MAC^{-1}$)	0.076† ± 0.015**	0.98	0.081‡ ± 0.024**	0.99	0.052 ± 0.012**	0.97	−0.0000082 ± 0.000021	0.0015	<0.001
TDL/MAC ($ms \cdot MAC^{-1}$)	3.95 ± 15.94	4.13 (S.E.E.)	−11.98* ± 8.62§	4.66 (S.E.E.)	−1.72 ± 10.09	3.79 (S.E.E.)	0.0383 ± 0.0324	1.3319	<0.02
MUVS/MAC ($L_{max} \cdot s^{-1} \cdot MAC^{-1}$)	0.95‡ ± 0.20**	0.97	0.90† ± 0.27**	0.99	0.61 ± 0.19**	0.97	−0.00030 ± 0.00051	0.0319	<0.001
TMUVS/MAC ($ms \cdot MAC^{-1}$)	18.06‡ ± 6.33**	0.87	15.75† ± 2.77**	0.87	9.25 ± 7.09¶	0.91	0.0024 ± 0.0136	0.6044	<0.001
−V/MAC ($L_{max} \cdot s^{-1} \cdot MAC^{-1}$)	2.44† ± 0.93**	0.98	2.33† ± 0.83**	0.98	1.33 ± 0.55**	0.97	−0.0014 ± 0.0036	0.0573	<0.001

DF = developed force; +dF/dt = maximal rate of rise of force; −dF/dt = maximal rate of fall of force; TPF = time to peak force; $RT\frac{1}{2}$ = time from peak force to half isometric relaxation; DL = peak shortening; TDL = time to peak shortening; MUVS = maximal unloaded velocity of shortening; TMUVS = time to maximal unloaded velocity of shortening; −V = maximal velocity of lengthening; S.E.E. = standard error of the estimate ($s_{y \cdot x}$).

Significant differences between halothane and isoflurane or between enflurane and isoflurane: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$. TDL/MAC differed significantly between halothane and enflurane ($P < 0.01$).

Significant differences between each anesthetic group and the non-anesthetic control: §$P < 0.05$; ¶$P < 0.01$; **$P < 0.001$.

and fractions or multiples of MAC may not be "equipotent" in terms of anesthetic effects. It is obvious from figures 3–5 that contractility was less decreased at 1 MAC of isoflurane than at 1 MAC of either halothane or enflurane.

Recovery from exposure to halothane, enflurane, and isoflurane was rapid. Most of the recovery occurred within the first few minutes after exposure to the anesthetic was discontinued. For all variables, at least 70% of the recovery was complete at 15 min. Thereafter, recovery toward control values was progressively slower, except for TDL and TPF, which displayed a different pattern. In order to determine the time at which recovery (defined as return to values at time zero) could be considered complete, values at 15, 30, and 60 min were compared to their respective control values at time zero in each group (Student's paired *t* test). Variables of relaxation (−dF/dt and −V, fig. 5) reached complete recovery at earlier times than did contraction variables (DF, +dF/dt in fig. 3; DL and MUVS in fig. 4). Recovery from exposure to enflurane was different in several respects. First, recovery of contractility after enflurane was incomplete even at 60 min, whereas, in the case of halothane and isoflurane, recovery reached completion at 30 min (except for DL). Second, recovery from exposure to enflurane was characterized by a further prolongation of the isometric twitch to beyond control values of TPF, whereas TPF returned to control early on (at 15 min) after exposure to halothane or isoflurane. The isotonic twitch was prolonged to beyond control values throughout recovery from exposure to any of the anesthetics. Yet, when values of contraction and relaxation variables (expressed as % of their respective value at time zero) after 60 min of recovery were compared between groups which included the non-anesthetic control, no statistically significant differences were found (one-way ANOVA, all variables $P > 0.1$).

TABLE 4. Anesthetic Concentrations (in MAC) Required to Reduce the Amplitude of Contractile Variables by 50% (ED50)

ED50 for	Halothane (MAC)	Enflurane (MAC)	Isoflurane (MAC)
DF	0.85 ± 0.03‡	0.86 ± 0.12‡	1.32 ± 0.33
+dF/dt	0.88 ± 0.07‡	0.95 ± 0.19‡	1.48 ± 0.32
−dF/dt	0.98 ± 0.17‡	1.02 ± 0.21‡	1.51 ± 0.12
DL	0.94 ± 0.11‡	0.96 ± 0.19‡	1.44 ± 0.25
MUVS	1.13 ± 0.10†	1.34 ± 0.28*	1.97 ± 0.66
−V	0.90 ± 0.16‡	0.89 ± 0.14‡	1.46 ± 0.36

Significant differences between halothane and isoflurane or between enflurane and isoflurane: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ *versus* isoflurane. There were no statistically significant differences between halothane and enflurane.

Discussion

This study clearly demonstrates that the negative inotropic effects of isoflurane are less than those of either halothane or enflurane at equipotent anesthetic concentrations in ferret ventricular myocardium. The negative inotropic effect of each of the three anesthetic agents was dose-dependent and reversible. In most previous studies, the inotropic effects of halothane, enflurane, and isoflurane were studied separately, and there were some differences in experimental protocols, which makes it difficult to validly compare the anesthetics. A series of classical studies from one laboratory on the inotropic effects of halothane,[17] enflurane,[22] and isoflurane[24] serve as one of the standard sources of information for comparison of inotropic effects of these anesthetics *in vitro*. It was reported that these anesthetics caused "direct myocardial depression in the following order of severity: halothane > isoflurane > · · · > enflurane."[24] MAC of humans was used to set equipotent anesthetic concentrations; the muscles studied had cross-sectional areas in excess of 1.1 mm^2 and may have suffered from core hypoxia.[24] Finally, comparisons are rendered particularly difficult because the different anesthetic agents were studied at different temperatures ranging from 22 to 37° C. In summary, there is no agreement on relative potencies of the three anesthetics regarding their myocardial depressant effect among *in vitro* studies on cat papillary muscle,[17,18,22,24] guinea pig papillary muscle,[25] and isolated rat atria.[38]

In this study, halothane and enflurane were significantly more depressant than isoflurane for both isometric (DF, +dF/dt, −dF/dt) and isotonic variables (DL, MUVS, −V) (table 3). Our observation that isoflurane is less of a myocardial depressant corroborates findings by Merin[10] who compared hemodynamic effects of halothane, enflurane, and isoflurane in identical, acute, and chronic dog preparations. Hemodynamics were similar except for: 1) significant increases in filling pressures (LVEDP) caused by high concentrations of halothane and enflurane, but not by isoflurane; and 2) a substantial decrease in afterload (SVR) caused by isoflurane, but not the other two anesthetics. Isoflurane produced a dose-dependent depression of ventricular function in the intact dog, but less so than either halothane or enflurane. This difference was attributed to a decreased afterload by isoflurane, and to a minor degree of cardiac sympathetic stimulation.[9,10] Since these factors do not play a role in this study, the lesser intrinsic depressant effects of isoflurane *in vitro* could well account for its smaller ventricular depressant effect *in vivo*.

The negative inotropic effects of volatile anesthetics have been attributed to interference with several steps involved in excitation-contraction coupling in ventricular myocardium, mechanisms which have been extensively discussed in a recent review.[39] In brief, halothane, enflurane, and isoflurane alter Ca^{2+} exchange at the sarcolemma and reduce entry of Ca^{2+} through the surface membrane.[25,40,41] Second, there is evidence from studies on isolated sarcoplasmic reticular (SR) vesicles,[42] skinned cardiac fibers with preserved SR function,[43–46] isolated dog ventricular fibers,[47] and isolated rat cardiac myocytes[48] that halothane, enflurane, and isoflurane inhibit Ca^{2+} sequestration by the SR and decrease the amount of Ca^{2+} for release. Third, halothane and isoflurane affect the contractile proteins. Halothane and isoflurane appeared to decrease the calcium sensitivity of the contractile proteins in functionally skinned right ventricular rabbit papillary muscle,[43,44,46] whereas enflurane did not.[45] Yet, in Triton X-100 skinned rat ventricular trabeculae, halothane, enflurane, and isoflurane caused a reversible dose-dependent decrease of the calcium responsiveness of the contractile proteins.§ Halothane caused a depression of actomyosin ATPase in dog heart myofibrils[49] and of myofibrillar ATPase of cat[50] and beef heart[51] at Ca^{2+} concentrations likely to be encountered in the myoplasm. Isoflurane's depressant effect on myofibrillar ATPase was less than that of halothane, whereas enflurane was reported to have no effect.[51] The troponin-tropomyosin complex seems to play a key role in the action of halothane, as its removal or inactivation rendered the enzyme system insensitive to both Ca^{2+} and halothane. Yet, in a recent study, it was found that halothane did not change the binding of calcium to isolated bovine cardiac troponin C,[52] although no differentiation was made between high-affinity and low-affinity binding sites, the latter being of physiological relevance. The relative magnitude of the anesthetic effects on isolated protein systems and on skinned fibers may have been modified by the procedures used to isolate them,[39,49] and it is therefore desirable to complement this information with studies on non-disrupted intact muscle preparations.

The production of force reflects the interaction between myosin cross-bridges and actin, and the amount of force is determined by the number of independently operating cross-bridges.[53] The formation of force-generating cross-bridges follows the binding of Ca^{2+} to troponin C, which in turn depends on the moment-to-moment availability of free myoplasmic calcium, and on the affinity of troponin C for Ca^{2+}. The decrease in the amplitude of contraction (developed force and/or shortening) is due to a decrease in the number of cross-bridges as a result of a reduced intracellular availability of calcium ions possibly compounded by changes in the contractile proteins themselves. A reduced intracellular calcium availability can be inferred from the recent observation that halothane caused a dose-dependent decrease of the amplitude of the intracellular calcium transient measured with ae-

§ Murat I, Vassort G: Personal communication.

quorin.[54] Evidence for an anesthetic-induced decrease in the Ca^{2+} responsiveness of the contractile proteins will be discussed later.

The maximal rate of turnover of myosin cross-bridges correlates with the activity of myosin ATPase, and is best approximated in intact muscle by the maximal velocity of shortening at zero load (MUVS).[55,56] The decrease of MUVS observed with the three anesthetics most likely reflects a slowing of the rate of turnover of cross-bridges, which is consistent, for halothane and isoflurane, with a reduction in actomyosin ATPase activity.[49-51] Unloaded velocity of shortening normally increases rapidly to reach its maximum value within 15–20% of the time to peak isometric force[57] (in cat papillary muscle). TMUVS in this study was 25.1 ± 3.7% of TPF (ferret papillary muscle, mean ± SD, n = 27). The intracellular calcium transient measured with aequorin reaches its peak at comparable times.[30,58] When the increase in the intracellular calcium transient is slowed, as with caffeine,[58] or with halothane,[54] unloaded velocity of shortening reaches a later peak as well.[57,59] The three anesthetics slowed the increase towards maximal unloaded velocity of shortening so that MUVS was reached at later times (fig. 6). This could be due to a slower increase of intracellular $[Ca^{2+}]$ as a consequence of reductions of Ca^{2+} entry through the surface membrane and of Ca^{2+} release from the SR. The anesthetic-induced increase in TMUVS (fig. 6) and delay of onset of shortening (fig. 7C) contrast sharply with the minimal anesthetic-induced delay in force development in the same conditions (fig. 7A). It is unlikely that the characteristics of the lever system cause this delay in shortening; if inertial forces would be responsible, one would expect the largest delay in the control twitch, the opposite of what was observed. An explanation for this paradox may rather be found in cross-bridge kinetics which differ in isometric *versus* isotonic contractions (*vide infra*).

Halothane, enflurane, and isoflurane abbreviated both time to peak force (TPF) and isometric relaxation (RT½). Even when corrected for the decrease in time to peak force, isometric relaxation was still abbreviated. This characteristic feature of volatile anesthetics is illustrated in figures 7A and B. The isometric force traces in figure 7A were obtained at control and different anesthetic concentrations of isoflurane and were scaled so that the relative rates of force development (+dF/dt) were the same to allow for comparisons of relative amplitude of force development at a same relative rate of force development. This construction is based on observations that the rate of force development correlates better than developed force with the peak of the intracellular Ca^{2+} transient.[60] It is obvious from figures 3 and 7A that peak force is slightly more depressed than the rate of force development (+dF/dt). Because of the less sustained period of force development, time to peak force occurred slightly earlier and peak force was somewhat more depressed than the maximal rate of force development (+dF/dt). The acceleration of isometric relaxation (figs. 3, 7B) is not necessarily a consequence of the concomitant decrease in peak force, as time to half isometric relaxation (RT½) was unchanged in control conditions over a range of extracellular calcium concentrations of 1.25–10 mM in cat right ventricular papillary muscle.[61] In contrast to isometric relaxation, there was little effect of isoflurane, halothane, or enflurane on the time course of isotonic relaxation (figs. 7C, D).

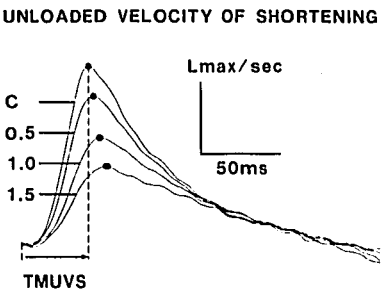

FIG. 6. Time course of velocity of shortening during a twitch contraction at zero load (zero-load-clamp) in a representative muscle exposed to isoflurane. Four contractions were superimposed, the control (C), and one at 0.5, 1.0, and 1.5 MAC of isoflurane. Volatile anesthetics caused a dose-dependent depression in MUVS (dots) and delay the attainment of the peak unloaded velocity of shortening (a vertical dotted line was drawn through MUVS at time zero). Same muscle as in figure 2.

In order to explain the differential effects of volatile anesthetics on isometric and isotonic relaxation, one needs to address their effects on the forces underlying myocardial relaxation. The decline of force in an isometric twitch depends on: 1) the fall of myoplasmic $[Ca^{2+}]$; 2) the affinity of troponin C for Ca^{2+}, which determines the rate at which Ca^{2+} ions become released from troponin C; 3) the response of myofilaments to a given level of occupancy of Ca^{2+}-binding sites on troponin C; and 4) dynamics of attachment and detachment of actomyosin cross-bridges. Whereas volatile anesthetics have an effect on the intracellular Ca^{2+} transient,[54] it is presently unclear whether they have an effect on cellular events downstream from the Ca^{2+} transient: on the rate of unbinding of Ca^{2+} from troponin C, on the conformational changes that occur in the troponin-tropomyosin complex, and on the actomyosin interaction. We will therefore address the above-mentioned mechanisms in a general sense as the "calcium responsiveness of the contractile apparatus." A decrease in calcium responsiveness (and specifically, a decrease in Ca^{2+} bound to troponin C) will cause less Ca^{2+} to remain bound during isometric relaxation, and the decrease in force will be faster and relaxation abbreviated. Decreases in calcium responsiveness of the contractile system are due to changes in the rate at which bound Ca^{2+} is released from troponin C, *i.e.*, the off-rate of binding of Ca^{2+} to troponin C increases.[62]

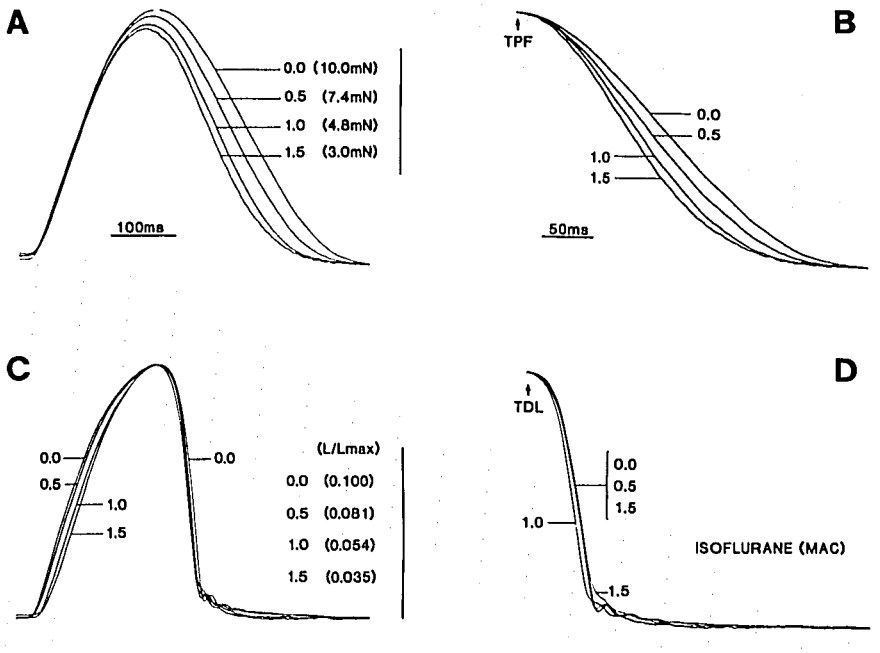

FIG. 7. Time course of force development during an isometric twitch (A) and of shortening during an isotonic twitch at the preload of L_{max} (C) of a representative muscle at time zero (0) and in 0.5, 1.0, and 1.5 MAC of isoflurane. The force traces were scaled vertically in panel A so that they had the same relative rate of force development. The vertical bar corresponds to 10, 7.4, 4.8, and 3.0 mN force, respectively, for the isometric twitches at 0, 0.5, 1.0, and 1.5 MAC. Shortening traces in panel C were scaled vertically to the same amplitude to allow for easy comparison of time course. The vertical bar corresponds to 0.100 L/L_{max}, 0.081 L/L_{max}, 0.054 L/L_{max}, and 0.035 L/L_{max} of shortening for the contractions at 0, 0.5, 1.0, and 1.5 MAC, respectively. Panel B shows the time course of isometric relaxation only, whereby the force traces were shifted horizontally and made to coincide at their respective time to peak force (TPF) to allow for easy comparison of the time courses of isometric relaxation. Panel D illustrates the time course of isotonic relaxation (lengthening) whereby the length traces were moved horizontally and made to coincide at their respective time to peak shortening (TDL) to allow for comparison of time course of lengthening. Isoflurane slightly abbreviated the time to peak force, and caused a dose-dependent abbreviation of isometric relaxation. On the other hand, the timing of relaxation of the isotonic twitch was hardly affected. The onset of shortening was delayed in a dose-dependent manner (see also fig. 6). Same muscle as for figure 2.

The abbreviation of isometric relaxation caused by volatile anesthetics can be explained by an increase in the rate of Ca^{2+} uptake by the SR and/or a decrease in the calcium responsiveness of the contractile proteins, mechanisms for which there is evidence from other studies. Halothane, enflurane, and isoflurane increased the initial rate of SR Ca^{2+} uptake in isolated rabbit SR vesicles, but decreased maximal Ca^{2+} uptake[42]; this could explain both the accelerated isometric relaxation and the negative inotropic effect. Yet a possible effect of an increased rate of SR Ca^{2+} uptake is not apparent from the time course of isotonic relaxation, that is either unchanged (isoflurane) (fig. 7) or even slowed at higher anesthetic concentrations (halothane, enflurane).[26] The abbreviation of isometric relaxation can also be explained by a decrease in calcium responsiveness of the contractile proteins, a hypothesis for which there is independent evidence from skinned fiber experiments.[43,46] In particular, in view of the fact that the period of force development is shortened relative to the rate of force development (fig. 7A), the rate of unbinding of Ca^{2+} from troponin C has increased more than the rate of binding of Ca^{2+} to troponin C has decreased. Therefore, such a reduction in calcium sensitivity should only have a moderate depressant effect on the rate and amount of force production, but a profound effect on the duration of decline of force. Our observations of the type shown in figures 7A and B are compatible with this view.

During shortening, however, the native calcium sensitivity of the contractile proteins may be different from that during force development. Indeed, in papillary muscles of cat, ferret, and frog, active shortening or the accompanying lack of force development was associated with a higher myoplasmic calcium concentration than at comparable times in isometric twitches.[63] During shortening, there may be less calcium being bound, or more calcium being released from contractile proteins as a consequence of a decreased affinity of troponin C for calcium at low force levels. The biochemical basis for this argument was first demonstrated by Bremel and Weber,[64] who showed that the formation of rigor complexes by adding myosin to reconstituted thin filaments increased the affinity of troponin for calcium. This may be true as well for ordinary force-generating cross-bridges in physiological twitch contractions of intact muscle.[65,66] In detergent-extracted bovine ventricular bundles (where troponin C was the only calcium-binding species), muscle fibers bound more Ca^{2+} over the same pCa range when generating force than when cross-bridge formation was inhibited by vanadate.[67,68] A decrease in calcium sensitivity of the contractile proteins by each of the three anesthetics will be more obvious and visible when the native myofibrillar calcium sensitivity is high (as during force development) than when it is already low (as during shortening). It is therefore not surprising that isometric relaxation is much more abbreviated than is isotonic relaxation, and our data

are entirely compatible with the hypothesis that these three anesthetics decrease calcium sensitivity of the contractile proteins.

The same mechanism of action of volatile anesthetics can also account for the paradoxical association of a much retarded onset of shortening and a very mildly (or not at all) slowed time course of force development: in isometric conditions, more cross-bridges attach than during shortening, troponin C acquires a higher Ca^{2+} affinity, and becomes a more effective Ca^{2+} "sink," which leads to even more cross-bridge formation. Changes in the time course of the intracellular Ca^{2+} transient will, therefore, have only a modest influence on the time course of the rise of force. On the other hand, during shortening, less cross-bridges are attached at any time, and the native Ca^{2+} affinity of troponin C is low such that shortening or velocity of shortening will more closely follow the time course of the intracellular Ca^{2+} transient. In view of halothane's effect to increase the time to peak of the intracellular Ca^{2+} transient,[54] our findings of a minimal slowing of force development (fig. 7A) and of a more pronounced delay in the onset of shortening (and, hence, a later peak velocity, fig. 6) are not unexpected.

In this study, differences among the three anesthetics were mostly qualitative, but all three had effects on contractility in the same direction. This contrasts with reports that enflurane had no influence on myofibrillar ATPase,[50] nor on the Ca^{2+}-sensitivity of contractile proteins in cardiac skinned fibers[45], whereas halothane and isoflurane did.[43,46] Although this discrepancy cannot be readily explained, it is possible that the procedures used to isolate myofibrillar preparations and to prepare skinned fibers have removed or partially inactivated fragile subcellular regulatory mechanisms, such as phosphorylation of contractile proteins.[39,69] Myofilament sensitivity in intact muscle is apparently considerably greater than that observed in skinned fibers.[70] Alternatively, it is conceivable that enflurane acts differently on the contractile proteins in ways that cannot be inferred from the present study.

Recovery from exposure to any of the three anesthetics was extremely rapid and most of it occurred in the first few minutes after the anesthetic was discontinued. During recovery, the transient prolongation of the isotonic contraction along with the still abbreviated isometric relaxation may suggest that recovery of intracellular calcium availability precedes the restoration of the native myofibrillar calcium sensitivity, a hypothesis that needs verification with the detection of the intracellular calcium transient in these conditions.

In summary, isoflurane is a less potent myocardial depressant than either halothane and enflurane. The negative inotropic effect results from actions on multiple sites. Halothane, enflurane, and isoflurane alter the intracellular availability of calcium ions for contraction, by interfering with the entry of Ca^{2+} through the surface membrane and release and uptake of Ca^{2+} by the SR. As our results suggest, halothane, enflurane, and isoflurane might also change the affinity of troponin C for Ca^{2+} and/or myofibrillar responsiveness to Ca^{2+}. Further studies are required to assess the relative importance of each of such actions.

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