We studied the electrical properties of intact muscle fibers from normal and malignant hyperthermia-susceptible (MHS) pigs. Resting membrane potentials, action potentials, and current-voltage relationships were measured with and without the presence of halothane. There were no changes in the resting potentials or the specific membrane conductances at any concentration of halothane in either the normal or MHS fibers. The current-voltage relationships of normal and MHS fibers did not differ. Contractures were observed in MHS muscle when the concentration of halothane was ≥0.8%. These halothane-induced contractures were not associated with depolarization of the surface membrane. Contractures were not observed in normal muscle even at concentrations of 6.0% halothane. In contrast, halothane altered the shape of the action potentials of both MHS and normal fibers. However, these changes were significantly greater in MHS fibers, occurred at much lower concentrations, and were partially prevented by preincubation in 10 μM dantrolene.

Key words: malignant hyperthermia • resting potentials • action potentials • contracture • halothane • dantrolene

MALIGNANT HYPERThERMIA: EFFECTS OF HALOTHANE ON THE SURFACE MEMBRANE

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Malignant hyperthermia is initiated in predisposed humans and animals by volatile anesthetics such as halothane. Episodes of malignant hyperthermia are characterized by hypermetabolism and stiffness of the skeletal muscle and may result in a life-threatening crisis. In intact fibers from muscles susceptible to malignant hyperthermia (MHS), intracellular Ca²⁺ increases on application of halothane, and this increase is well correlated with the force of a concomitantly developing contracture.¹⁰ The source of this increased intracellular Ca²⁺ is thought to be the sarcoplasmic reticulum, because studies on skinned fibers and on isolated vesicles of the sarcoplasmic reticulum have revealed disorders in the regulation of Ca²⁺.³,⁴,⁵,¹⁵,¹⁶ Besides this explanation of the pathomechanism of malignant hyperthermia, it has also been suggested that depolarization of the surface membrane by anesthetics may be the initiating event for the contractures.⁵,⁷,⁸ This hypothesis was based on a report that 2.0% halothane caused a significant depolarization of intact MHS muscle fibers.⁵ Yet, there are other reports of unchanged resting membrane potentials of MHS fibers upon halothane exposure.¹,¹³ Therefore, it was of interest to further investigate this hypothesis.

The present experiments were designed to measure the electrical parameters of MHS muscle fibers during halothane-induced contractures. We wanted to determine whether there are indeed any changes in these parameters associated with the development of a malignant hyperthermic reaction. The electrical parameters were measured in the presence of a wide range of halothane concentrations (from 0.0 to >4.0%) and contrasted with those of normal muscle.
MATERIALS AND METHODS

Biopsies of external intercostal muscle were taken from 15 purebred Pietrain MHS pigs and 12 normal Yorkshire pigs. In several cases, biopsies of the common digital extensor muscles were also taken.

Several weeks prior to the biopsies the animals were classified as MHS or normal by a halothane challenge. The biopsy procedures were performed while the animals were mechanically ventilated and anesthetized with thiopental (Bio-tal, Bioceutic Lab., St. Joseph, MO). From each muscle, several small bundles of 50–100 fibers, intact from tendon to tendon, were prepared. One of these bundles was then suspended in a plexiglas chamber (2.0 ml) with one tendon fixed and the other fastened to force transducer (Akers Horten, Norway). Subsequently, a preparation was stretched 10–20% beyond its rest length. Hence, the fibers had striation spacings between 2.2 and 2.5 μm. Prior to the study, each preparation was allowed to equilibrate in the experimental chamber for approximately 60 min. During this period, if a preparation elicited a spontaneous contracture it was discarded. Each preparation was visually inspected throughout the experiment. Those preparations which appeared to have many cut fibers or many fibers with clots were discarded.

The bathing solution contained 135.0 mM NaCl, 4.0 mM KCl, 0.85 mM MgCl₂, 2.35 mM CaCl₂, 1.0 mM NaH₂PO₄, 12.0 mM NaHCO₃, 5.5 mM glucose, 7.6 mM sucrose, and 9.6 mM sodium gluconate. The pH was adjusted to 7.2 by gassing this solution with a mixture of 95% O₂ and 5% CO₂. All experiments were conducted at 37°C. Halothane was applied to the bathing solution via a fluorothane vaporizer (Fluotec, Fraser, Sweatman Inc., Buffalo, NY). The concentration of halothane in the bathing solution was determined as partial pressure in the bathing solution (10–6 mbar) by using sonication and exhaustible sampler. Samples of the bathing solution were pooled. Contractures were not observed in normal muscles even when the concentration of halothane in the bath reached the maximum levels (4–6%)

RESULTS

Whenever MHS muscles were exposed to halothane at bath concentrations ≥0.8%, they developed contractures. The amplitudes of the contractures recorded from the intercostal bundles were greater than those recorded from the extensor muscle (which may indicate a difference in contracture threshold). However, the changes in the action potentials due to halothane administration recorded from both muscles were identical and independent of the contracture amplitude. Thus, the action potential data from both muscles were pooled. Contractures were not observed in normal muscles even when the concentration of halothane in the bath reached the maximum levels (4–6%).

Resting Membrane Potentials. The resting potentials of MHS muscle fibers in normal bathing solution were not significantly different from those of normal muscle fibers: mean values were -82.7 ± 4.9 mV (n = 308; X ± SD) for MHS fibers and -83.4 ± 4.8 mV (n = 206) for normal fibers. This is illustrated in Fig. 1, which shows the mean resting membrane potentials recorded from MHS and normal fibers upon increasing concentrations of halothane and after the washout of halothane. There was no significant difference in any of these mean potential values. This held true when the comparisons were intragroup between the various halothane concentration ranges or intergroup be-
between the animal types ($P$ values $>0.25$). Dantrolene (10 $\mu$M) had no effect on the resting potential of the MHS fibers ($P > 0.25$). The mean resting potentials were $-82.2 \pm 5.2$ mV ($n = 9$) in the presence of dantrolene alone, $-81.3 \pm 2.6$ mV ($n = 34$) in the presence of dantrolene and 1.2–3.2% halothane, and $-80.8 \pm 3.7$ mV ($n = 16$) in the presence of dantrolene and $>3.2%$ halothane.

### Action Potentials

In the absence of halothane there was no difference between action potentials recorded from normal fibers or those from MHS fibers. Halothane altered the shape of action potentials recorded from both MHS and normal fibers by decreasing the maximum rate of rise, the peak, and the maximum rate of fall (see Table 1). However, the concentration of halothane required to cause such changes was much lower for the MHS fibers (see Fig. 2). Halothane had a minimal

![Figure 1](image1.png)

**FIGURE 1.** No effects of halothane on resting membrane potentials in either MHS or normal muscle. The mean resting membrane potentials ($\bar{x} \pm SD$) were plotted at zero halothane and at six graded halothane concentrations (bath partial pressure, %). The mean values before, during, and after halothane were not statistically different for each group or between groups ($P > 0.25$). The solid squares refer to the MHS muscle fibers and open squares to the normal fibers. The numbers next to the symbols represent the numbers of fibers which were sampled.

![Figure 2](image2.png)

**FIGURE 2.** The effects of halothane on action potentials elicited in MHS (A) and normal (B) intercostal muscle fibers. At low concentrations of halothane, the conformation of the action potentials of MHS fibers was more affected than those of normal fibers. Action potentials of normal fibers were not significantly altered until the concentration of halothane was $>3.2%$. In general, halothane caused a decrease in the rate of rise, peak potential, and the rate of repolarization.

<table>
<thead>
<tr>
<th>Table 1. Effects of halothane on action potentials.</th>
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<tbody>
<tr>
<td>Animal type</td>
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<td>Control conditions</td>
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<tr>
<td>Normal</td>
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<tr>
<td>MHS</td>
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<tr>
<td>Halothane 1.75–2.7%</td>
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<td>Normal</td>
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<td>MHS</td>
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<td>Halothane 2.7–3.2%</td>
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<td>MHS</td>
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<td>Halothane 2.75–2.7% + 10 $\mu$M Dantrolene</td>
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<td>MHS</td>
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<tr>
<td>Halothane 2.7–3.2% + 10 $\mu$M Dantrolene</td>
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<td>MHS</td>
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Note: Values represent means $\pm$ SD. Numbers of values are given in parentheses.
effect on the maximum rate of rise in action potentials of normal fibers but significantly slowed the rate in MHS fibers \((P < 0.01)\). Thus, the mean rates of rise were significantly different between the action potentials of MHS and normal fibers at all halothane concentrations above 1.75\% \((P < 0.01)\). Similarly, halothane had very little effect on the peak of the action potentials of normal fibers, but significantly reduced the peak of the action potentials of MHS fibers \((P < 0.01)\).

Halothane reduced the rate of repolarization in both MHS and normal fibers. The maximum repolarization rates were significantly different between the MHS and normal fibers at halothane concentrations between 1.75 and 3.2\% \((P < 0.01)\). At higher halothane concentrations (>3.2\%) the maximum rate of repolarization was not significantly different between MHS and normal fibers, because at these levels even the rates in normal fibers were significantly decreased \((P < 0.01)\). The parameter which was affected most by halothane was the total time required for the membrane to repolarize to 90\% of the prestimulus value (Fig. 3). The time required for 90\% repolarization was significantly increased in normal fibers when the concentration of halothane was greater than 3\% \((P < 0.01)\), but much lower halothane levels (<1\%) induced similar changes in MHS fibers.

At halothane concentrations >2.0\% the MHS fibers required up to 8 msec to repolarize to 90\% of the prestimulus membrane potential (see Fig. 3). The mean 90\% repolarization times were significantly different between the MHS and normal action potentials at halothane concentrations greater than 1.75\% \((P < 0.01)\). All halothane-induced changes were fully reversible after wash-out (e.g., Fig. 3). Occasionally, movement artefacts were noted when an action potential was elicited, but these movements occurred after the membrane had already repolarized.

Dantrolene, which is known to reverse MH episodes in vivo, also attenuated the effects of halothane on action potentials in vitro. At a given concentration of halothane, action potentials elicited in those MHS fibers pretreated with 10 \(\mu\)M dantrolene had higher rates of rise and fall than those elicited in fibers which were not treated (Table 1). In two preparations, the presence of dantrolene prevented the contracture response to halothane but did not totally eliminate the effects of halothane (1.2–3.0\%) on the action potentials (see Fig. 3). At very high halothane concentrations (>3.2\%), dantrolene had little or no protective value; contractures were present and action potentials were significantly altered \((P < 0.01)\).

### Specific Membrane Conductances and Steady-State Current-Voltage Relationships

No differences were observed between the specific membrane conductances (reciprocal value of the specific membrane resistance) of normal and MHS fibers. Halothane caused a consistent but statistically non-significant decrease of the specific membrane conductance in both normal and MHS fibers. The mean conductance values were 122.0 \(\pm\) 29.0 \(\mu\)S/cm\(^2\) \((n = 22)\) for MHS fibers and 117 \(\pm\) 47.0 \(\mu\)S/cm\(^2\) \((n = 50)\) for normal fibers in normal solution, and 99.0 \(\pm\) 26.0 \(\mu\)S/cm\(^2\) \((n = 12)\) for MHS fibers and 94.0 \(\pm\) 37.0 \(\mu\)S/cm\(^2\) \((n = 12)\) for normal fibers exposed to 0.7–2.8\% halothane.

The steady-state current-voltage relationships were calculated over a wide range of potentials (from −152 to −24 mV). No significant differences were observed between the relationships of normal and MHS fibers in the absence of halothane (Fig. 4), which indicated no difference in Cl\(^-\) or K\(_r\) conductance. Halothane (0.7–2.8\%) had no effect on the current-voltage relationship of normal fibers, but decreased the slope mea-
FIGURE 4. Current-voltage relationships of normal and MHS muscle fibers with and without halothane (0.7–2.8%). There was no difference between the current-voltage relationships for normal fibers without halothane, normal fibers with halothane, or MHS fibers without halothane. Only the current-voltage relationship of MHS fibers in halothane, at potentials more negative than −80 mV (anomalous rectification), was altered. Plotted points represent mean values.

SKELETAL MUSCLES OF A HUMAN OR SWINE SUSCEPTIBLE TO MALIGNANT HYPERTHERMIA MAY GO INTO CONTRACTURES UPON EXPOSURE TO VARIOUS ANESTHETIC AGENTS. It has been suggested that this anesthetic-induced reaction involves an initial depolarization of the surface membrane. However, we observed no change in resting potentials of MHS fibers due to halothane exposure. This was true even when we exposed these fibers to very high concentrations of halothane. Our observation is contrary to reports by Gallant and coworkers, but is consistent with previous reports by Bradley et al., Lopez et al., and more recent studies by Gallant. In addition, under rest conditions we did not detect any differences in action potentials or the current-voltage relationships (including the inward K+ rectifier) between normal and MHS fibers. Thus, we also did not confirm the report of an abnormal specific membrane resistance in MHS fibers. The reasons for these discrepancies are not clear but may be related to the condition of the muscle fibers at the time of the measurements. One needs to continuously monitor the condition of MHS preparations, because this muscle is injury sensitive and deteriorates more rapidly than normal muscle. Thus, in the present study we used more than one criterion to determine the viability of a given preparation. For example, we monitored isometric force simultaneously and noted that upon washout of halothane the contractures completely reversed. Nevertheless, it is now apparent that depolarization is not required for contracture development in MHS fibers.

Halothane altered the shape of action potentials of both normal and MHS muscle. However, an elevated intracellular Ca2+ concentration above the mechanical threshold was not required to produce such changes; they were detected in the absence of contractures (e.g., in normal fibers or in MHS fibers in the presence of dantrolene). This result is consistent with those of others dealing with the effects of volatile anesthetics on action potential characteristics in normal muscle.

Halothane had an effect on the surface membrane which was independent of a malignant hyperthermic reaction (e.g., contractures), but the magnitude of these effects may be amplified by an episode. We detected significant differences in the effects of halothane on action potentials from MHS and normal fibers. Action potentials of normal fibers were not altered until higher halothane levels were achieved and the K+ currents and/or the inactivation of Na+ channels appeared to be most significantly affected.

In MHS fibers, low halothane concentrations significantly altered all phases of the action potentials (rise, peak, and fall). This would indicate that halothane alters both the time-dependent Na+ and K+ currents in these MHS fibers. It was previously reported that the extent of dysfunction related to malignant hyperthermia is different between the intercostal and common digital extensor muscles. However, we observed no difference in
the effects of halothane on the action potentials of these muscles, but we did note larger amplitude contractures in the intercostal preparations.

The most pronounced difference between the effects of halothane on the action potentials recorded from the normal and MHS fibers was in the rate of repolarization. This very slow repolarization (up to 8 msec) in MHS fibers could be due to one or more of the following possibilities: (1) an abnormal inactivation of Na+ channels, (2) an abnormal K+ current, or (3) an increased Ca2+ current. In addition, we suggest that more than one type of K+ current may be affected. For example, the difference in the current-voltage relationships between normal and MHS muscle fibers in the presence of halothane, in the range of potentials more negative than -80 mV, may indicate a reduced activity of the anomalous rectifier. In other words, halothane administration reduced transverse tubular potassium conductance without affecting the resting membrane potential.

We do not believe that these halothane-induced changes in action potentials of MHS fibers are involved in the initiation of malignant hyperthermic episodes. However, the slowing of the rates of rise and/or fall of the action potentials could contribute to the halothane-induced twitch potentiation observed in both MHS and normal muscles. In such prolonged action potentials, the time during which the membrane potential remains above the mechanical threshold would be lengthened, and this in turn would produce increased twitch tension.

We conclude that contracture development in MHS muscle is not related to alterations of the surface membrane by anesthetic agents. Halothane did not alter steady-state properties of MHS or normal surface membranes. Halothane altered the dynamic electrical properties of the excitable membrane, and these effects were more pronounced in MHS skeletal muscle fibers but were not related to the occurrence of a contracture.

REFERENCES