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Halothane, Enflurane, and Isoflurane Decrease Calcium Sensitivity and Maximal Force in Detergent-treated Rat Cardiac Fibers

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This study was designed to test the hypothesis that the volatile anesthetics directly affect cardiac contractile proteins. For this purpose, the effects of various anesthetic doses of halothane, enflurane, and isoflurane on myocardial calcium sensitivity and maximal calcium–activated force were examined in rat cardiac fibers skinned with Triton X-100. In this preparation, all membranes are chemically destroyed, and the sarcoplasmic reticulum is not functional. The three anesthetics shifted the pCa/tension curves (pCa = $-\log_{10}[Ca^{2+}]$) toward higher calcium concentrations and decreased pCa for half-maximum activation (pCa_{50}) in a dose-dependent and reversible fashion without changing the slope of this relationship (Hill coefficient). No differences between agents were observed at equipotent anesthetic concentrations. In addition, the three anesthetics decreased both maximal activated tension and tension at half-maximal activation in a dose-dependent fashion. Both the decrease in calcium sensitivity and the decrease in maximum activated tension may contribute to the negative inotropic effects of these agents. The relative importance of such effects compared with the other mechanisms of action remains to be determined, however. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Heart, contractile proteins: actin; myosin; troponin. Ions, calcium: sensitivity.)

HALOTHANE, ENFLURANE, AND ISOFLURANE directly depress myocardial contractility by interfering with several steps in excitation–contraction coupling, an effect that is dose dependent and reversible.[1] They act directly on the myocyte sarcolemma by increasing calcium binding to the plasma membrane[2] and by inhibiting calcium influx through the slow calcium channel,[3–5] both actions leading to decrease of the amount of calcium available for contractile activation. These agents also modify calcium handling by the sarcoplasmic reticulum. In mechanically disrupted rabbit ventricular fibers[6] and isolated sarcoplasmic reticulum,[7] volatile anesthetics seem to decrease the total capacity of sarcoplasmic reticulum for Ca^{2+} by reducing calcium uptake and calcium-induced calcium release. The resulting effect is a decreased amount of calcium available for internal storage and release. These two sites of action are considered the main targets of volatile anesthetics. However, a direct effect on the contractile proteins cannot be ruled out. Significant myofibrillar depression measured in terms of actomyosin ATPase activity was demonstrated only at anesthetic concentrations that are high compared with concentrations used in clinical practice.[8–12] All of these studies were recently reviewed by Rusy and Komai,[1] who pointed out the necessity of both knowing the gas phase concentration of anesthetic agent at equilibrium and of using preparations where myofibrillar regulation is preserved. In three separate studies,[6,13,14] Su *et al.* studied the effects of halothane, enflurane, and isoflurane on Ca^{2+}-activated tension development in mechanically disrupted right rabbit ventricular papillary muscles, where sarcoplasmic reticulum function was blunted by high concentrations of ethylene bis(oxyethylene-nitrilo)tetraacetic acid (EGTA). In these studies, halothane, enflurane, and isoflurane slightly decreased maximal Ca^{2+}-activated tension development in a dose-dependent fashion for both halothane and enflurane but not for isoflurane. A decrease of myocardial Ca^{2+} sensitivity was demonstrated only for high halothane concentrations (3 and 4%)[13] but was not observed with enflurane or isoflurane. In ferret papillary muscle, clinical anesthetic concentrations of halothane, enflurane, and isoflurane (0.5–2 MAC) caused a dose-dependent and reversible decrease in all parameters of contractility and impaired myocardial relaxation. The time course of contraction and relaxation in both isometric and isotonic twitches was also modified. These agents prolonged the duration of lengthening of an isotonic twitch, yet they abbreviated the duration of isometric relaxation in identical conditions.[15,16] This was taken as possibly resulting from reduced myofibrillar Ca^{2+} sensitivity, an effect that would appear to be a common feature of all of the three anesthetics. To test this hypothesis, the effects of various anesthetic doses of halothane, enflurane, and isoflurane on myocardial Ca^{2+} sensitivity and maximal Ca^{2+}-activated force were examined in rat cardiac fibers, skinned with Triton X-100. Each detergent-treated fiber was exposed in random order to various concentrations of each of the three anesthetics. In this preparation, all

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membranes are chemically destroyed and both sarcoplasmic reticulum, including T tubules, and mitochondria are not functional.[17,18] This preparation differs from mechanically disrupted fibers in which only sarcolemma is disrupted, whereas the sarcoplasmic reticulum is still functional and mitochondria are presumably intact. Detergent-treated fibers from rats were used, as the characteristics of this preparation, including biochemical[19] and ultrastructural data,[17,18] had been extensively studied. The calcium sensitivity of the cardiac contractile apparatus is identical in most adult mammalian species, including rat, rabbit, dog ventricle, and dog atria.[20] Furthermore, the Ca^{2+} sensitivity of detergent-treated skinned fibers is not dependent on the isoenzymatic composition of the myosin, as no differences were observed between fibers from pressure-overloaded rat and guinea pig hearts and sham-operated animals. This was the case despite chronic pressure overload that is characterized by an isoenzymatic shift of the main contractile protein, namely myosin, from V1 to the low ATPase isoform V3 in rats.[21] Evidence will be presented for a direct decrease of myocardial Ca^{2+} sensitivity and maximal force in clinical anesthetic concentrations of halothane, enflurane, and isoflurane.

Methods

Preparation of Skinned Fibers

Hearts were rapidly removed from rats (160–200 g body wt) that had been anesthetized with ethyl carbamate (2 g/kg ip) according to the recommendations of the Institutional Animal Care Committee (INSERM, Paris). Subendocardial left ventricular fibers were excised at room temperature in a zero-calcium Krebs solution. They were mounted between two stainless hooks, one connected to an AE801 transducer (AME, Horten, Norway). The length and diameter were determined optically with a binocular microscope and a micrometer. Ventricular fibers were assumed to be cylindric. After excision the muscles were slightly stretched and transferred for 60–90 min to a relaxing solution containing the nonionic detergent octyl phenoxy polyethoxyethanol (Triton X-100 1%). The duration of detergent treatment (ranging from 60 to 90 min) does not affect maximal activated tension within these limits, and this time is sufficient to destroy sarcoplasmic reticulum and mitochondrial and sarcolemmal membranes.[17,18] Before experiments, the preparation was stretched by about 20%, and the sarcomere length was adjusted with the use of laser diffraction to 2.0–2.1 μm (10 mW He-Ne laser, Spectra Physics, Inc., Mountain View, California), to avoid sarcomere length-dependent changes in Ca^{2+} sensitivity and to allow development of maximal force.[22] Two different bathing solutions, a relaxing solution (A) and an activating solution (B), were freshly prepared from frozen stock solutions. The compositions of the solutions used were calculated with the use of the computer program of Fabiato and Fabiato[23] and binding constants of Fabiato.[24] The following composition of the solutions was maintained constant: free Mg^{2+} 3.16 mM, MgATP 3.16 mM, creatine phosphate 12 mM, Na^{+} 30.6 mM, imidazole 30 mM, EGTA 10 mM, dithiothreitol 0.3 mM. Acetic acid was used to adjust *p*H at 7.1. Ionic strength was adjusted to 0.16 M with K acetate. Two solutions of extreme Ca^{2+} concentration were calculated: the relaxing solution (A), whose pCa was set at 9; and the activating solution (B), whose pCa was set at 4.5 ($pCa = -\log_{10}[\text{free } Ca^{2+}]$, where free calcium is expressed in molar concentration). Solutions of intermediate Ca^{2+} concentrations were obtained by mixing these two solutions (A and B). Experiments were performed at 22° C unless otherwise indicated, as binding constants are given at this temperature and the stability of the preparation is significantly greater than at a more physiologic temperature.

The muscles were immersed in small chambers containing 2.5 ml of solution. Eight chambers were arranged around a disk that could be moved under the muscle to change the solution as required. The disk was itself immersed in a temperature-controlled bath positioned on a magnetic stirrer. Each solution was vigorously stirred at high speed (>1,000 rpm) to facilitate diffusion of Ca, EGTA, and substrates into the muscle. The rate of stirring was chosen above the minimal rate, allowing development of maximal activated tension without interfering with data recording.

Data Analysis

For each muscle, a pCa tension curve was obtained in control conditions by stepwise exposure of the fibers to solutions with increasing Ca^{2+} concentrations and measurement of developed tension (fig. 1). Ca^{2+} concentration was expressed as pCa ($pCa = -\log_{10}[Ca^{2+}]$). Resting tension in the relaxing solution (A) was taken as zero tension, and maximal tension at pCa 4.5 (B) was taken as 100%. Intermediate values were expressed as a percentage of this maximal value. Data were analyzed with the use of a linearization of the Hill equation where F (relative force) $= [Ca]^{n_H}/(K + [Ca]^{n_H})$. The slope coefficient (n_H) and the pCa for half-maximal activation $pCa_{50} = (-\log_{10}K)/n_H$ were computed. Changes in tension of less than 10% or more than 90% of maximal activated tension were not plotted because these values are more heavily weighted than values near 50%.[25] At least three points were used for the determination of pCa_{50} and Hill coefficient (n_H). After exposure to different pCa in the presence of anesthetics, the fiber was then immersed in the control B solution to measure the relative decrease in maximal force

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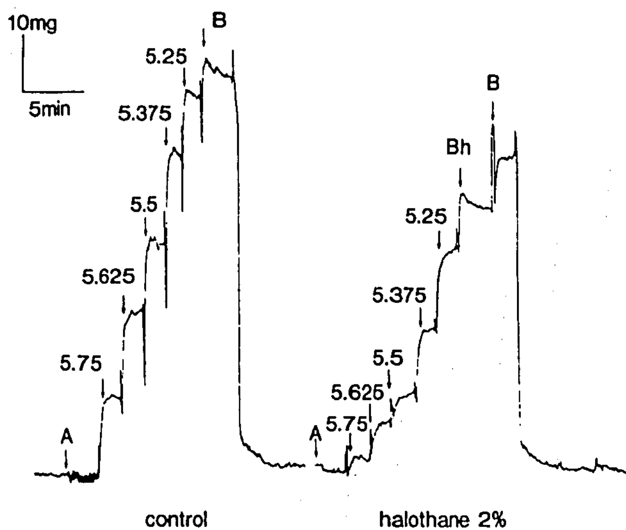

FIG. 1. Changes in tension related to changes in Ca^{2+} concentration expressed as pCa (pCa = $-\log_{10}$[Ca]). The fibers were immersed successively in the relaxing solution (A; pCa = 9), in intermediate solutions of increasing Ca^{2+} concentrations (pCa values are indicated on the figure), and in the activating solution (B; pCa = 4.5). The left panel shows the stepwise increases in tension obtained during control conditions; the right panel represents the changes in tension obtained with the same fiber during exposure to 2% halothane. At the end of each anesthetic exposure, the fiber was again immersed in the activating solution (B) free of anesthetic. In this particular experiment, 2 h elapsed between the two experimental conditions.

before relaxation. This procedure was chosen because it permits the measurement of maximal force to become time independent (see "Results" below).

EXPERIMENTAL DESIGN

The effects of halothane, enflurane, and isoflurane were assessed in two different series of experiments. For both experimental conditions, the test solutions were equilibrated in separate chambers by continuous and vigorous bubbling for 15 min with the chosen anesthetic. The carrier gas was 100% nitrogen (N_2), which flowed through calibrated vaporizers (Fluotec Mark III®, Enfluratec®, and isoflurane Draeger Vapor® 19.1). The anesthetic concentration in the gas phase was monitored with an infrared calibrated analyzer (Normac®, Datex, Finland). The anesthetic concentrations used were 0.5, 1.0, 2.0, and 3.0 vol% halothane; 0.75, 1.5, 3.0, and 4.5 vol% isoflurane; and 1.05, 2.1, and 4.2 vol% enflurane. These concentrations are roughly equivalent to 0.5, 1, 2, and 3 MAC multiples of halothane and isoflurane and 0.5, 1, and 2 MAC multiples of enflurane in adult rats at 37° C.[26,27] The anesthetic concentrations obtained in the solutions were measured by gas–liquid chromatography (GLC) to determine the exact amount of anesthetic present in the test solutions and to ensure that evaporation was minimal despite vigorous stirring necessary to achieve maximal Ca^{2+}-activated tension. A Varian 1400® gas chromatograph equipped with a flame ionization detector and a Porapack Q® 3.17 mm by 150 cm column was used for determination of anesthetic concentrations.[28,29] A 60-ml flask containing 100 μl of the solution equilibrated 15 min with the anesthetic was maintained at 60° C (above the boiling point of each anesthetic) for 20 min before injection of 1 ml of gas into the apparatus, previously calibrated with known concentrations of each anesthetic (head space technique). The anesthetic concentrations measured in the experimental solutions (n = 6) after 15 min of continuous bubbling at 1 MAC were as follows: 0.63 ± 0.06 mM for halothane, 1.09 ± 0.04 mM for enflurane, and 0.82 ± 0.01 mM for isoflurane. The evaporation was minimal as the concentrations in the muscle chamber measured at the third and the fifth minutes were decreased by less than 2%.

In all the experimental conditions described below, each of the three anesthetics was tested at all MAC multiples in the same fiber. In the first series of experiments, changes of tension at half-maximal activation (half-activated tension) were examined using the solution with a pCa that was close to the calculated pCa_{50}, obtained at the beginning of the study. Each fiber was exposed in a random order to test solutions of exactly identical composition equilibrated with 1, 2, and 3 MAC multiples of each anesthetic. Each test was bracketed by determination of half-activated tension with the control test solution, free of anesthetic. Results are expressed in percentage of these corresponding control values. The effects of anesthetics on maximal Ca^{2+}-activated tension were studied with a similar experimental protocol. Each fiber was exposed in a random order to the activating solution (solution B, pCa 4.5) equilibrated with 0.5, 1, 2, and 3 MAC multiples of each anesthetic. Each test was bracketed by determination of maximal Ca^{2+}-activated tension with the control B solution, free of anesthetic. Results are expressed in percentage of these corresponding control values.

In a second series of experiments, pCa-tension curves were obtained at each MAC multiple for each anesthetic in a random order in each fiber. Tension values were normalized as described above, so that the calcium sensitivity could be compared in different experimental conditions; half maximal pCa (pCa_{50}) and Hill coefficient (n_H) were computed. A control pCa-tension curve (with solutions of various Ca^{2+} concentrations but free of anesthetics) was again obtained at the end of the study, *i.e.*, 4–6 h after the reference curve had been determined.

STATISTICAL ANALYSIS

Comparisons between anesthetics and at equivalent MAC multiples were made by repeated measures analysis

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TABLE 1. Mean (±SEM) Values for pCa_{50} and Hill Coefficients during Control Conditions and at the Different MAC Multiples

	MAC	pCa_{50}	Hill Coefficient
Control	0	5.681 ± 0.043	3.27 ± 0.04
Halothane	0.5	5.620 ± 0.035*	3.55 ± 0.11
	1	5.538 ± 0.043*	3.77 ± 0.28
	2	5.501 ± 0.043*	3.50 ± 0.27
	3	5.445 ± 0.046*	3.02 ± 0.13
Isoflurane	0.5	5.579 ± 0.039*	3.33 ± 0.15
	1	5.535 ± 0.041*	3.39 ± 0.22
	2	5.500 ± 0.047*	2.94 ± 0.17
	3	5.475 ± 0.043*	3.14 ± 0.24
Enflurane	0.5	5.580 ± 0.039*	3.22 ± 0.26
	1	5.536 ± 0.042*	3.13 ± 0.18
	2	5.520 ± 0.050*	3.11 ± 0.38
Control end	0	5.663 ± 0.043	3.15 ± 0.15

* Indicates significant changes ($P < 0.001$) *versus* control.

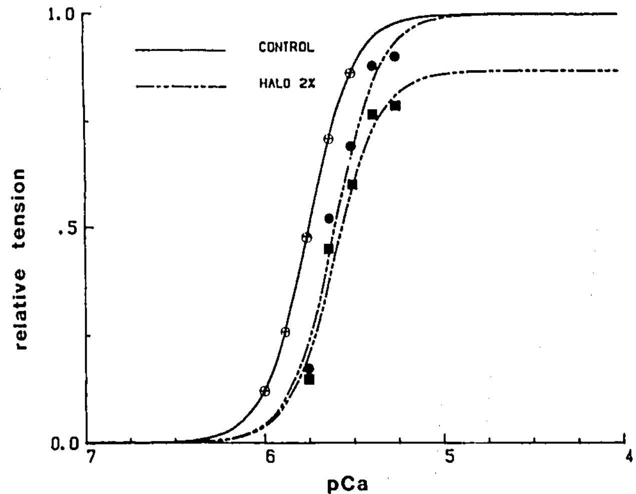

FIG. 2. pCa/tension curves obtained during control conditions (continuous line) and during exposure to 2% halothane (dashed lines). Both relative data (■) and normalized data (●) during anesthetic exposure are represented on the figure.

of variance with the use of a computer program (Stats, Statsoft 2.1, 1985). For multiple comparisons among groups where indicated by ANOVA results, paired *t* tests with the Bonferonni correction were used. Comparisons between control values obtained at the beginning and the end of each study were made with the use of Student's *t* test for paired data. Values of P 0.05 or less were regarded as significant. Results are expressed as mean ± standard error of the mean (SEM).

Results

The characteristics (±SD) of the fibers (n = 23) were as follows: mean length 1,210 ± 150 μm (range, 900–1,500), mean diameter 250 ± 60 μm (range, 120–450), resting tension/total tension ratio 0.07 ± 0.01, mean maximal activated tension 43.9 ± 8.0 mN/mm^2. Maximal active developed tension is similar to that developed by intact rat papillary muscles, but resting tension is lower. This results partly from the very low Ca^{2+} concentration of the relaxing solution (10^{-9} M).

The pCa–tension curves were determined in six fibers with each agent used in random order. Figure 1 illustrates the changes in tension observed with increasing Ca^{2+} concentration with solutions free of anesthetic (left panel) and with solutions previously equilibrated with 2% halothane (right panel). At the end of each pCa–tension curve obtained during anesthetic exposure, the fibers were again exposed to the control activating solution (B) (fig. 1, right panel), as maximal activated force decreased regularly during the study and represented 65% of the initial developed force at the end of the overall study. No significant differences for pCa_{50} and slope values were observed between the pCa–tension curves obtained at the beginning and at the end of the study, whereas at least 4 h elapsed between these two determinations (table 1). Tension changes after Ca changes were plotted, as shown in figure 2. Tensions obtained during anesthetic exposure were expressed first as a percentage of the maximal tension in the control B solution (relative plot) and were then normalized to their maximal value in the presence of anesthetics (normalized plot). Figure 2 illustrates the resulting plots of tension at each pCa during exposure to 2% halothane; both relative and normalized plots are represented. During anesthetic exposure, maximal activated tension decreased in most experiments when compared with maximal activated tension obtained with the control B solution at the same time (see below). However, the shift toward higher Ca^{2+} concentrations observed persisted when the curves were normalized for the same maximal activated tension. With increasing anesthetic concentration, a shift toward higher Ca^{2+} concentrations was observed. Figure 3 illustrates a typical family of curves obtained with increasing halothane concentration in the same fiber. Panel A shows the experimental points and the computed curves; maximal Ca^{2+}-activated tensions decreased in a dose-dependent fashion with increasing halothane concentration (compared with maximal Ca^{2+}-activated tension obtained at exactly the same time in the anesthetic-free solution). Panel B shows the dose-dependent shift to the right, which remains obvious even after the curves had been normalized for tension. The three anesthetics significantly shifted the pCa to tension curves to the right, as attested to by the significant decrease in pCa_{50} values with increasing anesthetic concentration ($P < 0.001$ for each agent), with no significant change in the Hill coefficient (table 1). This effect on Ca^{2+} sensitivity was dose dependent and immediately reversible as attested to by the absence of changes in both pCa_{50} and Hill coef-

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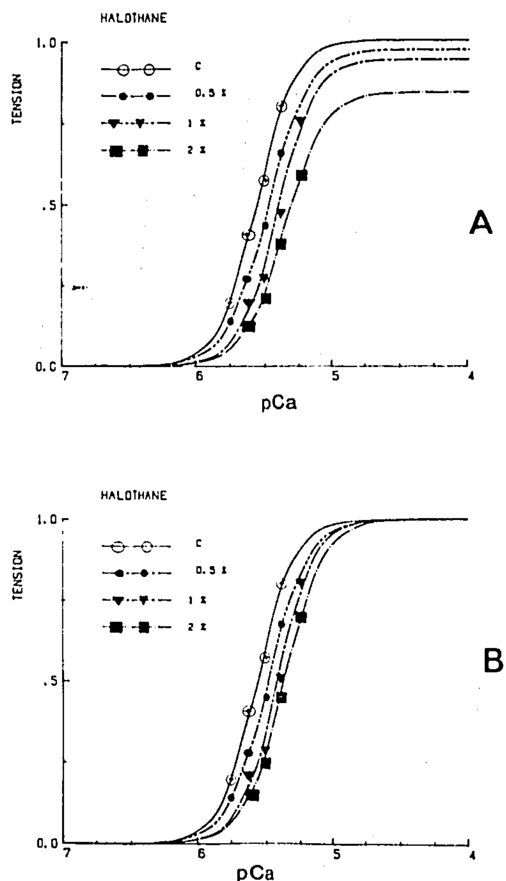

FIG. 3. Family of pCa/tension curves obtained in the same fiber in control conditions (C) and with increasing halothane concentrations (0.5, 1, and 2%). *A.* Experimental points and computed curves. Maximal developed tension decreased in a dose-dependent fashion (for these curves 100% represents maximal activation in anesthetic-free solution obtained at the same time). The curves are shifted in a dose-dependent fashion to the right. *B.* Same curves as in *A* but normalized to maximal tension in control B solution. The shift to the right remains obvious with increasing anesthetic concentrations; pCa_{50} decreases in a dose-dependent fashion, but the slope of each curve (Hill coefficient) remains unchanged. This indicates a decrease in the apparent Ca^{2+} sensitivity of the contractile proteins with increasing halothane concentration.

ficient of the control curves obtained at the end of study for each fiber (table 1). No differences between agents were observed at equivalent MAC multiples ($P > 0.10$). A lack of effect of volatile anesthetics on Ca^{2+} binding or on the affinity of EGTA for Ca^{2+} was verified by an Orion Ca^{2+} electrode. Similar Ca^{2+} activities were measured either in control solutions or in solutions exposed to 2% halothane for various pCa values ranging from 5.75 to 4.5.

The effects of anesthetics on maximal activated tension were determined in eight fibers. Maximal activated tension decreased significantly with increasing anesthetic concentration ($P < 0.001$ for each of the three agents) when compared with that obtained by immersing the muscle in the control B solution at the same time (table 2 and fig. 4). This effect was also immediately reversible, as shown in figure 4. No differences between agents were found at each MAC multiple.

The effects of halothane, enflurane, and isoflurane on half-maximal activated tension were determined in seven fibers. A pCa–tension curve was first established before anesthetic exposure to determine the pCa value corresponding to half-maximal activation. Fibers were equilibrated in solutions with that pCa_{50} and subsequently with various concentrations of each anesthetic in a random order. Half-maximal activated tension decreased significantly in a dose-dependent fashion with increasing concentrations of each anesthetic ($P < 0.001$ for each of the three agents) (table 2 and fig. 4). The effects of anesthetics on half-maximum activated tension were greater than those on maximal activated tension, as this is also the consequence of decreased calcium sensitivity. This effect was immediately reversible when switching from anesthetic-equilibrated solution to control solution of identical pCa. No significant differences between agents were observed at equivalent MAC multiples.

In additional experiments, the effects of one MAC multiple of halothane, enflurane, and isoflurane were studied at 22° C and 37° C, but only the effects of one anesthetic were examined in the same fiber. During anesthetic exposure, the curves were shifted to the right, the magnitude of this shift being identical at both temperatures when expressed in pCa units. The effects on half-maximal activated tension and maximal activated tension were also identical. Binding constants of the solutions were not corrected for temperature changes in these experiments. The results of these experiments are not included in the data presented in tables 1 and 2.

Discussion

This study clearly demonstrates that halothane, enflurane, and isoflurane decrease both maximal tension and the apparent Ca^{2+} sensitivity of detergent-treated cardiac rat fibers in a dose-dependent and reversible fashion. These changes are directly related to the effects of anesthetics because we have ruled out anesthetic effects on the affinity of EGTA for Ca^{2+}, as it was also previously reported by Merin *et al.*[9]

The preparation used in the present work, *i.e.*, detergent-treated skinned fibers, allowed us to study the direct effects of volatile anesthetics on cardiac contractile proteins. There is sufficient evidence that ultrastructural myofibrillar integrity is well preserved in such preparations and that all functional membranes, including transverse tubules, sarcoplasmic reticulum, and mitochondria, are totally destroyed.[17,18] Chemically skinned fibers were also demonstrated to be responsive to changes in substrate

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concentrations (creatine phosphate, ATP, ADP, Mg^{2+}) and other physiologic conditions such as intracellular *p*H, temperature, or sarcomere length.[30–32] However, as for other preparations, the exact state of phosphorylation is not known.

In our study, changes in myocardial Ca^{2+} sensitivity were apparent with anesthetic concentrations used in clinical practice. This differs from previous observations by Merin *et al.*,[9,10] where a decrease in actomyosin ATPase Ca^{2+} sensitivity was only observed for high halothane concentrations. Using a myofibrillar preparation from kitten papillary muscle, Ohnishi *et al.*[11] reported that 1% halothane (about 0.6 mM) produced significant depression in ATPase Ca^{2+} sensitivity. A critical review of these studies was recently published.[1] Among the possible reasons for these discrepancies is that the isolation process used to study myofibrillar ATPase activity may be responsible for such relative "insensitivity" of the preparations.[9,10] The difficulties in obtaining a steady-state equilibrium with volatile anesthetics were also reported. In the more recent study,[12] a significant depression of myofibrillar ATPase Ca^{2+} sensitivity was observed with 5.0 mM halothane and 4.4 mM isoflurane, but, surprisingly, enflurane had no effect on ATPase sensitivity even at a high concentration (4.4 mM).

Our results differ from those previously reported by Su *et al.* in mechanically disrupted cardiac fibers from rabbits.[6,13,14] They observed a decreased myocardial Ca^{2+} sensitivity only for high halothane concentrations (3% and 4%), but they could not demonstrate any effect with either isoflurane (1–3%) or enflurane (2.5–7.5%). Some differences in experimental conditions may help to explain these differences. First, as stated before, the exact role of residual membranes, including subsarcolemnal terminal cisterns, sarcoplasmic reticulum, and mitochondria, is not precisely known in these preparations, and it depends widely on experimental conditions and the method used to obtain skinned fibers.[33] Even with high EGTA concentrations, the effects of anesthetics on mitochondria may modify the overall response to anesthetics.[34] Second, the effects of the three anesthetics were not examined in the same fiber, and the pCa–tension curves were obtained by averaging the results of tension changes at different pCa values obtained in different preparations. Because calcium sensitivity may slightly differ within different fibers (see standard errors in control conditions on table 1), the effect of anesthetics may be not apparent because of these individual variations. The changes expressed in pCa units are small enough to be blunted if the same preparation is not used. Similar temperatures were used in these studies and in our study. The reasons for choosing this unphysiologic temperature were explained in the "Methods" section. At 37° C, the three anesthetics also caused an apparent decrease in Ca^{2+} sensitivity. A quantitative assessment was not done because the same experimental protocol was not carried out and binding constants had not been corrected for temperature changes. The MAC multiples used to compare the effects of anesthetics were obtained at 37° C.[26,27] As MAC values decrease with decreasing body temperature, this may lead to overestimates of the effects of anesthetics on apparent Ca^{2+} sensitivity. However, the results of our chromatographic measurements are close to anesthetic concentrations measured in blood in clinical conditions.[29]

TABLE 2. Mean (±SEM) Changes in Tension at Half Maximal Activation and Maximal Activation, Expressed as Percentage of the Immediately Preceding Control

Agent	MAC	Half-activated Tension % Decrease (n = 7)	Maximal Activated Tension % Decrease (n = 8)
Halothane	0.5		4.8 ± 1.3
	1	20.5 ± 6.0	8.1 ± 2.1
	2	33.5 ± 5.5	15.8 ± 2.3
	3	46.5 ± 7.0	20.5 ± 3.1
Isoflurane	0.5		4.2 ± 1.7
	1	20.2 ± 5.3	8.1 ± 2.8
	2	27.3 ± 4.6	14.2 ± 2.4
	3	33.4 ± 4.7	21.3 ± 2.1
Enflurane	0.5		2.2 ± 1.2
	1	15.4 ± 6.2	8.8 ± 2.3
	2	20.5 ± 5.8	13.2 ± 2.7

All values differed significantly from controls.

The pCa–tension relationships are thought to reflect Ca^{2+} binding to sites on troponin C, whereas Hill coeffi-

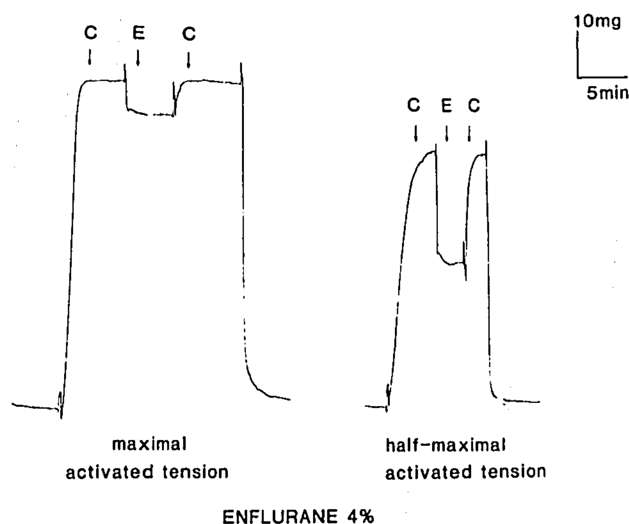

FIG. 4. Changes in maximal activated tension and half-maximal activated tension during exposure to 4% enflurane obtained in the same fiber. Each anesthetic exposure (E) was bracketed by two controls (C) with the use of solutions of exactly identical composition but free of anesthetic. For this fiber, calculated pCa_{50} during control condition was 5.672, and the pCa of the solution used for half-maximal activation was 5.625.

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cients have no simple relation to the number of Ca^{2+} binding sites and cannot be described simply by the binding properties of cardiac troponin C.[35] The changes in pCa_{50} values without changes in Hill coefficients as observed in our study are consistent with an apparent decrease in Ca^{2+} sensitivity of the contractile proteins. These results may partly explain the reversal of negative inotropic effects of halothane with increasing Ca^{2+} in the bathing medium observed in isolated papillary muscle.[36] This decrease in Ca^{2+} sensitivity may reflect a faster rate of release of Ca^{2+} by troponin upon withdrawal of Ca^{2+}, and this observation is consistent with the abbreviated time course of isometric relaxation in isolated ferret papillary muscle during exposure to halothane, isoflurane, and enflurane.[15,16] The exact cellular mechanism by which volatile anesthetics decrease Ca^{2+} sensitivity of the contractile proteins is not known. A direct interaction with the tropomyosin–troponin complex is probably involved, but the precise site of action of volatile anesthetics, if any, remains to be determined. A recently published abstract suggests that halothane does not modify calcium binding by troponin C.[37] More studies are needed to define more precisely the subcellular locus on contractile proteins involved.

In addition to their effects on Ca^{2+} sensitivity, the volatile anesthetics also decreased maximal activated force. This may reflect a decrease in the number of cross-bridges attached at any one moment or a decrease in the amount of force a cross-bridge is able to generate. Halothane was demonstrated to decrease maximal ATPase activity in a dose-dependent and reversible fashion[9,10] but only at high anesthetic concentrations. This effect was antagonized by very high Ca^{2+} concentrations (10^{-3} to 10^{-2} M). The present study did not investigate the effects of volatile anesthetics on ATPase activity, but there is no simple correlation between active developed tension and maximal ATPase activity.[30,35,38–40] Indeed, it was demonstrated that a decreased ATPase activity, as produced by a decrease in substrate concentration (MgATP), can produce an increase in maximal tension of skinned fibers.[25,31]

The changes in half-maximum activated tension are interesting because they are occurring at Ca^{2+} concentrations similar to those observed during normal contraction.[24] Intracellular free calcium increases from 10^{-7} to 10^{-5} M during normal activation. Thus, the decreased Ca^{2+} sensitivity as observed in our study, as well as the decrease in maximal activated tension, may participate in the negative inotropic effects of these agents. The relative importance of such effects compared with the other mechanisms cannot be assessed from this study, however. Nevertheless, our data suggest that any decrease in the rise in activator Ca caused by the volatile anesthetics[3–5] would be magnified by the decreased Ca sensitivity of the contractile proteins.

Another interesting conclusion of the present study is that volatile anesthetics directly interfere with protein systems. Traditionally, volatile anesthetics have been thought to act by perturbing the lipid bilayer portion of biologic membranes.[41] The principal argument for this theory comes from the observation of Meyer and Overton that the potency of a general anesthetic increases in proportion to its lipid solubility.[42] There is some evidence, however, that volatile anesthetics and especially halothane might also act directly on protein molecules.[43,44] This was demonstrated with the use of the firefly soluble protein luciferase and other proteins like hemoglobin or acetylcholine receptors.[45–47] The direct effect of volatile anesthetics on chemically skinned fibers is another argument that reinforces the possibility of multiple sites of action of volatile anesthetics.

In conclusion, this study demonstrates that clinical anesthetic concentrations of halothane, enflurane, and isoflurane decrease both maximal Ca^{2+}-activated tension and apparent myocardial Ca^{2+} sensitivity in a dose-dependent and reversible fashion. This effect may participate in the overall direct negative inotropic effect of these agents. These results help to explain the changes of myocardial relaxation observed in isolated papillary muscle with increasing anesthetic doses.[16]

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