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Isoform-dependent Effects of Halothane in Human Skinned Striated Fibers

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Background: **Reports of the effects of halothane on isoform contractile proteins of striated muscles are conflicting. To determine whether halothane affects cardiac and skeletal contractile proteins differently, the authors examined the effects of two doses of halothane (0.44 and 1.26 mM, equivalent to 0.75 and 2.25 vol%, respectively) on the Ca^{++} sensitivity and maximal force in human skinned cardiac, type I (slow twitch), and type II (fast twitch) skeletal muscle fibers.**

Methods: **Left ventricular muscle strips and skeletal muscle biopsy specimens were obtained from eight and ten patients undergoing cardiac and orthopedic surgery, respectively. Sarcolemma and sarcoplasmic reticulum were destroyed with ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid plus Brij 58. Ca^{++} sensitivity was studied by observing the isometric tension developed by skinned fibers challenged with increasing concentrations of Ca^{++}. Muscle fiber type was determined in each skeletal fiber by the difference in strontium-induced tension measurements.**

Results: **Halothane shifted the Ca^{++} tension curves toward higher Ca^{++} concentrations and increased the Ca^{++} concentration for half-maximal activation in both cardiac and type I skeletal muscle fibers (from 1.96 μM and 1.06 μM under control conditions to 2.92 μM and 1.71 μM in presence of 0.75 vol% halothane, respectively) without changing the slope of this relationship (Hill coefficient). In contrast, no significant effect was observed in type II fibers. Halothane also decreased the maximal activated tension in the three groups of fibers with a lesser effect in type II fibers.**

Conclusions: **Halothane decreases Ca^{++} sensitivity and maximal force in human skinned cardiac and type I fibers at 20°C. It is concluded that the negative inotropic effects of halothane depend on contractile proteins isoforms. (Key words: Anesthetics, volatile: halothane. Muscle, cardiac: skinned fibers. Muscle, skeletal: skinned fibers. Proteins, contractile: calcium sensitivity; isoforms; maximal force.)**

THE negative inotropic effect of halothane on cardiac muscle[1] usually is not observed on skeletal muscle.[2] Moreover, several studies have demonstrated a moderate positive inotropic effect in directly stimulated skeletal muscle.[3,4] The mechanisms underlying these different effects are not fully understood. Potential targets of halothane in both muscles are the structures involved in excitation-contraction coupling. To date, several pieces of evidence suggest that the effects of halothane on sarcolemma and sarcoplasmic reticulum may lead to a decrease of the amount of Ca^{++} available for contractile activation in cardiac but not skeletal muscle.[4–9] In addition, halothane has been shown to decrease Ca^{++} sensitivity and maximal force of the contractile proteins in cardiac muscle.[10,11] In skeletal muscle, the effect of halothane on the responsiveness of the contractile proteins to activation by Ca^{++} dependent on the fiber type remains to be determined. In fact, no data are available on the effects of halothane on different isoforms of human contractile proteins.

Cardiac and skeletal muscles are very similar with respect to sarcomere organization, contractile protein structure and function, and gene organization.[12] For example, type I skeletal muscle contains a similar isoform of troponin C as cardiac muscle.[13] In large mammals, including humans, the low adenosine triphosphatase (V3) isomyosin heavy chain is the predominant form in the ventricles and exhibits close similarities and comparable kinetics with that of slow skeletal isomyosin[12] (whereas the fast skeletal isomyosin is characterized by a higher adenosine triphosphatase activity, with a heavy chain different from the other myosin heavy chains). In contrast, striated muscle actin exists as two specific isoforms: α-cardiac and α-skeletal.[12]

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In the current study, we used the skinned fiber technique to investigate the effect of halothane on the interactions of Ca^{++} with human contractile proteins in three types of fiber preparations: type I (slow twitch, slow oxidative), type II (fast twitch, fast oxidative), skeletal fibers of vastus lateralis muscle, and left ventricular muscle. In our procedure, using both EGTA (ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) and Brij 58 detergent, all membranes are chemically destroyed and both sarcoplasmic reticulum (SR; including T tubules) and mitochondria are nonfunctional.[14] We hypothesized that if the action of halothane on contractile proteins of striated muscle was isoform-dependent, differences between various types of fibers should be observed, and could explain, in part, the different effects of halothane on cardiac and skeletal muscles.

Materials and Methods

The study protocol was approved by our University Studies Ethics Committee, and written informed consent was obtained from all patients before participating in the study. Left ventricular muscle strips were dissected from the endocardial surface from eight patients undergoing cardiac surgery for valvular heart disease. Left ventricular function, as assessed by cardiac catheterization (left ventricular size, wall thickness, ejection fraction, cardiac output) was within the normal range in all patients. The myocardial fragments removed were immediately placed in cardioplegic solution at room temperature and rapidly transported to the laboratory. Skeletal biopsy specimens were taken from the vastus lateralis muscle from ten patients undergoing elective surgery of the lower limbs. None of the patients were confined to bed before surgery. Bundles containing several hundred fibers were attached at their extremities to a wooden stick to maintain their resting length and immediately placed in a relaxing solution at room temperature and rapidly transported to the laboratory.

Skinned Fiber Preparation

Chemically skinned cardiac and skeletal fibers were prepared as previously described.[11,15] Chemical skinning with EGTA renders the muscle fiber sarcolemma freely permeable to external solutes. Segments of cardiac and skeletal muscle, containing several hundred fibers, were dissected free and immediately placed in a relaxing solution at 4°C for 24 h. The skinning solution was replaced with fresh solution after 1, 4, and 12 h. After 24 h, the segments were transferred to a skinning storage solution that was identical to the relaxing solution except for the addition of 50% glycerol and stored at −20°C until used (1 or 2 weeks). This technique is identical to that used by a number of other laboratories.[16,17] No change in skinned fiber properties could be noticed after 2 or 3 weeks of storage.

Bundles of cardiac muscle (which will be called fibers hereafter) or single skeletal fibers were isolated from the main fascicle under a 40-power Swift Model 31-400-00 binocular microscope (Tokyo, Japan). Each cardiac or skeletal fiber was mounted horizontally between two clamps in a muscle bath (0.8 ml) filled with a relaxing solution. One clamp was attached to a Grass Model FT-03 force displacement transducer (Quincy, MA). The muscle contracture was amplified and recorded on a Gould 2200 S device (Valley View, OH). The preparation was bathed for 15–20 min in a relaxing solution containing the nonionic detergent Brij 58 (2%), which irreversibly eliminates the capability of the SR to sequester Ca^{++} and to release it under appropriate stimulation, but does not affect the contractile proteins.[14] The length and diameter of the preparations were measured under a 400-power Olympus lens. Each skeletal fiber was straightened by adjusting the position of the transducer, and the resting tension was then applied by stretching the fiber by 20% of its initial length. For cardiac fibers, the initial straightening was made to a length at which an increase in resting tension was first detected, and the preparation was then stretched a further 20% of the initial length of the bundle, as previously described by Maughan *et al.*[18] Finally, the functional destruction of SR was confirmed by studying Ca^{++} release from SR with 40 mM caffeine after loading the SR with a known concentration of Ca^{++} (pCa 6.8) in the presence of adenosine triphosphate. Only preparations with no significant contracture to caffeine (*i.e.*, no functional SR) were included in the study.

For all experiments described, the length of the fibers was kept constant to avoid sarcomere length-dependent changes in Ca^{++} sensitivity. All experiments were performed at room temperature (20 ± 1°C).

Solutions and Vapor Anesthetics

The concentrations of the different components in the solutions were calculated using program 3 of Fabiato and Fabiato[19] to keep the ionic strength at 200 mM. The stability constants of Orentlicher *et al.*[14] were used in the calculations: K_{CaEGTA} 1.919×10^6/M, K_{CaATP}

5.0×10^3/M, K_{MgEGTA} 40/M, and K_{MgATP} 1.0×10^4/M. Composition of solutions has been previously published.[11,15]

To assess the effects of halothane, the test solutions were equilibrated by continuous bubbling with the anesthetic agent for 20 min. Halothane was mixed with 100% nitrogen by means of a calibrated vaporizer (Fluotec Mark III, Cyprane Keighley). The anesthetic concentrations in the gas phase were monitored with an infrared calibrated analyzer (Capnomac, Datex, Finland). The anesthetic concentrations used were 0.75 and 2.25 vol% halothane. These concentrations in gas phase are roughly equivalent to 1 and 3 minimum alveolar concentration multiples of halothane in humans at 37°C. The anesthetic concentrations obtained in the experimental chamber were measured by gas-liquid chromatography to determine the amount of anesthetic present in the solutions. A Varian 1400 gas chromatograph equipped with flame ionization detector and a Porapack Q 3.17 mm by 150 cm column (Palo Alto, CA) was used for determination of anesthetic concentrations.[20] A 60-ml flask containing 100 μl of the solution equilibrated for 20 min with the anesthetic was maintained at 60°C (above the boiling point for halothane) for 20 min before injecting 1 ml gas into the apparatus, previously calibrated with known concentrations of halothane (head space technique). The anesthetic concentrations measured in the experimental solution after 20 min of continuous bubbling were as follows: 0.44 ± 0.03 and 1.26 ± 0.08 mM for 0.75 and 2.25 vol% halothane (concentrations in the gas phase given by the calibrated analyzer), respectively.

Experimental Procedure

For each skinned cardiac or skeletal fiber, a pCa-tension curve was obtained under control conditions by stepwise exposure of the preparation to solutions with increasing Ca^{++} concentrations and measurements of developed tension (fig. 1). Ca^{++} concentrations ranged from pCa 6.4 ($[Ca^{++}] = 0.3$ μM) to pCa 4.8 ($[Ca^{++}] = 15.8$ μM) where $pCa = -\log_{10}[Ca^{++}]$. Intermediate tensions were expressed as a percentage of the maximal tension. Data were fitted using nonlinear regression analysis (Enzfitter, Elsevier Biosoft, Cambridge, UK) to the modified form of Hill's equation: $[Ca^{++}]^{nH}/(K_{50}{}^{nH}+[Ca^{++}]^{nH})$, where F is the relative tension, nH (Hill coefficient) is a measure of the slope of the relationship, and K_{50} is the Ca^{++} concentration (expressed in μM) that yields 50% of the maximal Ca^{++}-activated force.

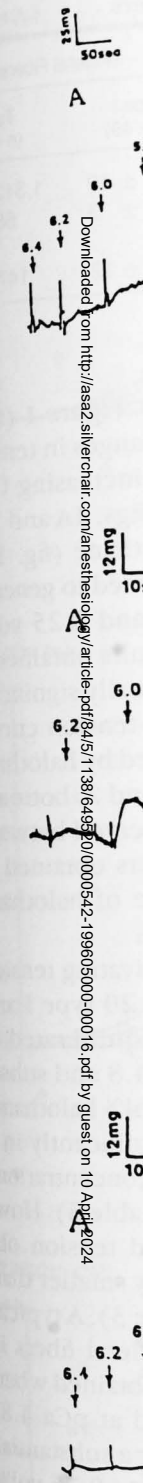
Under all experimental conditions, halothane was tested at 0.75 and 2.25 vol% on the same cardiac fibers. Hence, pCa-tension curves were obtained for the two concentrations studied in a random order in each fiber. A final pCa-tension curve was obtained with solutions free of anesthetic. Because maximal activated tension decreased regularly during the study and represented roughly 80–85% of the initial developed force at the end of the overall experiment, tension values were normalized to their maximal value at each anesthetic concentration, and then plotted to allow analysis of the sensitivity of the preparations to Ca^{++} in the presence of 0.75 and 2.25 vol% halothane. The mean values of the two control curves were used to assess the effects of halothane. To minimize the decline in maximal tension at <15%, the effects of each concentration of halothane on skeletal contractile proteins were studied in different fibers. Hence, after determination of the first control curve, each skinned fiber was exposed to 0.75 or 2.25 vol% halothane, and finally, the experiment was completed with a second control curve.

In a second series of experiments, changes of tension at maximal Ca^{++}-activated force were examined using a pCa 4.8 solution. Each fiber was exposed to test solutions equilibrated with 0.75 or 2.25 vol% halothane. Each test was immediately preceded and followed by determination of maximal Ca^{++}-activated tension with the control test solution (*i.e.*, free of anesthetic) so that no significant differences between controls were observed. Isometric tension development from baseline to steady state was compared between test solutions and the mean of the two control measurements. Results were expressed as a percentage of these corresponding control values.

In skeletal fibers, a final series of experiments examined changes of tension at half-maximal activation using solutions with concentrations of Ca^{++} that were close to the calculated K_{50}, obtained at the beginning of the study. Each fiber was exposed to test solution equilibrated with 0.75 vol% halothane. Each test was bracketed by determination of half-maximal activated tension with the control solution, free of halothane. No significant difference between controls was observed. Results were expressed as percentages of these corresponding control values.

Muscle Fiber Typing

All skinned skeletal fibers were tested with increasing concentrations of Sr^{++} as described by Takagi *et al.*[21] and validated in our laboratory.[15] For each fiber, a pSr-



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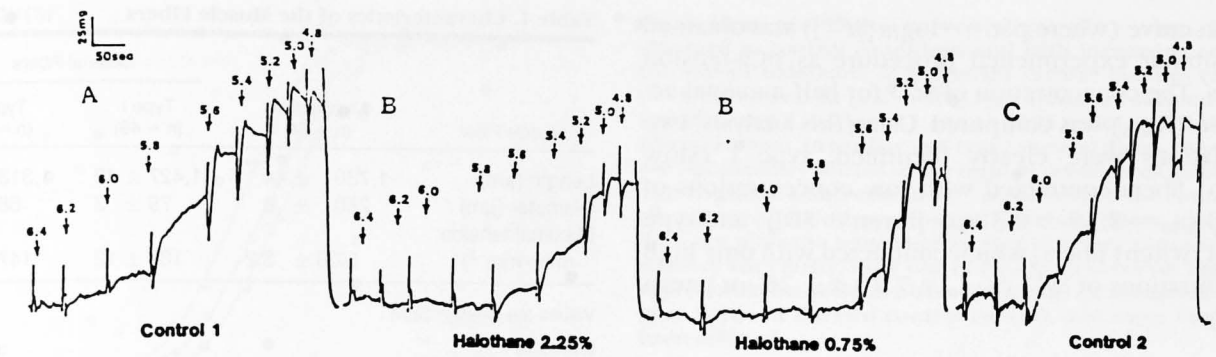
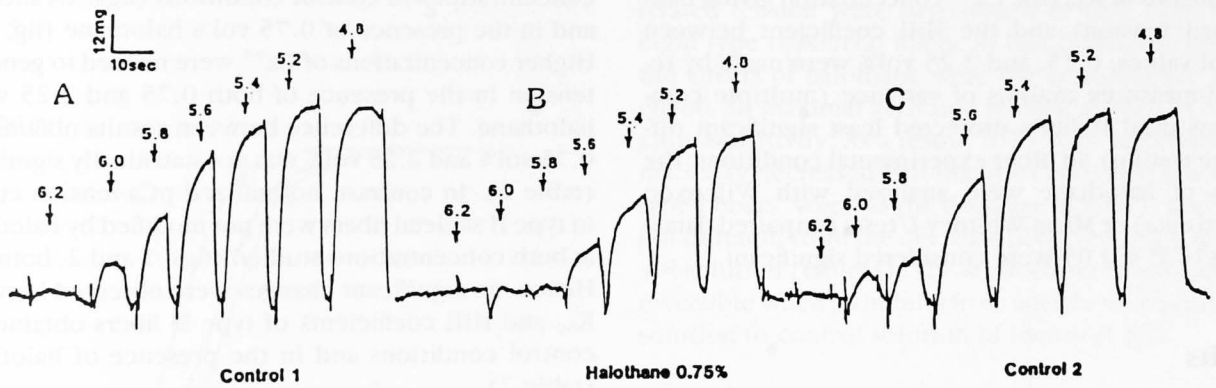




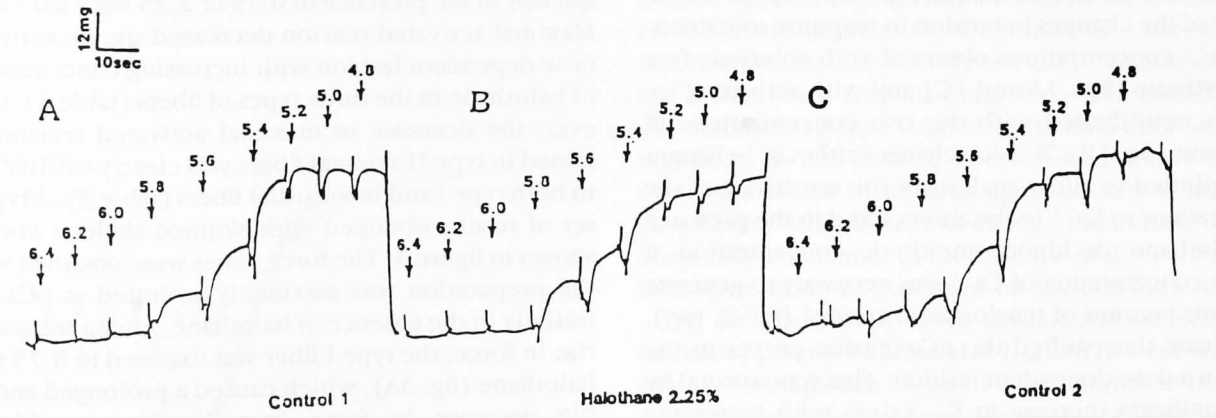


Fig. 1. Changes in tension related to increasing Ca^{++} concentration, expressed as pCa (where $pCa = -\log_{10}[Ca^{++}]$), obtained in a skinned cardiac bundle (*top*), a skinned type I skeletal fiber (*middle*), and a skinned type II skeletal fiber (*bottom*). (*A*) Changes in tension obtained in control solutions (in the absence of halothane). (*B*) Changes in tension obtained in the presence of halothane: in cardiac and type I skeletal fibers, 0.75 vol% halothane clearly shifts the threshold of Ca^{++} concentration to the right, whereas 2.25 vol% halothane does not exhibit any significant effect in type II skeletal fibers. (*C*) Changes in tension obtained in control solutions after anesthetic exposure (postanesthetic control). Thirty (cardiac bundles) and twenty (skeletal fibers) minutes elapsed between each experimental condition. Arrows = changes in Ca^{++} solutions.

tension curve (where $pSr = -\log_{10}[Sr^{++}]$) was obtained in a similar experimental procedure as pCa-tension curves. The concentration of Sr^{++} for half-maximal activation (K_{Sr50}) was computed. Using this analysis, two populations were clearly identified: type I (slow twitch) fibers contracted with low concentrations of Sr^{++} (K_{Sr50} = 1.35 ± 0.30 μM [mean ± SD]), and type II (fast twitch) fibers, which contracted with only high concentrations of Sr^{++} (K_{Sr50} = 7.20 ± 1.25 μM [mean ± SD]).

Statistical Analysis

Results were expressed as mean ± standard error of the mean. For pCa-tension curves in myocardial fibers, comparisons of K_{50} (the Ca^{++} concentration giving half-maximal tension) and the Hill coefficient between control values, 0.75, and 2.25 vol% were made by repeated-measures analysis of variance (multiple comparisons used Fisher's protected least significant difference testing). In other experimental conditions, the effects of halothane were analyzed with Wilcoxon (paired data) or Mann Whitney *U* tests (unpaired data). Values of $P < 0.05$ were considered significant.

Results

Characteristics of the muscle fibers for myocardium, type I and type II skeletal muscle are shown in table 1. The pCa-tension curves were determined in eight myocardial fibers with 0.75 and 2.25 vol% halothane used in a random order. Figure 1 (top) shows an example of the changes in tension in response to increasing Ca^{++} concentrations observed with solutions free of anesthetic (figs. 1A and 1C) and with activating solutions equilibrated with the two concentrations of halothane (fig. 1B). Tension changes after Ca^{++} changes were plotted to allow analysis of the sensitivity of the preparations to Ca^{++} in the absence and in the presence of halothane. At higher anesthetic concentration, a higher concentration of Ca^{++} was necessary to generate the same amount of tension as in control (fig. 2, top). Halothane thus shifted the pCa-tension curves to the right in a dose-dependent fashion. This was attested by the significant increase in K_{50} values with increasing halothane concentrations with no significant change in the Hill coefficient (table 2).

The pCa-tension curves in skeletal fibers were determined using muscle biopsy specimens obtained from ten patients. The effects of halothane on Ca^{++} sensitivity

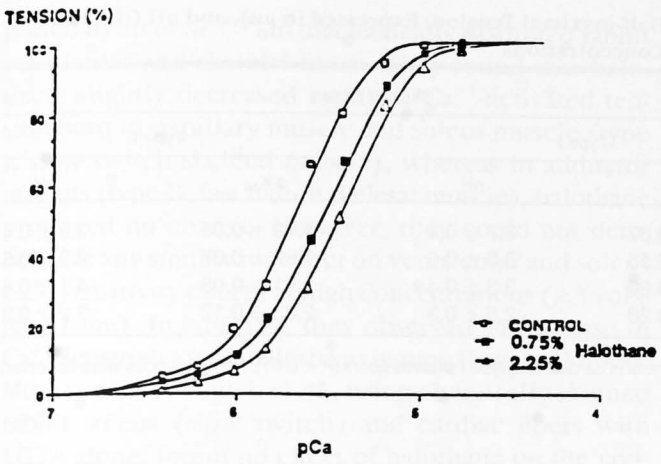
Table 1. Characteristics of the Muscle Fibers

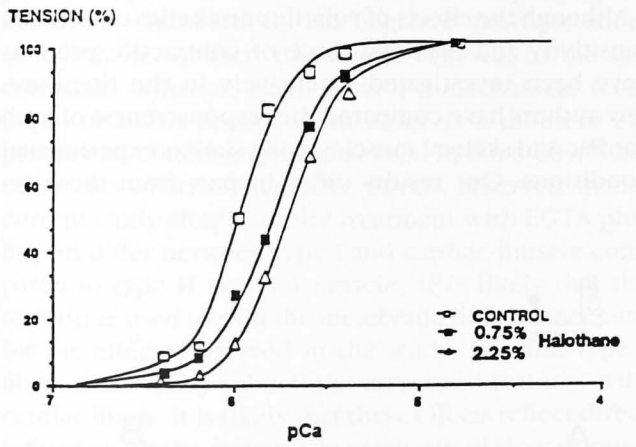
Muscle Fiber	Myocardium (n = 24)	Skeletal Fibers	
		Type I (n = 49)	Type II (n = 44)
Length (μm)	1,730 ± 44	1,427 ± 42	1,313 ± 29
Diameter (μm)	250 ± 6	79 ± 3	68 ± 3
Maximal tension ($mN \cdot mm^{-2}$)	52.8 ± 3.2	187 ± 12	147 ± 12

Values are mean ± SEM.

were dependent on muscle fiber types. Figure 1 (middle) shows a typical example of the changes in tension of type I skeletal fiber in response to increasing Ca^{++} concentrations in control conditions (figs. 1A and 1C) and in the presence of 0.75 vol% halothane (fig. 1B). Higher concentrations of Ca^{++} were needed to generate tension in the presence of both 0.75 and 2.25 vol.% halothane. The difference between results obtained at 0.75 vol% and 2.25 vol% was not statistically significant (table 2). In contrast, normalized pCa-tension curves in type II skeletal fibers were not modified by halothane at both concentrations studied (figs. 1 and 2, bottom). Hence, no significant changes were observed between K_{50} and Hill coefficients of type II fibers obtained in control conditions and in the presence of halothane (table 2).

The effect of halothane on maximal activating tension was determined in 16 cardiac bundles, 20 type I and 18 type II skeletal fibers. Fibers were equilibrated in a maximally activating solution at pCa 4.8 and subsequently in the presence of 0.75 or 2.25 vol% halothane. Maximal activated tension decreased significantly in a dose-dependent fashion with increasing concentrations of halothane in the three types of fibers (table 3). However, the decrease in maximal activated tension obtained in type II skeletal fibers was clearly smaller than in both type I and myocardial fibers (table 3). A typical set of results obtained with skinned skeletal fibers is shown in figure 3. The force traces were obtained when the preparation was maximally activated at pCa 4.8, initially in the absence of halothane. After a substantial rise in force, the type I fiber was exposed to 0.75 vol% halothane (fig. 3A), which caused a prolonged and stable decrease in force immediately reversible on switching from halothane-equilibrated solution to control solution of identical pCa. In contrast, the same experiment performed with 2.25 vol% halothane caused only a moderate decrease in tension of type II fibers (fig. 3B).





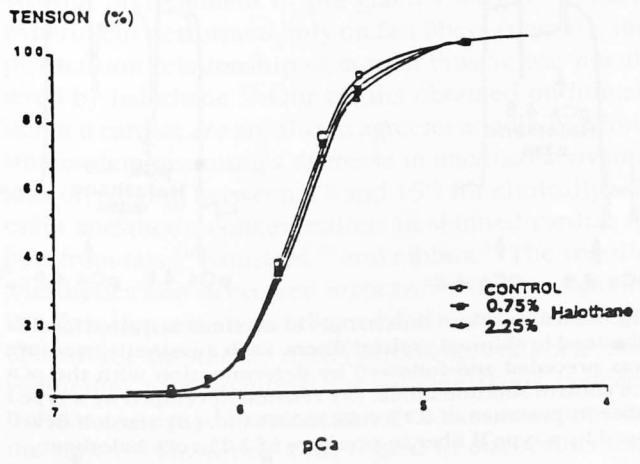


Fig. 2. Mean pCa-tension curves (where pCa = $-\log_{10}$ [Ca^{++}]) obtained in control conditions and with increasing concentrations of halothane in 8 skinned cardiac bundles (*top*), 8 (0.75 vol%), and 12 (2.25 vol%) type I skinned skeletal fibers (*middle*), and 7 (0.75 vol%), and 11 (2.25 vol%) type II skeletal fibers (*bottom*). In cardiac and type I skeletal fibers, the curves are significantly shifted to the right in presence of halothane. This indicates a decrease in Ca^{++} sensitivity of the contractile proteins with halothane. In type II skeletal fibers, halothane does not alter the tension-pCa curve at either concentration studied. For clarity, only one of two control curves has been represented for skeletal fibers (there was no statistical difference between the two control curves), and error bars have been omitted.

Finally, the effects of halothane on half-maximal activated tension were determined in nine type I, and eight type II skeletal fibers. At half-maximum tension, the effects of halothane were the consequence of the effects on maximal activated tension as well as on calcium sensitivity. As a result, 0.75 vol% halothane dramatically decreased half-maximal activated tension in type I fibers, whereas the decrease in type II fibers was not different from the decrease observed using the pCa 4.8 solution (table 3). These effects were immediately reversible when switching from anesthetic-equilibrated solution to control solution of identical pCa.

Discussion

We have used three human skinned fiber systems to investigate the possible role of cardiac and skeletal muscle-specific myofibrils isoforms in the mechanism of halothane depression of contractility. Our results demonstrate that the volatile anesthetic decreases both Ca^{++} sensitivity and maximal force in human slow (ventricular and type I skeletal) skinned fibers and has no effects in fast skinned fibers. Because most of the skeletal muscles in humans are mixtures of fast and slow fibers,[22] the absence of effects of halothane on fast fibers may contribute to the absence of direct negative inotropic action of this agent on skeletal muscle.

The Ca^{++} sensitivity of the cardiac and skeletal contractile apparatus expressed as K_{50} and Hill coefficients closely overlaps that found by other studies in humans using saponin pretreatment of trabeculae from normal heart[23] and EGTA + Brij skinned fibers obtained from lateral gastrocnemius muscle.[16] Type I fibers had a lower Ca^{++} threshold for tension development than type II fibers, and both had steeper calcium-tension relationships than myocardial fibers. Our technique

Table 2. Mean ± SEM Values for K_{50} (the Ca^{++} Concentration for Half-maximal Tension, Expressed in μM), and nH (the Hill Coefficient) in Absence of Halothane and at 0.75 and 2.25 vol % Concentrations

	Myocardium		Skeletal Fibers			
			Type I		Type II	
	K_{50}	nH	K_{50}	nH	K_{50}	nH
Control	1.96 ± 0.07	2.2 ± 0.1	1.06 ± 0.07	3.7 ± 0.4	1.70 ± 0.06	3.9 ± 0.4
0.75%	2.92 ± 0.09	2.2 ± 0.2	1.71 ± 0.15	3.3 ± 0.6	1.82 ± 0.07	3.9 ± 0.6
Control	1.96 ± 0.07	2.2 ± 0.1	1.18 ± 0.09	3.3 ± 0.4	1.91 ± 0.08	4.1 ± 0.4
2.25%	3.55 ± 0.12	2.3 ± 0.3	2.17 ± 0.28	2.5 ± 0.3	2.03 ± 0.12	3.2 ± 0.3

Increase in K_{50} values was significant at both concentrations of halothane *versus* control in cardiac and type I skeletal fibers ($P < 0.01$). No differences were observed between Hill coefficients.

characterizes the fiber type of skinned mammalian muscle fibers based on their relative sensitivities to strontium. It was assumed that type I fibers had high sensitivities to strontium, and that type II fibers had low sensitivities to strontium.[21] This method has been validated by correlating it with standard histochemical staining in a large number of muscle fibers.[15]

Our study uses a skinned fiber preparation because it allows rapid application and removal of particular agents, which influences the myoplasmic Ca^{++} concentrations. Such a preparation has been widely used to study contractile apparatus itself.[14,16] The technique uncouples T tubules from SR structures with EGTA and then uses Brij 58 to destroy the SR membranes. However, the skinned fiber techniques were demonstrated to be highly sensitive to experimental conditions such as temperature, intracellular *p*H, or changes in surrounding substrate concentrations.[24] The technique allows the diffusion outside the cell of low molecular-weight proteins, and also may induce inadvertent proteolysis, alterations in myosin light chain phosphorylation, and changes in cross-bridge kinetics.[25] This could partly explain the finding that the myofilament sensitivity to Ca^{++} appears to be lower in skinned preparation than in intact muscle.

Although the effects of volatile anesthetics on calcium sensitivity and maximal force of contractile proteins have been investigated extensively in the literature, few authors have compared the responsiveness of both cardiac and skeletal muscles using similar experimental conditions. Our results differ in part from those re-

Table 3. Changes in Tension at Maximal Activation and at Half-maximal Activation (Mean ± SEM), Expressed as Percentage of Control Values

	Halothane (vol %)	Myocardium	Skeletal Fibers	
			Type I	Type II
Maximal tension	0.75	−17.7 ± 1.04	−13.2 ± 1.8	−3.6 ± 1.74*
	2.25	−35.6 ± 2.3	−20.9 ± 1.8	−6.9 ± 2.52*
Half-maximal tension	0.75	—	−67.3 ± 3.9	−4.3 ± 2.5*

All values are significantly different from control ($P < 0.01$).

* $P < 0.01$: at each halothane concentration, values obtained in type II skeletal fibers are significantly lower than in the other types.

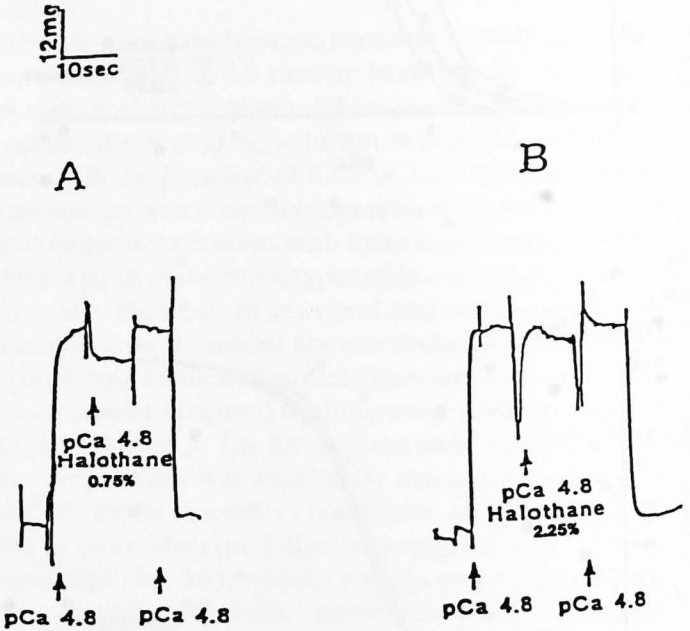


Fig. 3. An example of the changes in maximal activated tension obtained in skinned skeletal fibers. Each anesthetic exposure was preceded and followed by determination with the pCa 4.8 control solution alone. (*A*) Tension developed by a type I fiber in presence of 0.75 vol% halothane. (*B*) Tension developed by a type II fiber in presence of 2.25 vol% halothane.

ported by Su *et al.*[26,27] in mechanically disrupted rabbit ventricular and skeletal fibers. They found that halothane slightly decreased maximal Ca^{++}-activated tension both in papillary muscle and soleus muscle (type I, slow twitch skeletal muscle), whereas in adductor magnus (type II, fast twitch skeletal muscle), halothane produced no change. However, they could not demonstrate any significant effect on ventricular and soleus Ca^{++} sensitivity except at high concentrations (≥3 vol% halothane). In addition, they observed an increase in Ca^{++} sensitivity with halothane in type II skeletal fibers. More recently, Blanck *et al.*, using chemically skinned rabbit soleus (slow twitch) and cardiac fibers with EGTA alone, found no effect of halothane on the contractile apparatus.[17] These authors concluded from their observations on skinned fibers and isolated troponin C that halothane does not alter the affinity of cardiac troponin C for Ca^{++} and that the myofibrils are not an important site for the negative inotropic effect of halothane. In fact, the simple removal of the sarcolemmal diffusion barriers with a mechanical technique or EGTA pretreatment alone is a different experimental condition than that used in our study.[28] Because the volatile anesthetic effects observed in the current study after a similar treatment with EGTA plus Brij 58 differ between type I and cardiac muscle compared to type II skeletal muscle, it is likely that the technique used to skin the membrane does not account for the effects observed in the study. Because type I fibers share many subcellular structural features with cardiac fibers, it is likely that these effects reflect direct influences on the contractile apparatus of slow skinned fibers.

Our results obtained on human type II skinned fibers are consistent with those reported by Ohta *et al.* in saponin pretreatment of pig gracilis muscle. In their experiment performed only on fast fibers (type II), the pCa-tension relationship of normal muscle was not altered by halothane.[29] Our results obtained on human skinned cardiac are are also in agreement with previous studies demonstrating a decrease in maximal activated tension ranging between 5% and 15% for clinically relevant anesthetic concentrations in skinned cardiac fibers from rats,[10] hamsters,[30] and rabbits.[31] The volatile anesthetics also decreased myocardial Ca^{++} sensitivity in a dose-dependent and reversible fashion. The effects of the three anesthetics used (halothane, enflurane, and isoflurane) were identical for equianesthetic concentrations expressed in minimum alveolar concentration multiples.[10] However, with regard to our results and to our previous study on myocardial proteins,[11] the major shift in Ca^{++} sensitivity appears to occur with 0.75 vol%, with very little (although significant) further shift being present with a threefold increase in concentration. This behavior suggests some sort of saturable phenomenon.

In several respects, the conditions of our experiments were different from those encountered in the anesthetized human. To preserve the viability of the preparations, it was necessary to work at a temperature lower than 37C, as is commonly done with skinned preparations. Because there could be temperature-dependent differences (*i.e.*, greater depression at a lower temperature) in the negative inotropic effect of the volatile anesthetics,[32] and because minimum alveolar concentration values decrease with decreasing body temperature,[33] our data may overestimate the effects of anesthetics on Ca^{++} sensitivity.

To date, the exact cellular mechanism by which halothane decreases Ca^{++} sensitivity and maximal force of the contractile proteins is not known. According to previous studies, the effects on Ca^{++} sensitivity may involve the troponin-tropomyosin system[10,27,31] (but not directly tropinin C[17]). Our results suggest that troponin I may not be a direct target of halothane, because cardiac and skeletal (type I and type II) isoforms are clearly different. Alternatively, the decrease in Ca^{++} sensitivity could be directly related to the decrease in maximum Ca^{++}-activated force, as previous studies have shown that myosin cross-bridges binding enhances Ca^{++} binding to tropinin C and may thus play a key role in activation of the thin filament. Combined decreases in maximum tension and Ca^{++} sensitivity also are observed with agents such as 2,3-butanedione monoxime, which affects the biochemical states of the cross-bridges during the working cycle, resulting in a reduction in the number of cross-bridges in a force-generating state.[34,35] With halothane, the decrease in maximal force also could involve processes of attachment and detachment of actomyosin cross-bridges, leading to a decrease in the number of cross-bridges involved in force generation[36] as well as the amount of force developed by individual cross-bridges.[37] Additional studies are needed to define the exact cellular mechanism by which halothane interferes with contractile proteins.

In conclusion, the current study demonstrates that halothane decreases both calcium sensitivity and maximal force of contractile proteins in human cardiac and type I skeletal skinned muscle fibers and has no sig-

nificant effect in type II skeletal skinned muscle fibers. Such significant effects depending on contractile protein isoforms have not been described previously. If these results can be extrapolated to *in vivo* conditions, they may partly explain the difference in the overall inotropic action of halothane between cardiac and skeletal muscle.

References

1. Rusy BF, Komai H: Anesthetic depression of myocardial contractility: A review of possible mechanisms. ANESTHESIOLOGY 1987; 67:745–66

2. Gallant EM, Godt RE, Gronert GA: Mechanical properties of normal and malignant hyperthemia susceptible porcine muscle: Effects of halothane and other drugs. J Pharmacol Exp Ther 1980; 213: 91–6

3. Waud BE, Waud DR: Effects of volatile anesthetics on directly and indirectly stimulated skeletal muscle. ANESTHESIOLOGY 1979; 50: 103–10

4. Williams JH, Holland M, Lee JC, Ward CW, Davy KP: Effects of BAY K 8644, nifedipine, and low Ca^{++} on halothane and caffeine potentiation. J Appl Physiol 1991; 71:721–6

5. Bosnjak ZJ, Kampine JP: Effects of halothane on transmembrane potentials, Ca^{++} transients, and papillary muscle tension in the cat. Am J Physiol 1986; 251:H374–81

6. Blanck TJJ, Peterson CV, Baroody B, Tegazzin V, Lou J: Halothane, enflurane, and isoflurane stimulate calcium leakage from rabbit sarcoplasmic reticulum. ANESTHESIOLOGY 1992; 76:813–21

7. Frazer MJ, Lynch C III: Halothane and isoflurane effects on Ca^{++} fluxes of isolated myocardial sarcoplasmic reticulum. ANESTHESIOLOGY 1992; 77:316–23

8. Connelly TJ, Hayek RE, Rusy BF, Coronado R: Volatile anesthetics selectively alter [^{3}H] ryanodine binding to skeletal and cardiac ryanodine receptors. Biochem Biophys Res Commun 1992; 186:595–600

9. Louis CF, Roghair T, Mickelson JR: Volatile anesthetics inhibit dihydropyridine binding to malignant hyperthermia-susceptible and normal pig skeletal muscle membranes. ANESTHESIOLOGY 1994; 80: 618–24

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

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

12. Swynghedauw B: Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. Physiol Rev 1986; 66:710–71

13. Parmacek MS, Leiden JM: Structure, function, and regulation of troponin C. Circulation 1991; 84:991–1003

14. Orentlicher M, Reuben JP, Grundfest H, Brandt PW: Calcium binding and tension development in detergent-treated muscle fibers. J Gen Physiol 1974; 63:168–86

15. Adnet PJ, Bromberg NL, Haudecoeur G, Krivosic I, Adamantidis MM, Reyford H, Bello N, Krivosic-Horber RM: Fiber-type caffeine sensitivities in skinned muscle fibers from humans susceptible to malignant hyperthermia. ANESTHESIOLOGY 1993; 78:168–77

16. Ruff RL: Calcium sensitivity of fast- and slow-twitch human muscle fibers. Muscle Nerve 1989; 12:32–7

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

18. Maughan D, Low E, Litten R III, Brayden J, Alpert N: Calcium-activated muscle from hypertrophied rabbit hearts. Mechanical and correlated biochemical changes. Circ Res 1979; 44:279–87

19. Fabiato A, Fabiato F: Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (Paris) 1979; 75:463–505

20. Imbenotte M, Erb F, Goldstein P, Erb C, Scherpereel Ph: Halothane and enflurane metabolite elimination during anaesthesia in man. Eur J Anaesth 1987; 4:175–82

21. Takagi A, Yonemoto K, Sugita H: Single-skinned human muscle fibers: Activation by calcium and strontium. Neurology 1978; 28: 497–9

22. Johnson MA, Polgar J, Weightman D, Appleton D: Data on the distribution of fibre types in thirty-six human muscles: An autopsy study. J Neurol Sci 1973; 18:111–29

23. Hajjar RJ, Gwathmey JK, Briggs GM, Morgan JP: Differential effect of DPI 201-106 on the sensitivity of the myofilaments to Ca^{++} in intact and skinned trabeculae from control and myopathic human hearts. J Clin Invest 1988; 82:1578–84

24. Winegrad S: Regulation of cardiac contractile proteins: Correlations between physiology and biochemistry. Circ Res 1984; 55: 565–74

25. Gao WD, Backx PH, Azan-Backx M, Marban E: Myofilament Ca^{++} sensitivity in intact versus skinned rat ventricular muscle. Circ Res 1994; 74:408–15

26. Su JY, Kerrick WGL: Effects of halothane on Ca^{++}-activated tension development in mechanically disrupted rabbit myocardial fibers. Pflügers Arch 1978; 375:111–17

27. Su JY, Bell JG: Intracellular mechanism of action of isoflurane and halothane on striated muscle of the rabbit. Anesth Analg 1986; 65:457–62

28. Herland JS, Julian FJ, Stephenson DG: Effects of halothane, enflurane, and isoflurane on skinned rat myocardium activated by Ca^{++}. Am J Physiol 1993; 264:H224–32

29. Ohta T, Endo M, Nakano T, Morohoshi Y, Wanikawa K, Ohga A: Ca-induced Ca release in malignant hyperthermia-susceptible pig skeletal muscle. Am J Physiol 1989; 256:C358–67

30. Murat I, Veksler VI, ventura-Clapier R: Effects of halothane on cardiac skinned fibers from cardiomyopathic animals. J Mol Cell Cardiol 1989; 21:1293–304

31. Murat I, Hoerter J, ventura-Clapier R: Developmental changes in effects of halothane and isoflurane on contractile properties of rabbit cardiac skinned fibers. ANESTHESIOLOGY 1990; 73:137–45

32. Miao N, Lynch C III: Effects of temperature on volatile anesthetic depression of myocardial contractions. Anesth Analg 1993; 76:366–71

33. Antognini JF: Hypothermia eliminates isoflurane requirements at 20°C. ANESTHESIOLOGY 1993; 78:1152–6

34. Herrmann C, Wray J, Travers F, Barman T: Effects of 2,3-butanedione monoxime on myosin and myofibrillar ATPases. An example of an uncompetitive inhibitor. Biochemistry 1992; 31:12 227–32

35. Backx PH, Gao WD, Azan-Backx MD, Marban E: Mechanism of force inhibition by 2,3-butanedione monoxime in rat cardiac muscle: roles of $[Ca^{2+}]_i$ and cross-bridge kinetics. J Physiol (Lond) 1994; 476:487–500

36. Shibata T, Blanck TJJ, Sagawa K, Hunter W: The effects of halothane, enflurane, and isoflurane on the dynamic stiffness of rabbit papillary muscle. ANESTHESIOLOGY 1989; 70:496–502

37. Murat I, Lechene P, Ventura-Clapier R: Effects of volatile anesthetics on mechanical properties of rat cardiac skinned fibers. ANESTHESIOLOGY 1990; 73:73–81