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# *Halothane and Isoflurane Decrease Calcium Sensitivity and Maximal Force in Human Skinned Cardiac Fibers*

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***Background:*** **Reports of the direct effects of volatile anesthetics on cardiac myofibrils, studied in various mammalian species but not in humans, have conflicted. To determine whether volatile anesthetics directly affect cardiac contractile proteins in humans, we examined the effects of various equianesthetic doses of halothane (0.46, 0.83, and 1.23 mM, equivalent to 0.75, 1.50, and 2.25%, respectively) and isoflurane (0.63, 1.22, and 1.93 mM, equivalent to 1.15, 2.30, and 3.50%, respectively) on the $Ca^{2+}$ sensitivity and maximal force in human skinned cardiac fibers.**

***Methods:*** **Left ventricular muscle strips were obtained from seven patients undergoing cardiac surgery. Sarcolemma was disrupted with EGTA (ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid), and sarcoplasmic reticulum was destroyed with EGTA plus BRIJ 58 detergent. $Ca^{2+}$ sensitivity was studied by observing the isometric tension developed by skinned fiber bundles challenged with solutions of increasing $Ca^{2+}$ concentrations expressed in *p*Ca (where *p*Ca = $-\log10[Ca^{2+}]$). Maximal force was measured with a *p*Ca 4.8 solution.**

***Results:*** **Both anesthetics shifted the *p*Ca–tension curves toward higher $Ca^{2+}$ concentrations and decreased *p*Ca for half-maximal activation in a dose-dependent and reversible fashion (from 5.71 for control to 5.56 and 5.55 for 1 MAC halothane and isoflurane, respectively) without changing the slope of this relationship (Hill coefficient). No differences between agents were observed at equianesthetic concentrations. The two agents also decreased the maximal activated tension in a dose-dependent fashion (−27 and −28% *vs.* control for 2 MAC halothane and isoflurane, respectively).**

***Conclusions:*** **The current study indicates that halothane and isoflurane decrease $Ca^{2+}$ sensitivity and maximal force in human skinned cardiac fibers at 20°C. If these effects extend to higher temperatures, they may contribute to the negative inotropic effect of these agents. (Key words: Anesthetics, volatile: halothane; isoflurane. Heart, contractility: contractile proteins; skinned fibers.)**

VOLATILE anesthetics at clinically useful concentrations depress the contractile force of the heart.[1] These agents produce dose-dependent decreases of indices of contractility in isolated atria and ventricles of various mammalian[2–4] species, in experimental animals,[5] and in humans.[6,7] The mechanisms underlying the negative inotropic effects of the volatile anesthetics are not fully understood. To date, accumulating evidence suggests that the volatile anesthetics act in a number of specific ways, including (1) effects on myocyte sarcolemmal flux of $Ca^{2+}$,[8–11] (2) alterations in $Ca^{2+}$ handling by the sarcoplasmic reticulum (SR),[12] and (3) modification of the responsiveness of the contractile proteins to activation by $Ca^{2+}$.[13,14] The two former sites of action are considered to be the main targets of volatile anesthetics. However, a direct effect on the contractile proteins cannot be ruled out despite conflicting reports in the literature. In intact ventricular muscle, clinical concentrations of isoflurane have been reported to decrease myofibrillar responsiveness to $Ca^{2+}$,[14,15] but this effect was less pronounced,[16] or not observed[15] with equianesthetic concentrations of halothane. Using mechanically "skinned" myocardial bundles, Su and Kerrick[17,18] and Su and Bell[19] found a slight dose-dependent depression of maximal $Ca^{2+}$-activated force but no reduction of myofilament $Ca^{2+}$ sensitivity except for high concentrations of halothane. More recently,

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Murat *et al.*[13] observed a significant effect of volatile anesthetics on contractile proteins using chemically "skinned" bundles in which both the sarcolemma and the SR were not able to modulate myoplasmic $Ca^{2+}$ concentration. Conversely, Blanck *et al.* conducted studies using chemically "skinned" myocardial bundles from rabbits and found that halothane had no effect on both myofilament $Ca^{2+}$ sensitivity and maximal $Ca^{2+}$-activated force.[20] Moreover, Herland *et al.* reported complex effects (including an increase in $Ca^{2+}$ sensitivity) of volatile anesthetic on components normally modulating force in mammalian myocardium.[21]

It is well known that some problems arise when interpreting the results of experiments performed using "skinned" preparations and it is possible that the effects of the volatile anesthetic agents depend on the technique(s) used to skin the preparations.[21] Hence, it seems obvious that care must be taken when comparing results obtained using different skinning techniques. However, the discrepancies between these different findings may also depend on differences among species used.[20]

The skinned myocardial bundles technique has been successfully applied in human to investigate the effects of various agents on myofilament $Ca^{2+}$ sensitivity.[22–24] A limited biopsy of left ventricular myocardium is easily performed during open heart surgery and allows preparations of skinned fibers from humans.

Therefore, to study a direct effect of volatile anesthetics on human myocardial contractile apparatus, we investigated the effects of equianesthetic concentrations of halothane and isoflurane on the $Ca^{2+}$ sensitivity and maximal $Ca^{2+}$-activated force in "skinned" myocardial bundles. In our procedure, using both EGTA (ethylene glycol bis ($\beta$-aminoethyl ether)-N,N,N′,N′-tetraacetic acid) and BRIJ 58 detergent, all membranes are chemically destroyed and both SR (including T-tubules) and mitochondria are not functional.[25,26] Evidence will be presented for a direct decrease of myocardial $Ca^{2+}$ sensitivity and maximal force with clinical anesthetic concentrations of halothane and isoflurane.

## Materials and Methods

Left ventricular muscle-strips were dissected from the endocardial surface from seven patients undergoing cardiac surgery for valvular heart disease. The study protocol was approved by the Lille University Studies Ethics Committee, and written informed consent was obtained from all patients before participating in the study. Left ventricular function, as assessed by cardiac catheterization (left ventricular size, wall thickness, ejection fraction, cardiac output), was in the normal range in all patients, except for one patient with moderate enlarged left ventricle (table 1). The patients were anesthetized with sufentanil and pancuronium bromide. The myocardial fragments removed were immediately placed in cardioplegic solution at room temperature and rapidly (within 5 min) transported to the laboratory.

### *Skinned Fiber Preparation*

Chemically skinned fibers were prepared as previously described by Wood *et al.*[27] Chemical skinning with EGTA renders the muscle fiber sarcolemma freely permeable to external solutes.[28] Segments of muscle, containing several hundreds of fibers, were dissected free, and immediately placed in a relaxing solution at 4°C for 24 h (table 2). The skinning solution was replaced after 1, 4, and 12 h with fresh solution. 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 (1–2 weeks). This technique is identical to that used by a number of other laboratories.[20,26,29] No change in skinned cardiac bundles properties could be noticed after 2–3 weeks of storage.

Bundles were isolated from the main fascicle with the help of a 40 power swift Model 31-400-00 binocular microscope. Each skinned fiber bundle 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 C force displacement transducer. The muscle contracture was amplified and recorded on a Gould 2200 S. The fibers were bathed for 10–15 min in a relaxing solution containing

**Table 1. Characteristics of the Patients Studied**

| Patient No. | Age (yr) | Sex | Cardiac Disease | LVEF (%) |
|---|---|---|---|---|
| 1 | 61 | F | MR | 73 |
| 2 | 49 | M | MS | 68 |
| 3 | 73 | F | MR + MS | 75 |
| 4 | 77 | M | MR | 78 |
| 5 | 68 | F | AS | 66 |
| 6 | 68 | F | MS + AR | 64 |
| 7 | 58 | M | MR + MS | 52 |

MR = mitral regurgitation; MS = mitral stenosis; AR = aortic regurgitation; AS = aortic stenosis; LVEF = left ventricular ejection fraction. Values are from ventriculogram at time of catheterization.

**Table 2. Constituents of Solution (mM)**

| Solution | K-Propionate | Mg-Acetate | K2-EGTA | MOPS | Ca-EGTA |
|---|---|---|---|---|---|
| Relaxing solution | 170 | 2.50 | 5.00 | 10 | — |
| Wash solution | 185 | 2.50 | 0.00 | 10 | — |
| $Ca^{2+}$ solution | | | | | |
| pCa 6.8 = $1.6 \times 10^{-4}$ | 172 | 2.46 | 3.85 | 10 | 1.152 |
| pCa 6.4 = $4.0 \times 10^{-4}$ | 172 | 2.44 | 2.85 | 10 | 2.159 |
| pCa 6.2 = $6.3 \times 10^{-4}$ | 172 | 2.44 | 2.28 | 10 | 2.72 |
| pCa 6.0 = $1.0 \times 10^{-3}$ | 172 | 2.42 | 1.73 | 10 | 3.272 |
| pCa 5.8 = $1.6 \times 10^{-3}$ | 172 | 2.42 | 1.25 | 10 | 3.752 |
| pCa 5.6 = $2.5 \times 10^{-3}$ | 172 | 2.4 | 0.86 | 10 | 4.144 |
| pCa 5.4 = $4.0 \times 10^{-3}$ | 172 | 2.4 | 0.57 | 10 | 4.432 |
| pCa 5.2 = $6.3 \times 10^{-3}$ | 172 | 2.4 | 0.36 | 10 | 4.64 |
| pCa 5.0 = $1.0 \times 10^{-2}$ | 172 | 2.4 | 0.22 | 10 | 4.784 |
| pCa 4.8 = $1.6 \times 10^{-2}$ | 172 | 2.4 | 0.11 | 10 | 4.888 |

MOPS = morpholinopropanesulfonic acid. Each solution contains adenosine triphosphate (2.5 mM). The chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). *p*H = 7.00 ± 0.01 for all solutions.

the nonionic detergent BRIJ 58 (2%), which irreversibly eliminates the capability of the SR to sequester $Ca^{2+}$ and to release it under appropriate stimulation, but does not affect the contractile proteins.[25] The preparation was then straightened to a length at which an increase in resting tension was first detected, and then stretched an additional 20% of the initial length of the bundle to compensate for internal shortening during activation. This technique was described by Maughan *et al.*[30] and used by authors studying human cardiac bundles.[23,31] The length and diameter of the skinned fiber bundles were measured under a 400X-power Olympus lens. Finally, the functional destruction of SR was confirmed by studying $Ca^{2+}$ release from SR with 40 mM caffeine after loading the SR with a known concentration of $Ca^{2+}$ (*p*Ca 6.8) in the presence of adenosine triphosphate. Only bundles with no contracture to caffeine (*i.e.*, no functional SR) were included in the study.

For all experiments described below, the length of the preparations was kept constant to avoid sarcomere length-dependent changes in $Ca^{2+}$ 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[32] to keep the ionic strength at 200 mM. The stability constants of Orentlicher *et al.*[25] were used in the calculations: $K_{CaEGTA}$ $1.919 \times 10^6$/M, $K_{CaATP}$ $5.0 \times 10^3$/M, $K_{MgEGTA}$ 40/M, and $K_{MgATP}$ $1.0 \times 10^4$/M. Composition of solutions is shown in table 2.

To assess the effects of halothane and isoflurane, the test solutions were equilibrated by continuous bubbling for 20 min with the chosen anesthetic agent. Halothane and isoflurane were mixed with 100% nitrogen by means of calibrated vaporizers (Fluotec Mark III and Isotec Mark III). The anesthetic concentrations in the gas phase were monitored with an infrared calibrated analyzer (Normac®, Datex, Finland). The anesthetic concentrations used were 0.75, 1.5, and 2.25 vol% halothane and 1.15, 2.30, and 3.50 vol% isoflurane. These concentrations are roughly equivalent to 1, 2, and 3 MAC multiples of halothane and isoflurane 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 a flame ionization detector and a Porapack Q® 3.17 mm by 150 cm column was used for determination of anesthetic concentrations.[33] 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 each anesthetic) for 20 min before injecting 1 ml gas into the apparatus, previously calibrated with known concentrations of each anesthetic (head space technique).

The anesthetic concentrations measured in the experimental solution (n = 3) after 20 min of continuous bubbling at equivalent multiples of MAC were as follows: halothane 0.46 ± 0.04, 0.83 ± 0.07, and

1.23 ± 0.10 mM for 0.75, 1.50, and 2.25%, respectively; and isoflurane 0.63 ± 0.07, 1.22 ± 0.09, and 1.93 ± 0.15 mM for 1.15, 2.30, and 3.50%, respectively.

*Experimental Procedure*

For each skinned cardiac bundle, a *p*Ca–tension curve was obtained in control conditions by stepwise exposure of the fibers to solutions with increasing $Ca^{2+}$ concentrations and measurements of developed tension (fig. 1A). $Ca^{2+}$ concentrations ranged from *p*Ca 6.4 to *p*Ca 4.8 where $pCa = -\log10[Ca^{2+}]$ (table 2). Intermediate tensions were expressed as a percentage of the maximal tension. Data were analyzed by linearizing Hill's equation, where relative tension = $[Ca]^{nH}/(K + [Ca]^{nH})$. The slope coefficient (nH) and the concentration of $Ca^{2+}$ for half-maximal activation ($pCa_{50}$) were computed.

In all the experimental conditions, each of the two anesthetics was tested at all MAC multiples in the same fibers. Hence, *p*Ca–tension curves were obtained at each MAC multiple for each anesthetic in a random order in each fiber. A final *p*Ca–tension curve was obtained with solutions free of anesthetics. Because maximal activated tension decreased regularly during the study, 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 $Ca^{2+}$ in the presence of different MAC multiple concentrations of anesthetics. The mean values of the two control curves were used to assess the effects of the two anesthetics studied.

In a second series of experiments, changes of tension at maximal $Ca^{2+}$-activated force were examined using a *p*Ca 4.8 solution. Each fiber was exposed in random order to test solutions equilibrated with 1, 2, and 3 MAC multiples of either halothane or isoflurane. Each test was preceded and followed by determination of maximal $Ca^{2+}$-activated tension with the control test solution (*i.e.*, free of anesthetic). 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.

*Statistical Analysis*

Comparisons between anesthetics and at equivalent MAC multiples were made by repeated measures analysis of variance. For multiple comparisons among groups where indicated by results of analysis of variance, Duncan's multiple-range test was used. Comparisons between control values obtained at the beginning and the end of each study were made using Student's *t* test for paired data. Values of $P < 0.05$ were regarded as significant. Results are expressed as mean ± standard error of the mean.

## Results

The characteristics of the 14 bundles studied were as follows: length (mean ± SEM) 2,097 ± 158 μm (range 1,400–2,800 μm); diameter (mean ± SEM) 236 ± 8.6 μm (range 200–280 μm); ratio of resting tension to total tension 0.07 ± 0.015; maximal activated tension (mean ± SEM) 42.8 ± 2.4 mN/mm$^2$. The *p*Ca–tension curves were determined in seven fibers with halothane and isoflurane used in a random order. Figure 1 shows a typical

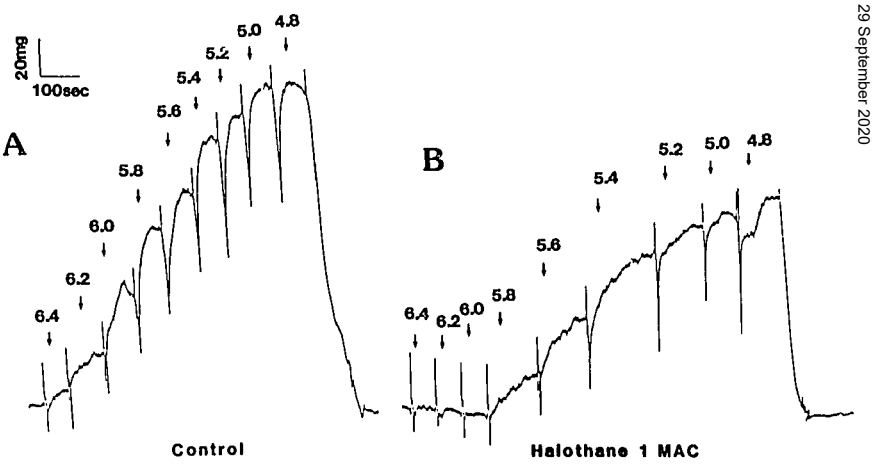


**Fig. 1. Changes in tension related to increasing $Ca^{2+}$ concentration, expressed as *p*Ca (where $pCa = -\log_{10}[Ca^{2+}]$) obtained with the same skinned cardiac bundle. (*A*) The changes in tension obtained in control solutions (in the absence of volatile anesthetics). (*B*) The changes in tension obtained in the presence of 1 MAC halothane. Twenty minutes elapsed between the two experimental conditions. Arrows = changes in $Ca^{2+}$ solutions.**

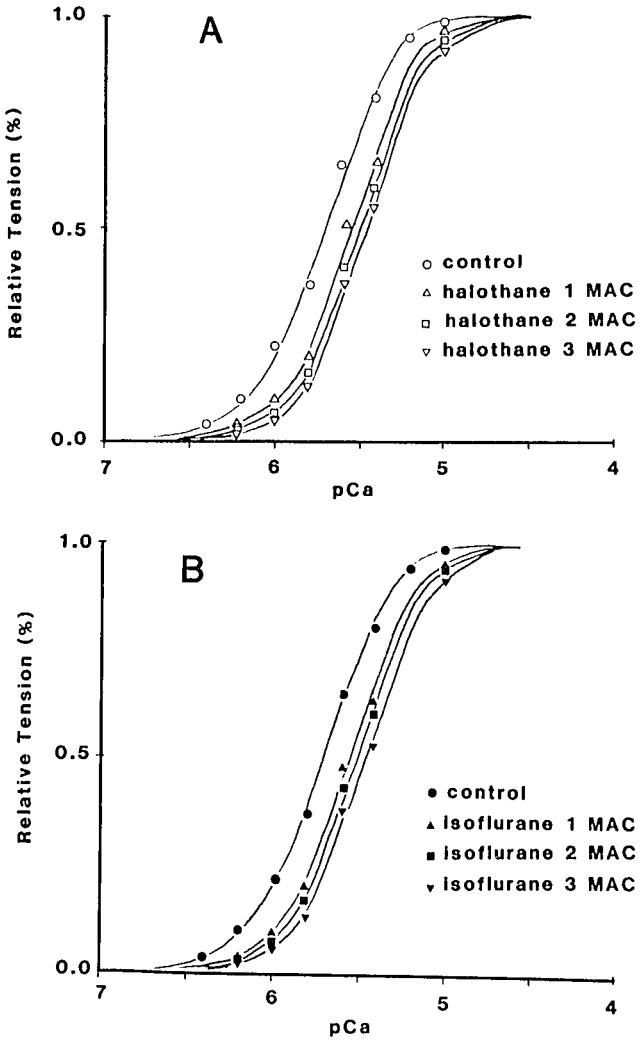


**Fig. 2. Mean $p$Ca–tension curves (where $p\text{Ca} = -\log_{10}[\text{Ca}^{2+}]$) obtained in control conditions and with increasing concentrations of halothane (*A*) and isoflurane (*B*) in seven skinned cardiac bundles from seven patients. The curves are significantly shifted, in a dose-dependent fashion, to the right; the concentration of $Ca^{2+}$ for half-maximal activation ($p\text{Ca}_{50}$) decreases, also in a dose-dependent manner. This indicates a decrease in the $Ca^{2+}$ sensitivity of the contractile proteins with increasing concentrations of halothane and isoflurane. For clarity, error bars have been omitted.**

example of the changes in tension in response to increasing $Ca^{2+}$ concentrations observed with solutions free of anesthetic (fig. 1A) and with activating solutions equilibrated with 1 MAC halothane (fig. 1B). As maximal activated force decreased regularly during the experiment and represented 70% of the initial developed force at the end of the overall experiment, tensions obtained during anesthetic exposure were normalized to their maximal value at each concentration of anesthetics. Tension changes after $Ca^{2+}$ changes were then plotted to allow analysis of the sensitivity of the preparations to $Ca^{2+}$ in the presence of anesthetics. With increasing anesthetic concentrations, a shift toward higher $Ca^{2+}$ concentrations was observed. Figure 2 illustrates a typical family of normalized curves obtained with equianesthetic concentrations of either halothane (fig. 2A) or isoflurane (fig. 2B). The dose-dependent shift to the right was already significant at 1 MAC equianesthetic concentration and remained apparent after the curves had been normalized for tensions. Both halothane and isoflurane significantly shifted the $p$Ca–tension curves to the right. This was attested by the significant decrease in $p\text{Ca}_{50}$ values with increasing anesthetic concentrations ($P < 0.01$ for the two agents), with no significant change in the Hill coefficient (table 3). No differences between halothane and isoflurane were observed at equivalent MAC multiples. The effects of the two anesthetics on $Ca^{2+}$ sensitivity were dose dependent ($P < 0.01$ between 1 MAC and 3 MAC multiples for the two agents) and reversible. Indeed, no significant differences for $p\text{Ca}_{50}$ and Hill coefficient were observed between the $p$Ca–tension curves obtained at the beginning and at the end of the study.

The effects of halothane and isoflurane on maximal activating tension were determined in seven fibers. Fibers were equilibrated in a maximally activating solution at $p$Ca 4.8 and subsequently with various concentrations of each anesthetic in a random order. Maximal activated tension decreased significantly in a dose-dependent fashion with increasing concentrations of each anesthetic ($P < 0.01$ for each of the two agents) (table 4). No differences between agents were found

**Table 3. Mean ± SEM Values for pCa 50 and Hill Coefficients in Absence of Anesthetics and at the Different MAC Multiples**

| | MAC | pCa 50 | Hill Coefficient |
|---|---|---|---|
| Control | 0 | 5.71 ± 0.047 | 2.08 ± 0.18 |
| Halothane | 1 | 5.56 ± 0.043 | 2.22 ± 0.20 |
| | 2 | 5.50 ± 0.044 | 2.29 ± 0.20 |
| | 3 | 5.47 ± 0.039 | 2.07 ± 0.12 |
| Isoflurane | 1 | 5.55 ± 0.046 | 2.06 ± 0.28 |
| | 2 | 5.52 ± 0.046 | 2.27 ± 0.34 |
| | 3 | 5.46 ± 0.047 | 2.05 ± 0.17 |

Decreases in pCa 50 values were significant at all MAC multiples *versus* control and at 3 MAC *versus* 1 MAC multiple for the two agents ($P < 0.01$). No differences were observed between Hill coefficients.

**Table 4. Mean ± SEM Changes in Maximal $Ca^{2+}$-activated Tension, Expressed as Percentage of the Mean of the Bracketing Controls**

| Anesthetic | MAC | Maximal Tension (% decrease) |
|---|---|---|
| Halothane | 1 | 14.7 ± 1.54 |
| | 2 | 27.8 ± 3.75 |
| | 3 | 37.5 ± 3.4 |
| Isoflurane | 1 | 14.6 ± 1.08 |
| | 2 | 28.6 ± 3.3 |
| | 3 | 37.8 ± 4.68 |

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

at each MAC multiple. A typical set of results obtained with skinned cardiac bundles is shown in figure 3. The force trace was obtained when the preparation was maximally activated at *p*Ca 4.8, initially in the absence of a volatile agent. After a substantial rise in force occurred, the preparation was exposed to 2 MAC halothane (fig. 3A), which caused a prolonged decrease in force level remaining stable for 60–100 s, and was immediately reversible upon switching from halothane-equilibrated solution to control solution of identical *p*Ca. The same experiment was then performed with 2 MAC isoflurane on the same bundle (fig. 3B).

## Discussion

This study demonstrates that halothane and isoflurane decrease both $Ca^{2+}$ sensitivity and maximal force of left ventricular skinned fibers from humans. The effects of both agents were similar in a dose-dependent and reversible fashion at equianesthetic concentrations.

The $Ca^{2+}$ sensitivity of the contractile apparatus expressed as $pCa_{50}$ values and Hill coefficients closely overlaps that found by other studies in humans using saponin pretreatment of trabeculae from normal heart.[23,31] In our study, only one patient had moderately low cardiac catheterization values but *in vitro* results recorded did not differ from the other patients.

Our study uses a skinned fiber preparation as it allows rapid application and removal of particular agents, which influences the myoplasmic $Ca^{2+}$ concentrations.[27,28] Such a preparation has been widely used to study contractile apparatus itself.[25,34] The technique uncouples T-tubules from SR structures with EGTA and then uses BRIJ 58 to destroy the SR membranes. The efficacy of BRIJ 58 application was verified in each cardiac bundle tested by applying a 40 mM caffeine solution after loading the SR. Only bundles with nonfunctional SR (*i.e.*, no response to caffeine) were included in the experiment. 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.[26,35] The technique allows the diffusion outside the cell of low-molecular-weight proteins, which may play a role in the modulation of muscle contractile function.[21,26] This could explain in part the fact that the myofilament sensitivity to $Ca^{2+}$ appears to be lower in skinned preparation than in intact muscle. Hence, care must be taken when interpreting results obtained using different skinning techniques.

Our results differ from those reported by Su and Kerrick[17,18] and Su and Bell[19] in mechanically disrupted rabbit ventricular fibers. They found that halothane, enflurane, and isoflurane slightly decrease maximal $Ca^{2+}$-activated tension, but they could not demonstrate any effect on myocardial $Ca^{2+}$ sensitivity except for high concentrations of halothane. Likewise, Blanck *et al.*, in chemically skinned rabbit cardiac fibers with EGTA alone, found no effect of halothane on the contractile apparatus.[20] Recently, Herland *et al.*[21] reported complex effects (including an increase in $Ca^{2+}$ sensitivity) of volatile anesthetics on components normally modulating force in skinned rat myocardium. However, because the lowest dose of halothane used in this study was 1.9 mM (4.6 MAC), and the highest 9.4 mM (22 MAC), the clinical relevance of their findings is questionable. Nevertheless, their study suggested that the effects of the volatile anesthetics on the $Ca^{2+}$-activated force responses of skinned cardiac muscle appear to depend to a large extent on the technique used to skin the preparation. Indeed, 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. Because the use of EGTA plus BRIJ 58 as well as the use of nonionic detergent Triton X-100 are known to fully disrupt and effectively remove all cellular membrane components,[26,36] it is likely that the volatile anesthetics effects observed in the current study after treatment with EGTA plus BRIJ 58 reflect direct influences on the contractile apparatus. In addition, when studying the direct effects of volatile anesthetics on the contractile apparatus, it is likely that the functional destruction of SR avoids the $Ca^{2+}$-induced $Ca^{2+}$ release mechanism that could be activated when bathing the fibers in successive solutions containing $Ca^{2+}$.

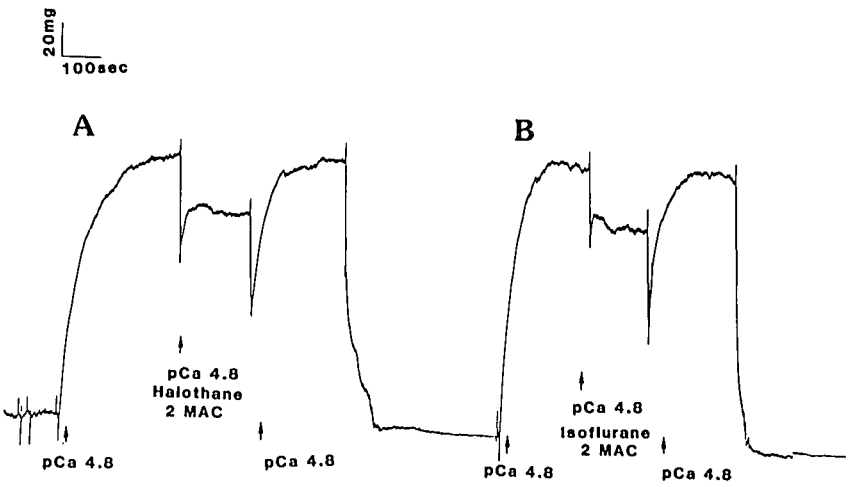


**Fig. 3. An example of the changes in maximal activated tension obtained in the same skinned cardiac bundle during exposure to 2 MAC halothane (*A*) and isoflurane (*B*) at *p*Ca 4.8 ($p\text{Ca} = -\log_{10}[\text{Ca}^{2+}]$). Each anesthetic exposure was preceded and followed by determination with the *p*Ca 4.8 control solution alone.**

Our results obtained on human skinned cardiac bundles are consistent with previous animal studies demonstrating a reduction in maximal actomyosin adenosine triphosphatase activity and adenosine triphosphatase $Ca^{2+}$ sensitivity in the presence of high concentrations of halothane (greater than 0.9 mM)[37] or isoflurane.[38] The relative insensitivity of the myofibrils (*i.e.*, an effect observed only with high concentrations of volatile agents) may be secondary to the isolation process used.[37] Our findings are in agreement with those obtained by Murat *et al.* in skinned cardiac fibers with a nonionic detergent Triton X-100 (1%) from rat,[13] hamster,[39] and rabbit.[40] They found a decrease in maximal activated tension ranging between 5 and 15% for clinically relevant anesthetic concentrations. The volatile anesthetics also decreased myocardial $Ca^{2+}$ sensitivity in a dose-dependent (between 0.5 and 2 MAC) and reversible fashion. The effects of the three anesthetics used (halothane, enflurane, and isoflurane) were identical for equianesthetic concentrations expressed in MAC multiples.

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 37°C, as is commonly done with skinned cardiac preparations.[13,17–21,23] Because there could be temperature-dependent differences (*i.e.*, greater depression at a lower temperature) in the negative inotropic effect of the volatile anesthetics,[41] and because MAC values decrease with decreasing body temperature, our data may overestimate the effects of anesthetics on $Ca^{2+}$ sensitivity.

To date, the exact cellular mechanism by which volatile anesthetics decrease $Ca^{2+}$ sensitivity of the contractile proteins is not known. The *p*Ca–tension relationships are thought to reflect $Ca^{2+}$ binding to sites on troponin C,[42] whereas the Hill coefficient, which has been used as an index of cooperation between actin and myosin myofilaments, does not reflect the number of $Ca^{2+}$ binding sites on the troponin complex.[24] The decrease in $p\text{Ca}_{50}$ values without modifications of Hill coefficients are consistent with an apparent decrease in $Ca^{2+}$ sensitivity of the contractile proteins. With regard to our results, the major shift in $Ca^{2+}$ sensitivity appears to occur with the 1 MAC concentration with very little (although significant) further shift being present with a tripling of concentration. This behavior suggests some sort of saturable phenomenon. A recent study suggests that halothane does not modify $Ca^{2+}$ binding by isolated troponin C.[20] A direct interaction of halothane at a locus beyond troponin C in the contractile process is probably involved, but the precise site of action of volatile anesthetics remains to be determined.

Nevertheless, the decrease in $Ca^{2+}$ sensitivity, as well as the decrease in maximal activated tension, may participate in the negative inotropic effects of halothane and isoflurane. The relative importance of such effects compared with the other mechanisms involved cannot be assessed from our study. However, our data, as well as previous reports,[4,13,14] suggest that the decreased $Ca^{2+}$ sensitivity of the contractile proteins may magnify the effects of these agents on the sarcolemma and SR, both actions leading to decrease of the amount of $Ca^{2+}$ available for contractile activation.

In conclusion, the current study demonstrates that, in skinned cardiac fibers from humans, clinical anesthetic concentrations of halothane and isoflurane decrease both maximal $Ca^{2+}$-activated tension and apparent myocardial $Ca^{2+}$ sensitivity in a dose-dependent and reversible fashion. Such significant effects on maximal $Ca^{2+}$-activated tension (27 and 28% for 2 MAC halothane and isoflurane, respectively) have not been described previously. If these results can be extrapolated to *in vivo* conditions, we conclude that these effects may contribute in the overall direct negative inotropic action of these agents.

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