

## Halothane Cooling Contractures of Skinned Mammalian Muscle Fibers

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The effects of halothane or cooling on  $\text{Ca}^{2+}$ -activated tensions and on the uptake and release of  $\text{Ca}^{2+}$  by the sarcoplasmic reticulum were investigated in chemically skinned fibers of the extensor digitorum longus muscle of adult rabbits. At 22° C, halothane (>0.46 mM) induced  $\text{Ca}^{2+}$  release from the SR of  $\text{Ca}^{2+}$ -loaded skinned fibers that resulted in transient tensions. Higher concentrations of halothane (>4.65 mM) reduced the steady-state accumulation of  $\text{Ca}^{2+}$  in the SR at 22° C. Cooling (to <10° C) elicited transient contractures (cooling-induced contractures [CC]) in  $\text{Ca}^{2+}$ -loaded skinned fibers, despite the fact that the tensions elicited by adding  $\text{Ca}^{2+}$  to the bath were depressed at these low temperatures. The skinned fibers did not develop CCs at 12–16° C. Halothane cooling contractures could be elicited at these temperatures by exposing the fibers to halothane concentrations that failed to elicit  $\text{Ca}^{2+}$  release at 22° C. The halothane cooling contractures were blocked by procaine but not by lidocaine. It was concluded that these contractures resulted from a synergistic interaction between halothane and cooling that stimulates  $\text{Ca}^{2+}$  release from, and reduces  $\text{Ca}^{2+}$  uptake by, the sarcoplasmic reticulum. (Key words: Anesthetics, volatile: halothane. Calcium-activated tensions. Muscle: contractures; sarcoplasmic reticulum; skinned fibers. Temperature: dependence of halothane-induced tensions.)

PREVIOUS STUDIES from our laboratory<sup>1-3</sup> have shown that when isolated intact skeletal muscles are superfused with cooled solutions containing halothane concentrations equivalent to those present clinically, they develop large transient contractures. Halothane cooling contractures (HCCs) can also be induced in fascicles dissected from muscle biopsies taken from patients undergoing elective surgery under halothane anesthesia.<sup>3</sup> We suggested that the HCCs provide a simple experimental paradigm for investigating the effects of halothane on the  $\text{Ca}^{2+}$  homeostasis and contractility of skeletal muscles. Because the HCCs can be elicited in the presence of clinically relevant concentrations of halothane,<sup>3</sup> drugs that inhibit these contractures may prove to be of potential value in the

management of the muscle contractures associated with halothane-induced malignant hyperthermia episodes.

In the studies with intact muscles, the effects of halothane and cooling on the sarcoplasmic reticulum (SR) could not be distinguished from possible effects on the sarcolemma or the contractile proteins. In the current study, the sarcolemma is functionally eliminated by chemical skinning,<sup>4,5</sup> and the experiments were designed to investigate the influence of halothane or low temperature on the  $\text{Ca}^{2+}$  affinity for the contractile proteins and on the uptake and release of  $\text{Ca}^{2+}$  by the SR. The data show that HCCs can be elicited in  $\text{Ca}^{2+}$ -loaded, chemically skinned mammalian muscle fibers when they are exposed to clinically relevant concentrations of halothane.

### Methods

This study was approved by the Animal Care Committee of the Programa de Medicina Experimental (PIMED) of the Federal University of Rio de Janeiro.

### SKINNED FIBER PREPARATION

Rabbits were killed by cervical dislocation, and small bundles of fibers were dissected from the extensor digitorum longus (EDL) muscle. The fibers were chemically skinned<sup>4</sup> and stored at -20° C in the presence of glycerol until used. As previously described,<sup>4,5</sup> overnight exposure at 0° C to a "skinning" solution (table 1) eliminated the sarcolemma as a diffusion barrier to large solutes, making it possible to modify the composition of the medium bathing the myofibrils and the SR membranes. On the day of the experiment, a single skinned fiber was dissected from a bundle and mounted in a lucite chamber containing 0.5 ml of the skinning solution and provided with stirring and temperature control. The temperature of the bathing medium was maintained at  $22 \pm 0.5^\circ \text{C}$  except during the cooling periods. For cooling, the temperature control system was turned off, and the fiber was superfused with precooled solution. The flow rate was adjusted to provide the required temperature in the chamber. For recording isometric tension, one end of the fiber was attached to a fixed clamp connected to a force-displacement transducer (Grass, model FT-03, Quincy, MA), and the other end was wrapped around a hook connected to a micromanipulator that was used to stretch the fiber to about 120% of its slack length. The transducer signals were amplified and recorded on a Grass polygraph (model 7).

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TABLE 1. Composition of the Experimental Saline Solutions

Components	Solutions	
	Skinning/Relaxing (R) (mM)	Washing (W) (mM)
K propionate	175	185
Mg acetate	2.5	2.5
K <sub>2</sub> Na <sub>2</sub> ATP	2.5	2.5
K <sub>2</sub> EGTA	5	0.05
Imidazol propionate	10	10

pH = 7.0.

## SOLUTIONS

The composition of the control bathing medium (washing solution, designated W) is shown in table 1; 50  $\mu$ M K<sub>2</sub> ethylene glycol-bis (beta-amino-ethyl ether)-N,N'-tetracetic acid (EGTA) was added to solution W to reduce the concentration of contaminating Ca<sup>2+</sup>. The relaxing solution (designated R) had a composition identical to that of the skinning solution (table 1). Solutions used to load Ca<sup>2+</sup> into the SR (loading solutions) or to activate the myofibrils directly (activating solutions) were prepared by adding CaK<sub>2</sub>EGTA to solution R to obtain different ratios of K<sub>2</sub>EGTA/CaK<sub>2</sub>EGTA while keeping the total [EGTA] constant at 5 mM. The pCa of the loading solution was typically 7.0, while the solutions used to activate the myofibrils directly had pCa values in the range of 5.2–7 (table 2). Association constants for CaEGTA and magnesium adenosine triphosphatase (MgATP) were those reported by Orentlicher *et al.*<sup>6</sup>; for all other ligands, the constants given by Fabiato and Fabiato<sup>7</sup> were used. In a series of experiments, K oxalate (5 mM) was added to the loading solution; the equilibria involving potassium and oxalate had negligible effects on free Ca<sup>2+</sup> but significantly affected the calculations for ATP and Mg species as discussed by Sorenson *et al.*<sup>8</sup>

Caffeine, procaine HCl, and lidocaine HCl (all from Sigma Chemical Co., St. Louis, MO) were stored in stock solutions and added to the experimental solutions as required. Halothane (Central de Medicamentos, Brazil) was added to the experimental solutions in sealed vials immediately before use. The solutions containing halothane (0.093–46.5 mM) were allowed to flow continuously into the experimental chamber.

## EXPERIMENTAL PROTOCOLS

At the beginning and at the end of each experiment, P<sub>O</sub>, the maximal contractile response of the fibers when challenged with 0.5 mM CaCl<sub>2</sub>, was recorded. Experiments during which P<sub>O</sub> declined by 20% or more were discarded. Each experimental protocol was repeated on a minimum of five fibers.

The procedures used to investigate the effects of cooling and of halothane on the uptake and release of Ca<sup>2+</sup> by the SR were adapted from those previously described.<sup>4,8–10</sup> Ca<sup>2+</sup> was loaded into the SR by two different procedures. The procedure used in most experiments consisted of exposing the fiber initially to 20 mM caffeine in solution R to release all Ca<sup>2+</sup> remaining in the SR. After two washes with W, the fibers were soaked in a loading solution of pCa 7.0 during 1 or 2 min, followed by two washes with W at 30-s intervals. The extent of loading was then evaluated by challenging the fibers with 20 mM caffeine in solution W. In other experiments, the challenge was provided by halothane or cooling to evaluate their ability to cause release of Ca<sup>2+</sup> stored in the SR. After the challenge with halothane or cooling, the fibers were soaked at room temperature in solution R (10–15 s), followed by two washes with W (15–30 s). A different protocol was used to load Ca oxalate into the SR.<sup>8</sup> For this purpose, the fibers were soaked for 30 min in a loading solution of pCa 7.0 to which 5 mM K oxalate was added, followed by two washes with solution W. The amounts of Ca<sup>2+</sup> accumulated into the SR with and without oxalate are significantly different. Total fiber Ca concentration increases from 1–3 mM in the absence of oxalate to 20–170 mM when oxalate is present in the loading solution, and crystals of Ca oxalate appear inside the SR.<sup>11</sup>

The influence of halothane on the steady-state accumulation of Ca<sup>2+</sup> in the SR was studied by adding the anesthetic to the loading solution (pCa, 7.0; no oxalate). Control experiments have shown that in the absence of halothane, the fibers are maximally loaded after 1 min soaking in this loading solution. After two washes with solution W, the fibers were challenged with 20 mM caffeine, and the amplitude of the caffeine-induced tension was used to estimate the amount of Ca<sup>2+</sup> stored in the SR.

To study the ability of halothane or cooling to induce release of SR-stored Ca<sup>2+</sup>, the fibers were either exposed to solution W premixed with halothane or they were superfused with precooled solutions in the absence or presence of halothane. After the challenge with halothane or cooling, the fibers were soaked at room temperature in

TABLE 2. Ca<sup>2+</sup> Concentrations in the Loading and Activating Solutions

pCa	CaEGTA <sup>2-</sup> (mM)	K <sub>2</sub> EGTA <sup>2-</sup> (mM)	Ca <sup>2+</sup> ( $\mu$ M)
7.0	0.8	4.2	0.0993
6.8	1.15	3.85	0.156
6.4	2.15	2.85	0.395
6.0	3.26	1.74	0.98
5.6	4.14	0.86	2.4
5.2	4.64	0.36	6.3

pH = 7.0

solution R (10–15 s), followed by two washes with W (15–30 s). In experiments in which the  $\text{Ca}^{2+}$  loading solutions did not contain oxalate, a brief exposure (15–30 s) to solution R containing 20 mM caffeine was interspersed before the first wash with W to release the  $\text{Ca}^{2+}$  remaining in the SR. A new  $\text{Ca}^{2+}$  loading period could then be initiated.

#### $\text{Ca}^{2+}$ -activated Tensions:

Skinned fibers pretreated with the nonionic detergent Brij-58 (0.5% w/v in solution R for 30–60 min) were used for the study of the effects of halothane and low temperature on the tensions caused by directly activating the myofibrils with buffered  $\text{Ca}^{2+}$ . This treatment disrupts the SR membranes but does not affect the ability of the fibers to contract when challenged with  $\text{Ca}^{2+}$ .<sup>6</sup> The damage to the SR membranes was confirmed by the inhibition (>90%) of the caffeine-induced contractures after exposure to Brij-58.

## Results

### HALOTHANE-INDUCED TENSIONS

Halothane elicited  $\text{Ca}^{2+}$  release from the SR of  $\text{Ca}^{2+}$ -loaded rabbit EDL skinned fibers studied at room temperature under the experimental conditions shown in figure 1. The experiments were initiated by recording the  $P_0$  (fig. 1A), followed by exposure to solution R to cause complete relaxation. The fiber was then soaked in a loading solution of  $p\text{Ca}$  7.0, and the functional status of the SR was evaluated by exposure to 20 mM caffeine (fig. 1B).  $\text{Ca}^{2+}$  accumulated by the SR during exposure to the loading solution was released, resulting in nearly maximal tension. The  $\text{Ca}^{2+}$  loading cycle was repeated successively, and the fiber was challenged with halothane in increasing concentrations. Figure 1C shows the contractile response elicited by 4.6 mM halothane. At the end of the experiment,  $P_0$  was determined again (fig. 1D). Data from 20 single fibers were used to construct the plot shown in figure 1, in which the ordinate represents the percent of fibers that developed contractures of peak amplitude greater than 5%  $P_0$  when challenged with the halothane concentrations indicated on the abscissa. The minimal concentration of halothane required for this effect varied among different fibers, the most sensitive of which developed detectable tensions in the presence of 0.46 mM halothane. All fibers contracted when exposed to 6.5 or 9.3 mM halothane.

The maximal halothane-induced tensions exhibited peak amplitudes in the range of 60–80% of  $P_0$ , which is comparable to the maximal caffeine-induced tensions of the skinned fibers under the same experimental conditions (fig. 1B). Under the  $\text{Ca}^{2+}$  loading conditions of figure 1, the halothane-induced tensions declined while the fibers

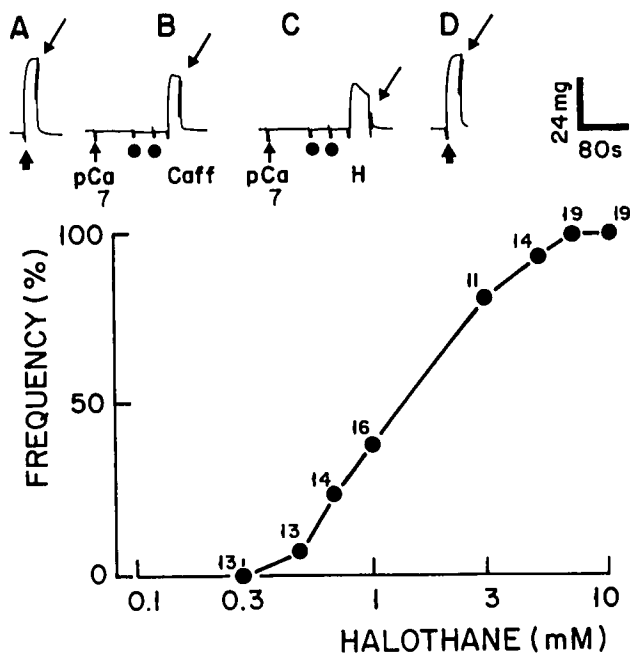


FIG. 1. Contractures elicited by halothane at 22° C. The tension recordings in the upper part show the experimental protocol. (A, D)  $P_0$ , elicited by 0.5 mM  $\text{CaCl}_2$ . Solution R was used to relax the fiber (slanted arrows). In B and C,  $\text{Ca}^{2+}$  was loaded into the SR by soaking the fiber in a solution of  $p\text{Ca}$  7.0 for 1 min. After two washes with W (filled circles), 20 mM caffeine (B) or 4.6 mM halothane (H) (C) was used to release the  $\text{Ca}^{2+}$  stored in the SR. Graph shows the cumulative frequency of contractile responses of 20  $\text{Ca}^{2+}$ -loaded fibers to halothane. The ordinate represents the per cent of fibers that developed contractures of peak amplitude greater than 5%  $P_0$  when challenged with the halothane concentrations indicated on the abscissa. Vertical arrows indicate the addition of 0.5 mM  $\text{CaCl}_2$  to the medium.

were still exposed to the anesthetic. Similar results were previously observed in human skinned fibers.<sup>10,12</sup> In contrast, fibers whose SR was loaded with Ca oxalate sustained the halothane-induced contractures at peak amplitude for several minutes (not shown).

The effects of halothane on the steady-state accumulation of  $\text{Ca}^{2+}$  in the SR are presented in table 3. The  $\text{Ca}^{2+}$  accumulation was significantly reduced in a dose-dependent manner for halothane concentrations in the range of 4.6–46 mM, but no effect was observed with 0.46 or 0.93 mM halothane. The inhibition of  $\text{Ca}^{2+}$  accumulation was irreversible in fibers exposed to 46 mM; this was not accompanied by changes in  $P_0$ .

### HALOTHANE COOLING CONTRACTURES

We investigated initially whether cooling *per se* elicited  $\text{Ca}^{2+}$  release in  $\text{Ca}^{2+}$ -loaded skinned EDL fibers. Cooling-induced contractures (CCs) were consistently observed at temperatures below 10° C (n = 10), but were generally absent above 12° C. (Only 1 of 10 fibers responded when tested at 12–16° C.) Figure 2 shows the results from two

TABLE 3. Effects of Halothane on the Steady-state Accumulation of Ca<sup>2+</sup> in the SR of Skinned EDL Fibers

Halothane Concentration (mM)	Control (a)	Experimental (a)	P
0.93	85 ± 4	81 ± 4	NS
4.65	85 ± 3	69 ± 5	0.05
9.30	81 ± 3	58 ± 6	0.01
46.50	88 ± 3	21 ± 9	0.01

a = Amplitude (% of P<sub>O</sub>; mean ± SEM) of the maximum caffeine-induced tensions of Ca<sup>2+</sup>-loaded fibers. Ca<sup>2+</sup> loading was obtained by soaking the fibers for 1 min in a solution of pCa 7.0, in the absence (control) or in the presence (experimental) of halothane. For each concentration of halothane, 4–6 fibers were used; each fiber served as its own control.

P = Student's two-tailed t test for paired data.

fibers that illustrate these observations. Fiber A did not contract when cooled to 12° C, whereas fiber B developed a transient CC upon cooling to 8° C.

Intact mammalian muscles develop HCCs when exposed to halothane concentrations that induce no tension at room temperature.<sup>2,3</sup> Accordingly, in the current study, we observed that HCCs could be elicited in skinned fibers during exposure to concentrations of halothane that in the same fibers had no contractile effect when tested at 22° C. Because of the variable sensitivity of the fibers to halothane at 22° C (fig. 1), concentrations of the anesthetic in the range of 0.3–1.0 mM were used for eliciting the HCCs in different experiments. As previously reported for intact mammalian muscles,<sup>2,3</sup> the HCCs of the skinned fibers appeared either as potentiation of the CC in preparations (n = 8) that contracted upon cooling *per se* (fig. 2B) or as the development of tension in fibers (n = 12) that, in the absence of halothane, did not contract when cooled to a given temperature (fig. 2A).

The effects of halothane on the contractile responses of the skinned fibers to cooling were easily reversed by washing out the preparations and could be reproduced several times in the same fiber by reexposure to the anesthetic (figs. 3 and 4).

Under the experimental conditions of figures 1 and 2, in which the Ca<sup>2+</sup> loading of the SR was performed in the absence of oxalate, the HCCs were transient and relaxation occurred during the cooling period. However, fibers that were allowed to accumulate Ca oxalate in the SR sustained the HCCs at peak amplitude while being superfused with cooling solutions containing halothane. The recordings shown in figure 3 were obtained from a fiber that was preloaded with Ca oxalate and subsequently challenged with halothane (0.93 mM) at room temperature (fig. 3A) or at 12° C (figs. 3B and C). After each challenge, the fiber was immersed in solution R at 22° C for 30 s and then returned to solution W until the next exposure to halothane; halothane did not elicit tension when applied at 22° C, but HCCs were recorded at 12°

C. The HCCs were sustained throughout the cooling period but relaxed on introduction of solution R. As previously reported for caffeine,<sup>8</sup> fibers preloaded with Ca oxalate were capable of developing successive HCCs without the need of interposing Ca<sup>2+</sup> loading periods after each contracture (figs. 3C and D).

#### EFFECTS OF PROCAINE AND LIDOCAINE ON THE HCCs

Procaine (2–6 mM) inhibited the CCs and the HCCs of the skinned muscle fibers in a dose-dependent manner. With 6 mM procaine, these contractures were abolished (fig. 4B). In contrast, lidocaine (4–6 mM) potentiated the contractures elicited by cooling both in the presence (fig. 4C) and in the absence of halothane (not shown). The effects of the two local anesthetics were readily reversible on washout (fig. 4D).

#### EFFECTS OF HALOTHANE AND COOLING ON THE CA<sup>2+</sup>-INDUCED TENSIONS

To investigate whether the potentiation of the cooling-induced contractures by halothane involved changes in the sensitivity of the contractile proteins to the activator Ca<sup>2+</sup>, Brij-treated fibers were used. The Ca<sup>2+</sup>-induced tensions were depressed by lowering the temperature of the medium and were nearly abolished at 4° C. At 13 ± 1° C, the maximal Ca<sup>2+</sup>-induced tension was reduced to 40–50% of its value at 22° C (n = 4). Halothane (0.093–

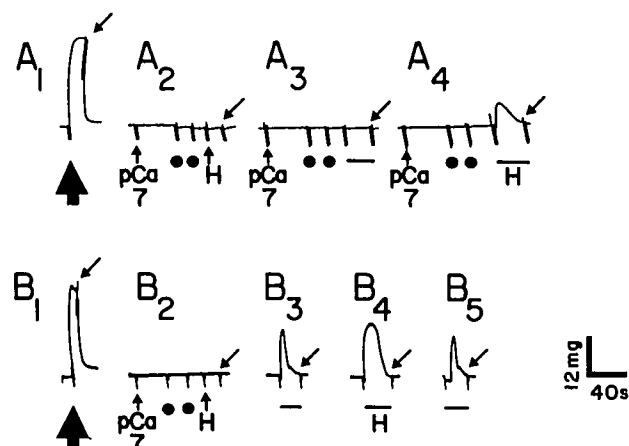


FIG. 2. CCs and HCCs in skinned muscle fibers. (A, B) Tension recordings from two different fibers studied at 22° C, except during the cooling periods, which are indicated by the bars under the recordings. Vertical arrows indicate the addition of 0.5 mM CaCl<sub>2</sub> to the medium. (A<sub>1</sub>, B<sub>1</sub>) P<sub>O</sub> elicited by 0.5 mM CaCl<sub>2</sub>. Solution R was used to relax the fiber (slanted arrows). In each of the other panels, Ca<sup>2+</sup> was loaded into the SR during a 1-min soaking in pCa 7.0. The loading period is not shown in B<sub>3</sub>–B<sub>5</sub>. After two washes with W (filled circles), 0.65 mM halothane (H) and/or cooling (12° C in A and 8° C in B) was used to release the SR-stored Ca<sup>2+</sup>. Further description is given in the text.

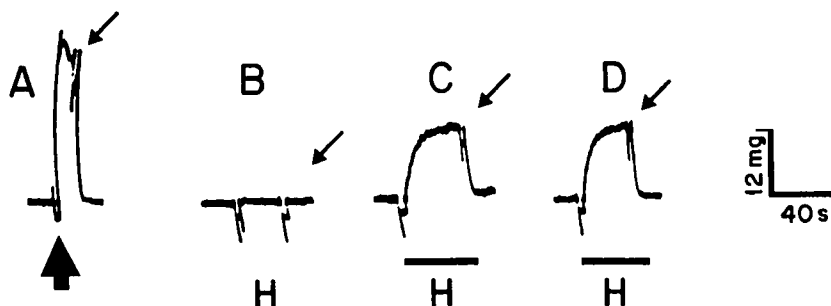


FIG. 3. HCCs in a skinned fiber loaded with Ca oxalate. (A)  $P_{O_2}$  elicited by 0.5 mM  $CaCl_2$  (vertical arrow). Solution R was used to relax the fiber (slanted arrows). Between A and B, the fiber was soaked for 30 min in a solution of  $pCa$  7.0, containing 5 mM K oxalate. (B–D) Effects of 0.93 mM halothane (H) at 22° C (B) or at 12° C (C, D; the cooling period is indicated by the horizontal bars). Further description is given in the text.

9.3 mM) had no significant effect on the amplitude or time course of submaximal ( $pCa$ , 5.6)  $Ca^{2+}$ -induced tensions studied at 8–14° C ( $n = 4$ ).

### Discussion

The current results confirm previous observations on other mammalian skinned muscle fibers<sup>10,12,13</sup> that halothane can elicit  $Ca^{2+}$  release from the SR of  $Ca^{2+}$ -loaded fibers. In our experiments, the most sensitive rabbit EDL fibers developed contractures when exposed at room temperature to 0.46 mM halothane. Halothane-induced contractures of mammalian skinned fibers are thought to result from release of SR-stored  $Ca^{2+}$ , possibly by facilitation of the  $Ca^{2+}$ -induced  $Ca^{2+}$  release mechanism.<sup>10,12</sup> Although halothane can inhibit the ATP-dependent uptake of  $Ca^{2+}$  by the SR of rabbit skeletal muscles,<sup>13,14</sup> our data suggest that this effect plays a negligible role in the halothane-induced contractures of skinned fibers. Thus, in our experiments, reduction of the steady-state accumulation of  $Ca^{2+}$  into the SR required halothane concentrations above 4.6 mM, whereas 80% of the fibers con-

tracted when exposed to 2.8 mM halothane. Endo *et al.*<sup>10</sup> reported that 0.01% v/v halothane (0.93 mM) caused a small reduction of the steady-state accumulation of  $Ca^{2+}$  in the SR of human skinned fibers and attributed this to the enhancement of the  $Ca^{2+}$ -induced  $Ca^{2+}$  release mechanism. A similar explanation might account for our results.

Cooling *per se* elicited tension in rabbit skinned muscle fibers. Models proposed to explain rapid cooling contractures assume that tension development is modulated by the rates of  $Ca^{2+}$  uptake and release by the SR at low temperature.<sup>15,§</sup> Tensions will occur whenever the cooling-induced increase of the rate of  $Ca^{2+}$  release exceeds the rate of the ATP-dependent  $Ca^{2+}$  uptake, which is reduced by cooling.<sup>15,16</sup> Our results indicate that in rabbit EDL this occurs at temperatures below 8–10° C, and accordingly, CC can be elicited at these temperatures. This is in striking contrast with frog skinned fibers that failed to develop CCs at 2° C; in frog fibers, however, contractures could be elicited upon cooling in the presence of caffeine, which increases  $Ca^{2+}$  release from the SR at low temperatures.<sup>14</sup>

Halothane concentrations that had no contractile effects at room temperature potentiated the CCs of the skinned EDL fibers, and HCCs could thus be elicited. The time course of these HCCs depended on the  $Ca^{2+}$  loading protocol used. Transient contractures were observed when the SR was loaded with  $Ca^{2+}$  in the absence of K oxalate. This may be attributed to depletion of halothane-releasable  $Ca^{2+}$  in the SR.<sup>2,3</sup> Consistent with this interpretation are our observations that skinned fibers loaded with Ca oxalate sustained the HCCs at peak amplitude and were capable of developing HCCs repeatedly without the need of additional  $Ca^{2+}$  loading. Because the SR in fibers loaded with Ca oxalate has a much higher Ca content than in fibers loaded without Ca oxalate,<sup>8,11</sup>  $Ca^{2+}$  depletion is unlikely to occur during the HCCs. A similar situation was previously reported in relation to the caffeine-induced contractures of rabbit skinned EDL muscle fibers.<sup>8</sup>

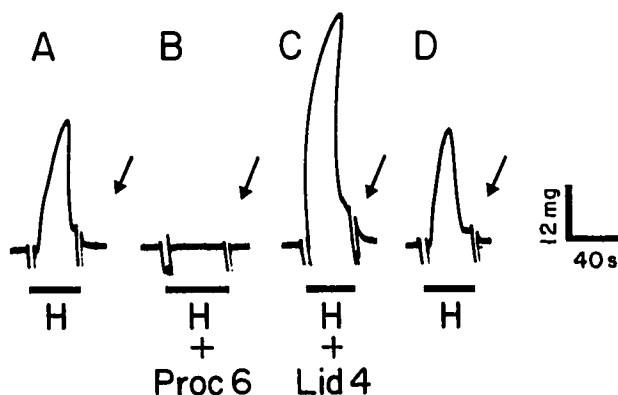


FIG. 4. Effects of procaine and lidocaine on the HCCs. HCCs elicited by exposure of  $Ca^{2+}$ -loaded fibers to 0.65 mM halothane (H, horizontal bars) at 13° C.  $Ca^{2+}$  loading was obtained by soaking the fiber for 1 min at 22° C in a solution of  $pCa$  7.0, before each challenge with halothane. Solution R (slanted arrows) was used to relax the fiber after the HCC. (A, D) Control HCC. (B, C) 6 mM procaine (Proc6) (B) or 6 mM lidocaine, (Lid4) (C) was applied for 30 sec before and also during the exposure to halothane. Further description is given in the text.

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The blockade of the HCCs of the skinned fibers by procaine and their potentiation by lidocaine are consistent with the effects of these local anesthetics on the HCCs of intact frog and mammalian muscles.<sup>1-3</sup> Procaine is a potent inhibitor of the Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release mechanism,<sup>17</sup> and this effect is thought to account for the block of cooling-induced contractures in the absence of halothane<sup>15,¶</sup> and for the inhibition of the halothane-induced tensions at room temperature.<sup>12</sup> The current study does not provide direct evidence for a similar mechanism being responsible for the blockade of the HCC of the skinned fibers by procaine. It is interesting, however, that the HCCs were potentiated rather than inhibited by lidocaine, which does not mimic the inhibitory effects of procaine on the Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release mechanism.<sup>17</sup> We emphasize that the concentrations of procaine and lidocaine required for modifying the HCCs of the skinned fibers (2-6 mM) are considerably higher than those present clinically.

The HCCs of the skinned fibers cannot be ascribed to an increased response of the contractile proteins to Ca<sup>2+</sup> because cooling *per se* inhibited the Ca<sup>2+</sup>-induced tensions whereas halothane did not affect the tensions induced by buffered Ca<sup>2+</sup> solutions at low temperatures. The latter observation is consistent with previous results from skinned skeletal muscle fibers studied at room temperature.<sup>10,12</sup>

In conclusion, our results show that HCCs can be elicited in mammalian skinned muscle fibers exposed to halothane concentrations within the range employed for general anesthesia in humans. These HCCs exhibit a number of characteristics typical of the HCCs of intact mammalian muscles, including those from humans.<sup>3</sup> Halothane is the anesthetic most frequently involved in the triggering of episodes of malignant hyperthermia (MH) in susceptible individuals. A distinctive characteristic of the HCCs is that they occur in the presence of clinically relevant concentrations of halothane. Because the SR of MH-susceptible muscles is more sensitive to the Ca<sup>2+</sup> releasing effects of halothane than normal muscle<sup>10</sup> and because cooling markedly enhances this effect of halothane, we would predict that lower concentrations of halothane, possibly equivalent to those present clinically, will elicit HCCs in MH-susceptible muscle, as compared to normal muscle. We are currently testing this prediction by comparing the results of the halothane-contraction test for MH performed at 37° C<sup>18,19</sup> with the ability of muscle strips or skinned fibers from the same human muscle biopsies to develop HCCs.

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