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Shortening velocity of skeletal muscle from humans with malignant hyperthermia susceptibility: effects of halothane

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Abstract

The aim of this investigation was to assess the effect of halothane on the velocity of shortening and lengthening of muscle from normal subjects and from patients with malignant hyperthermia susceptibility. Strips were mounted horizontally at optimal length in normal Krebs–Ringer's solution and mechanical parameters were obtained before and after exposure to 3 vol.% halothane. The maximun shortening velocity at zero load (V_{max}) was determined by using Hill's characteristic equation. The contraction and relaxation indices were measured under isotonic and isometric conditions: maximum shortening and lengthening velocities ($\max V_c$ and $\max V_r$, respectively); isometric peak twitch tension; peak of the positive ($+dP/dt_{max}$) and negative ($-dP/dt_{max}$) twitch tension derivative; ratio R1 = $\max V_c/\max V_r$ and ratio R2 = $(+dP/dt_{max})/(-dP/dt_{max})$. In normal muscle, halothane markedly increased V_{max}, $\max V_c$ and peak twitch tension by $30 \pm 10\%$, $30 \pm 5\%$ and $40 \pm 15\%$, respectively. The $\max V_r$ values increased concomitantly with the $\max V_c$ values, such that no change in the ratio R1 was observed. Both $+dP/dt_{max}$ and $-dP/dt_{max}$ increased such that the ratio R2 did not vary. In malignant hyperthermia susceptibility muscle, halothane induced a significant decrease in V_{max} ($-30 \pm 10\%$) and $\max V_r$ ($-45 \pm 15\%$) without changing $\max V_c$. The decrease in $\max V_r$ was greater than that of $\max V_c$, such that the ratio R1 increased significantly. Peak twitch tension and $+dP/dt_{max}$ remained unchanged whereas $-dP/dt_{max}$ decreased significantly; the ratio R2 increased by $40 \pm 10\%$. These results suggest that halothane alters the contractile properties of malignant hyperthermia susceptibility muscle.

Keywords: Malignant hyperthermia; Shortening velocity; Skeletal muscle

1. Introduction

Crises of malignant hyperthermia are characterized by hypercatabolism and stiffness of the skeletal muscle. They can be observed in predisposed humans and animals exposed to triggering agents such as halothane or succinylcholine (Adnet et al., 1991; Williams et al., 1991). Muscle fiber bundles exhibit a lower threshold of contracture in response to halothane or caffeine in humans or animals that are susceptible to malignant hyperthermia but not in normal humans or animals (Williams et al., 1991; Adnet et al., 1993). Abnormal intracellular calcium regulation has been proposed as the primary defect in malignant hyperthermia susceptibility muscle (Mickelson et al., 1988; Salviati et al., 1989; Shomer et al., 1993).

In vivo, muscle works against various conditions of tension. Most interventions such as neurohumoral and other factors cause concomitant changes in muscle length and muscle force development. Such interventions may have an effect on power output. However, the in vitro diagnostic test for malignant hyperthermia susceptibility has been extensively performed under isometric twitch conditions (European Malignant Hyperpyrexia Group, 1984; Adnet et al., 1991) that are quite dissimilar from those in vivo. In isolated muscle preparations in which muscle length and load are independently controlled, power output depends not only on the isometric tension generated, but also on the speed of muscle shortening. Likewise, the tension developed by skeletal muscle during shortening or lengthening is modified by changes in muscle length (Huxley, 1957; Jewel and Wilkie, 1960; Coirault et al., 1994). Indeed, there is little information concerning the tension changes that occur during the lengthening and

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shortening of malignant hyperthermia susceptibility muscle.

The purpose of this study was to define the mechanical determinant of contraction and relaxation over the entire load continuum in malignant hyperthermia susceptibility muscle. We established the effect of halothane on shortening and lengthening velocities of strips of vastus external muscle from malignant hyperthermia susceptibility patients.

2. Materials and methods

2.1. In vitro contracture tests

Fourteen patients were selected for diagnostic muscle biopsy based on suspicion of susceptibility to malignant hyperthermia. After approval by the Lille University Human Studies Committee and informed consent was obtained from the patients, extra muscle was removed. Halothane and caffeine contracture tests were performed as previously described (Adnet et al., 1991). The muscle used for the diagnostic test was the vastus lateralis. All patients were investigated according to the protocol supported by the European Malignant Hyperpyrexia Group. The criteria of malignant hyperthermia susceptibility were an increase in resting tension of at least 0.2 g both with a halothane threshold concentration $\leq 2\%$ (in the gas phase) and a caffeine threshold concentration ≤ 2 mM. A normal response was defined as a halothane threshold $\geq 2\%$ and a caffeine threshold ≥ 2 mM. Other results (i.e., one abnormal response either with halothane or with caffeine) were classified as malignant hyperthermia equivocal, but these patients were not included in this study since the significance of these results is not known.

2.2. Solutions

For all experiments, a Krebs–Ringer solution containing (in mM) 118.1 NaCl, 3.4 KCl, 2.5 $CaCl_2$, 0.8 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 glucose was used. The pH was 7.35 ± 0.05 and the solution was bubbled with preheated 5% carbon dioxide in oxygen. The halothane concentration was administered by bubbling the muscle bath with a calibrated vaporizer (Fluotec Mark III). The anesthetic concentration in the gas phase was monitored continuously with an infrared calibrated analyzer (Normac R, Datex). The anesthetic concentration in the Krebs–Ringer solution was measured by gas–liquid chromatography (GLC), using a varian 1400 R gas chromatograph equipped with a flame ionization detector and a Porapack QR 3.17 mm by 150 cm column (Imbenotte et al., 1987).

The anesthetic concentration measured in the experimental solution after continuous bubbling was 3 vol.% or 0.70 ± 0.05 mM.

2.3. Muscle preparation

The specimens were taken from the vastus lateralis muscle. Segments of muscle bundles (18–22 mm in length and 2–3 mm in diameter) were carefully dissected from the specimens. Since transected fibers were used for the study, pre-drug resting membrane potentials were recorded in each muscle fiber bundle as previously described (Adnet et al., 1992). All fiber segments tested (at least 15 per muscle bundle) had resting membrane potentials ranging between -80 and -85 mV, indicating that there was no significant transection-induced depolarization of the fibers. Muscle bundles were then mounted in a temperature-controlled (37°C) muscle chamber (4 ml), which was perfused continuously (4–5 $ml.min^{-1}$) with Krebs–Ringer solution. One end of the muscle bundle was pinned to the silicon bottom of the muscle chamber and the other end was attached by a thin silk thread to a force transducer and a servo controlled motor (Bioscience Dynamometer UFI and Biological Amplifier 120). The preparations were stimulated directly by means of silver electrodes with rectangular current pulses of 1 ms duration delivered at a frequency of 0.2 Hz by a stimulator CEA-DAM model GPI-GE2198. Length displacement was recorded on a Gould model type 4035 oscillographic recording system. The L_{max} was obtained by varying the preload imposed on the muscle and, hence, the length and by measuring the resultant isometric tetanic tension; L_{max} was defined as the length at which P_t developed. The isometric tetanic tension was obtained by stimulation at 20 Hz (train duration 300 ms).

2.4. Experimental set up

Surplus muscles were used to perform this study. After dissection and mounting, muscles were equilibrated in Krebs–Ringer for 30 min. Strips were then set at L_{max} and the shortening velocity was measured against 8–10 different levels of loads (P), ranging from preload up to the fully isometric contraction. After completion of the initial series of loaded contractions, the bath was bubbled with 3 vol.% halothane in Krebs–Ringer solution. After a 10- to 15-min equilibration period, a second series of 8–10 isotonic contractions was measured. A final series of 8–10 afterload contractions was measured with a solution free of halothane. The contracture that occurs during exposure of malignant hyperthermia susceptibility muscle to halothane disappears in a solution free of halothane. The intrinsic instability of the muscle varied slightly over 2-h intervals, the time required to complete an experiment. For data quality control, we selected only experiments in which the initial load was $< 10\%$ at the end of the experiments. The mechanical parameters characterizing the contraction and relaxation phases under isotonic and isometric conditions are defined as follows (Fig. 1) : maximum shortening and lengthening velocities ($\max V_c$ and $\max V_r$, L_{max} s^{-1}, respectively); maximal amplitude of shortening (Δl_{max});

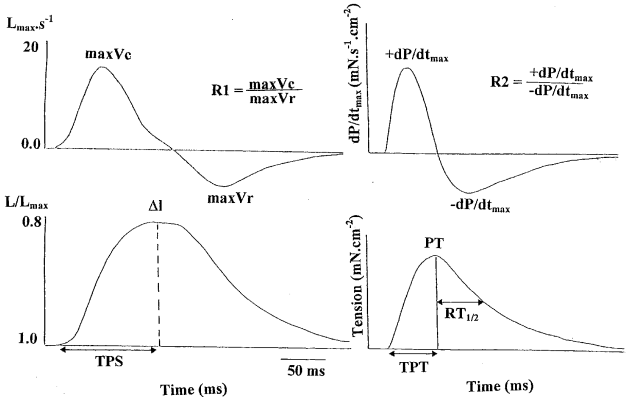

Fig. 1. The method used to obtain mechanical parameters for normal and malignant hyperthermia susceptibility muscle (left, under isotonic conditions); $\max V_c$ and $\max V_r$, maximum shortening and lengthening velocities, respectively; Δl, maximal amplitude of shortening; TPS, time to peak shortening of the isotonic twitch (right, under isometric conditions); PT, isometric peak twitch tension; TPT, time to peak tension; $RT_{1/2}$, half-relaxation time. dP/dt_{max}, peak of isometric force derivative (+, positive and −, negative). Coefficients R1 and R2 reflect contraction–relaxation coupling at low and heavy load, respectively.

time to peak shortening of the isotonic twitch (TPS, ms); isometric peak twitch tension (PT) normalized per cross-sectional area (PT, $mN.cm^{-2}$); peak of the positive ($+dP/dt_{max}$, $mN\ s^{-1}\ cm^{-2}$) and negative ($-dP/dt_{max}$, $mN\ s^{-1}\ cm^{-2}$) twitch tension derivative per cross-sectional area; time to peak tension (TPT, ms); the half-relaxation time ($RT_{1/2}$, ms) from the time of isometric peak active tension to the time to 50% decline of the isometric peak active twitch tension. Since the relaxation rate depends on the contraction rate, simultaneous variations in the two phases should be considered in order to quantify changes in the relaxation phase. Hence, two indices were used to quantify contraction–relaxation coupling, namely the coefficient $R1 = \max V_c / \max V_r$ and the coefficient $R2 = (+dP/dt_{max})/(-dP/dt_{max})$. The coefficients R1 and R2 reflected contraction–relaxation coupling at low and heavy load, respectively (Hervé et al., 1988).

The maximum shortening velocity ($\max V_c$) obtained from a series of afterload contractions was plotted against the isotonic total P normalized for the value of the fully isometric (P_t). Data were fitted according to Hill's characteristic equation (Hill, 1938)

$$(P + a)(\max Vc + b) = (P_t + a)b$$

where P is the total isotonic value, P_t is the value of the fully isometric twitch tension, $-a$ and $-b$ are the asymptotes of Hill's hyperbolar. Maximum unloaded shortening velocity (V_{max}, $L_{max}\ s^{-1}$) was obtained indirectly by extrapolation of the force–velocity curve to zero load.

At the end of the experiments, each muscle strip was blotted dry and weighed and its cross-sectional area was calculated as the ratio of muscle weight to muscle length at L_{max}, assuming a muscle density of 1.06. The characteristics of the studied muscle strips were as follows: L_{max}, 20.5 ± 0.5 mm; cross-sectional area, 0.07 ± 0.01 cm^2, respectively. Isometric tetanic tension at L_{max} was 11.5 ± 1.5 N cm^{-2}. No difference were observed between normal and malignant hyperthermia susceptibility muscle strips. Data are expressed as means ± S.E.; after analysis of variance, Student's paired and unpaired t-tests were used to compare mean values. Values of $P < 0.05$ were regarded as significant.

3. Results

The contracture test results of the 14 patients are shown in Table 1. Six of the 14 patients were diagnosed as being malignant hyperthermia susceptibility on the basis of halothane and caffeine-induced muscle bundle contracture. In normal muscle, halothane did not induce contracture, whereas malignant hyperthermia susceptibility muscle developed a contracture after exposure to increasing concentrations of halothane (0.5 to 3 vol.%) (Fig. 2).

3.1. Mechanical properties under control conditions and during halothane exposure at preload only

The main mechanical parameters are listed in Table 2. In the absence of halothane, isometric peak twitch tension, time to peak tension, maximum shortening velocity, time to peak shortening of the isotonic twitch and peak of the positive twitch isometric tension derivative were comparable between groups of normal and malignant hyperthermia susceptibility muscle strips. Peak shortened length of the isotonic twitch contraction loaded with preload only at L_{max} was 0.30 ± 0.07% and 0.40 ± 0.10% L_{max} in normal and malignant hyperthermia susceptibility muscle strips, respectively. When the relaxation phases of the two groups were compared, the maximal lengthening velocity, the half-relaxation time and the peak of the negative twitch isometric tension derivative were similar in normal and

Table 1
Contracture responses of patients diagnosed as normal muscle or malignant hyperthermia susceptibility muscle by halothane and caffeine contracture test
Values are means ± S.E.M.; n, number of patients. Halothane I represents the maximun contracture after exposure to 2 vol.% halothane performed according to the European malignant hypertermia protocol. Caffeine I represents the maximum contracture after exposure to 2 mM caffeine. MHS, muscle susceptible to malignant hyperthermia; MHN, normal muscle.

Diagnosis (n)	Contracture (g)	
	Halothane I	Caffeine I
MHN (8)	0.0	0.0
MHS (6)	0.80 ± 0.10[a]	0.4 ± 0.3[a]

[a] $P < 0.05$, significantly different from control value.

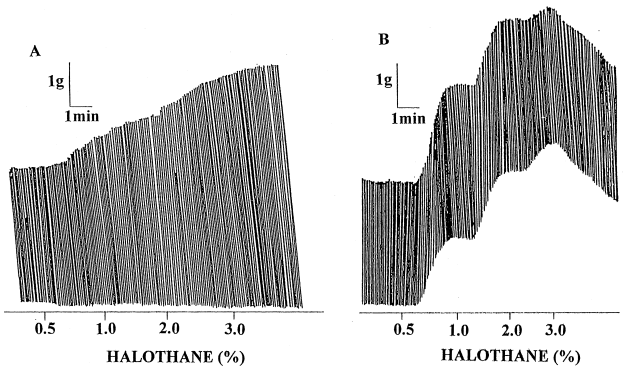

Fig. 2. Typical curve for the effect of halothane on skeletal muscle in vitro: (A) in normal muscle halothane exposure did not induce muscle contracture, (B) the malignant hyperthermia susceptibility muscle bundle developed a significant contracture (≥ 0.2 g) at 2 vol.% halothane.

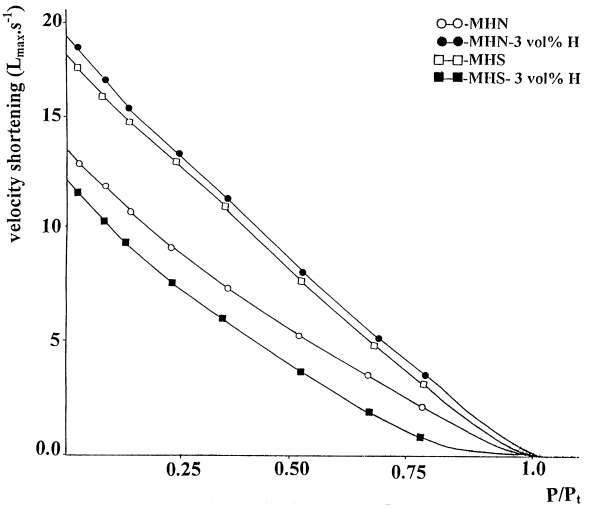

Fig. 3. Isotonic force–velocity relationships in a representative muscle strip before halothane (circle open), after exposure to 3 vol.% halothane (circle close) in normal muscle group (MHN), and before halothane (square open), after exposure to 3 vol.% halothane (square close) in malignant hyperthermia susceptibility muscle group (MHS). Maximum shortening velocity (maxV_c) obtained from a series of afterload contractions was plotted against the isotonic total P normalized to the value of the fully isometric (P_t); V_{max}, maximun unloaded shortening velocity obtained by extrapolation of the force–velocity curve to zero load. Values are means.

malignant hyperthermia susceptibility muscles. Maximal shortening velocity was higher than maximal lengthening velocity, resulting in a ratio R1 of 2.6 ± 0.3 and 2.5 ± 0.2 in malignant hyperthermia susceptibility and normal muscle strips, respectively. The peak of the negative twitch isometric tension derivative was lower than the peak of the positive twitch isometric tension derivative, as resulting a ratio R2 of 2.20 ± 0.16 and 1.96 ± 0.14 in malignant hyperthermia susceptibility and normal muscle strips, respectively.

Exposure to 3 vol.% halothane markedly increased the contractile performance in normal muscle. After 10 min, both shortening and lengthening velocities increased in the same proportion, such that the ratio R1 did not vary significantly. The isometric peak twitch tension increased by 140 ± 15%, whereas the peak shortened length of the isotonic twitch did not change. The peak of the positive twitch isometric tension derivative values increased concomitantly with the peak of the negative twitch isometric

Table 2
Contractility, contraction–relaxation coupling values before and after exposure to 3 vol.% halothane at preload only
Values are means ± S.E. obtained before and after exposure to 3 vol.% halothane. Halothane values were measured 10 min after exposure to 3 vol.% halothane. See text for explanation of abbreviation.

	MHN ($n = 8$)		MHS ($n = 6$)	
	Before	After	Before	After
Contraction phase				
V_{max}, L_{max} s^{-1}	14 ± 2	18 ± 1^a	17 ± 2	13 ± 2^a
maxV_c, L_{max} s^{-1}	11.20 ± 2.30	15.20 ± 2.0^a	15.40 ± 3.60	13.80 ± 3.50
Δl, % L_{max}	0.30 ± 0.07	0.50 ± 0.08^a	0.44 ± 0.10	0.42 ± 0.12
TPS, ms	92 ± 4	110 ± 7	92 ± 4	102 ± 6
TPT, ms	80 ± 5	82 ± 5	79 ± 4	80 ± 5
PT, mN cm^{-2}	452 ± 60	602 ± 63^a	408 ± 60	423 ± 44
$+dP/dt_{max}$, mN s^{-1} cm^{-2}	7611 ± 812	$10{,}374 \pm 1003^a$	7406 ± 1321	8019 ± 1025
Relaxation phase				
maxV_r, L_{max} s^{-1}	4.70 ± 1.10	6.30 ± 1.10^a	6.90 ± 2.0	3.50 ± 1.30^a
$-dP/dt_{max}$, mN s^{-1} cm^{-2}	3663 ± 347	5036 ± 594^a	3362 ± 547	2505 ± 294^{ab}
$RT_{1/2}$, ms	80 ± 8	82 ± 4	78 ± 7	117 ± 11^{ab}
Contraction–relaxation coupling				
R1	2.50 ± 0.20	2.30 ± 0.20	2.60 ± 0.30	3.60 ± 0.40^{ab}
R2	1.96 ± 0.14	2.14 ± 0.15	2.20 ± 0.16	3.18 ± 0.24^{ab}

[a] $P < 0.05$ vs. control within group of either normal muscle (MHN) or malignant hyperthermia susceptibility muscle (MHS).
[b] $P < 0.05$ between group of MHN and MHS.

tension derivative values; thus, no change in the ratio R2 was observed (Table 2). In malignant hyperthermia susceptibility muscle, no change of isometric contraction parameters was observed, whereas halothane significantly decreased both the shortening and the lengthening velocities (Table 2). The decrease in lengthening velocity appeared to be much greater than that in shortening velocity, so that the ratio R1 increased from 2.6 ± 0.3 to 3.6 ± 0.4. Also, the ratio R2 significantly increased from 2.20 ± 0.16 to 3.18 ± 0.24.

3.2. Effect of halothane on the force–velocity relationship

There was an inverse relationship between afterload and shortening velocity, both before and after halothane (Fig. 3). In malignant hyperthermia susceptibility muscle, the shortening velocity obtained at each afterload was higher than that obtained in normal muscle. In normal muscle, halothane changed the force–velocity relationship by increasing the shortening velocity over a wide range of relative afterloads. The extrapolated maximun unloading shortening velocity (V_{max}) increased significantly from 14 ± 2 to 18 ± 1 L_{max} s^{-1} (Fig. 3). In malignant hyperthermia susceptibility muscle, exposure to 3 vol.% halothane significantly decreased the extrapolated maximun unloading shortening velocity (V_{max}) from 17 ± 2 to 13 ± 2 L_{max} s^{-1}.

4. Discussion

The purpose of this study was to investigate the contractile behavior of malignant hyperthermia susceptibility muscle under isotonic and isometric loading conditions. Our results indicated that malignant hyperthermia susceptibility muscles generated and maintained high shortening and lengthening velocities with additional load. The maximun unloaded shortening velocity (V_{max}) in malignant hyperthermia susceptibility muscle was significantly higher than that obtained in normal muscle. However, in malignant hyperthermia susceptibility muscle, halothane exposure delayed the time course of relaxation without modifying the contraction phase, so that the ratios of contraction–relaxation increased.

In malignant hyperthermia susceptibility muscle, the low value of maxV_r and the increase in R1 strongly suggested an impairment of calcium movement through the membrane system, particularly the sarcoplasmic reticulum (Hervé et al., 1988). Indeed, the abnormal intracellular Ca^{2+} exchange (Ca-induced Ca release mechanism) reported by Endoh et al. (1983) would explain the increased sensitivity and rate of Ca^{2+} release from the sarcoplasmic reticulum in malignant hyperthermia susceptibility fibers (Ohta et al., 1989). Hence, elevated levels of residual Ca^{2+} could exist free or bound to Ca-binding proteins such as troponin. Thus, the increase in residual calcium concentrations in the myoplasm secondary to halothane exposure could explain the progressive decline in the relaxation rate correlate with the progressive change in resting tension (contracture) (Iaizzo et al., 1988; Adnet et al., 1998). This could contribute to increased passive stiffness according to the passive length–tension relation. The decrease in both lengthening velocity and maximal rate of isometric tension decline of malignant hyperthermia susceptibility muscle might be in part due to a decrease in calcium uptake by the sarcoplasmic reticulum. These alterations in sarcoplasmic reticulum uptake could be related to the increase in the calcium content of terminal cisternae of the sarcoplasmic reticulum in malignant hyperthermia susceptibility fibers, since calcium overload in the sarcoplasmic reticulum inhibits its pumping activity (Vitres et al., 1984). In addition, the intracellular acidosis caused by halothane-induced malignant hyperthermia susceptibility muscle contracture might also inhibit sarcoplasmic reticulum ATPase (Metzger and Fitts, 1987). It is unlikely that a decrease in the rate of Ca^{2+}-uptake by the sarcoplasmic reticulum contributes to the effect of halothane in decreasing the lengthening velocity and the maximal rate of tension decline because the slope of the initial part of loading time — Ca^{2+}-uptake curve is neither increased nor shifted to the left by halothane (Ohta et al., 1989). Likewise, a study performed on sarcoplasmic reticulum vesicles isolated from malignant hyperthermia susceptibility muscle and normal pigs showed that halothane stimulated the uptake of calcium by sarcoplasmic reticulum vesicles in malignant hyperthermia susceptibility muscle and no difference in the sensitivity of the two types of sarcoplasmic reticulum vesicles to halothane was observed (Louis et al., 1992).

Conversely, in normal muscle, the positive inotropic effect elicited by halothane was associated with a proportional improvement of lengthening velocity. Thus, no change in the R1 ratio was observed, indicating that there was a proportional increase in shortening and lengthening velocities. Indeed, the effect of halothane on the magnitude of the increase in the shortening velocity obtained under each load was higher than that obtained without halothane exposure. Muscle behavior under heavy loading conditions was not similar to that observed under low loading conditions. This mechanical property reflects the intrinsic capacity of muscle to control contractility according to the level of load. Both shortening and lengthening velocities increased linearly with the changes in loading condition : the lighter the load, the greater the extent of shortening and the faster the relaxation (Lecarpentier et al., 1979; Brutsaert et al., 1980; Coirault et al., 1994). Indeed, during a twitch, the ability to bear a given load is limited if the muscle is allowed to shorten: during shortening the affinity of troponin-C for calcium decreases, which results in less calcium being bound to myofilaments at the end of the shortening phase than at comparable time in the entire isometric contraction. Our results suggest that, during halothane-induced twitch potentiation, the muscle active state is enhanced.

It has been demonstrated that halothane causes a concentration-dependent positive inotropic effect in humans and in a variety of other mammalian species (Adnet et al., 1991; Williams et al., 1991; Etchrivi et al., 1994). Its positive inotropic effect has been attributed to interference with transsarcolemmal Ca^{2+} influx, but other findings argue against the notion that the effects of halothane are influenced, in part, by extracellular Ca^{2+}. Indeed, halothane has been reported to augment calcium transport across the sarcolemmal membrane of skeletal muscle and to increase calcium flux across the sarcoplasmic reticulum during muscle contraction (Ohta et al., 1989; Mickelson and Louis, 1996). Skeletal muscle shortening velocity depends on the rapidity of cross-bridge cycling which is, in turn a function of the level of contractile protein activation (Huxley, 1957; Brenner, 1988). It is likely that these effects combine to augment the activation of contractile proteins and are thought to account for the ability of halothane to augment muscle force output. However, both in malignant hyperthermia susceptibility and in normal muscles, it is unlikely that increased myofilament sensitivity to Ca^{2+} contributes to the inotropic and relaxant effects of halothane because the pCa–tension relationship is neither increased nor shifted to the left by halothane (Ohta et al., 1989). Thus during shortening if the sarcoplasmic reticulum capacity is intact, the ealier decline of the myoplasmic calcium transient would lead to an earlier relaxation (Housmans et al., 1983; Hofmann and Fuchs, 1987).

Our results differ from those of previous reports that indicate that halothane does not affect the twitch tension in normal muscle and potentiates it in MHS muscle (Gallant and Goettl, 1989; Gallant et al., 1980, 1986). This potentiation resulted from an increased rate of tension with no change in the time to peak tension. However, no significant effect was observed on the relaxation phase of twitchresponses (Gallant and Goettl, 1989). Only the tetanus relaxation of malignant hyperthermia susceptibility muscle was slowed by halothane, which led the authors to suggest that the calcium sequestrating mechanisms of malignant hyperthermia susceptibility pig muscles are adequate for removal of calcium released by a single stimulus but became overloaded or saturated during tetanus (Gallant et al., 1980).

Our method differs mainly by the use of cut fiber preparations. Hence, our finding that halothane delayed the time course of relaxation without modifying the contraction phase of malignant hyperthermia susceptibility muscle may be related less to the excitation–contraction coupling process per se than to the fatiguability of these cut fiber preparations. However, it has been demonstrated recently that such muscle cells may reseal with time and that these preparations may be far more physiological than appreciated in the past (Lehmann-Horn and Iaizoo, 1990).

Some limitations of our study have to be discussed. For technical reasons, maximum unloaded shortening velocity (V_{max}) cannot be measured directly by load clamping at zero load. V_{max} is obtained by extrapolation of force–velocity curves and is therefore sensitive to small changes in the slope of the force–velocity relation. The influence of the load on the effects of halothane on malignant hyperthermia susceptibility muscle contractility may critically depend on both experimental conditions and the model used. Experiments with fiber bundles from the vastus lateralis of the quadriceps have been shown to be physiologically relevant (European Malignant Hyperpyrexia Group, 1984; Adnet et al., 1991; Coirault et al., 1994), and the conditions of temperature and stimulation frequency used in our study were those commonly used in the in vitro diagnostic tests (European Malignant Hyperpyrexia Group, 1984; Adnet et al., 1991, 1992). Taking into account the close mechanical coupling that exists between contraction and relaxation, our results indicated that the relaxation phase would be more sensitive to halothane in malignant hyperthermia susceptibility muscle. The finding that V_{max} changed during halothane-induced contracture strongly suggests that the kinetics of cross-bridge function are indeed affected and that this change in cross-bridge performance may be attributed to a failure of the contractile system itself in malignant hyperthermia. However, we cannot exclude that intracellular metabolic changes that occur during muscle contracture, in particular the acccumulation of breakdown products of ATP, such as Mg-ADP, inorganic phosphate and hydrogen ions, may alter contractile performance. Taking into account these observations, malignant hyperthermia could cause a prolonged increase of intracellular calcium concentrations near to those needed to generate the force required to break cross-bridges.

To summarize, we demonstrated that the contractile properties of normal muscle are improved after exposure to halothane. Conversely in malignant hyperthermia susceptibility muscle, halothane caused an impairment of relaxation. This suggests that malignant hyperthermia susceptibility muscle will not return to its resting length rapidly enough, leading to an increase in the functional residual capacity. The mechanical abnormalities observed in this study might be related to sarcoplasmic reticulum dysfunction in malignant hyperthermia.

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