

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 $\geq 0.8\%$. 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 $10 \mu M$ dantrolene.

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

MUSCLE & NERVE 12:178-183 1989

MALIGNANT HYPERTHERMIA: EFFECTS OF HALOTHANE ON THE SURFACE MEMBRANE

PAUL A. IAIZZO, PhD, FRANK LEHMANN-HORN, MD, MS, STUART R. TAYLOR, PhD, and ESTHER M. GALLANT, PhD

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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} .^{3,9,15,16} 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.

From the Department of Pharmacology, Mayo Foundation, Rochester, MN, 55905 USA (Drs. Iaizzo, Lehmann-Horn, and Taylor), and the Department of Veterinary Biology, University of Minnesota, St. Paul, Minnesota 55108 (Drs. Lehmann-Horn and Gallant).

Acknowledgments: We are very grateful to Dr. R.A. Van Dyke for the determination of the halothane concentrations, to Dr. W.E. Rempel for the supply of the halothane-tested pigs, and to Dr. R. Rüdell for his comments on the manuscript. Dr. Lehmann-Horn was a Visiting Associate Professor at the University of Minnesota and a Visiting Scientist at the Mayo Foundation. This work was supported by the Wilhelm-Sander-Stiftung, the University of Minnesota Graduate School and Swine Center and NIH grants HL 07111 and NS 22369.

Address reprint requests to Dr. Lehmann-Horn at Neurologische Klinik der Technischen Universität München, Möhlstrasse 28, D-8000 München 80, FRG.

Accepted for publication May 3, 1988.

0148-639X/1203/0178 \$04.00/6
© 1989 John Wiley & Sons, Inc.

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.¹⁴ The biopsy procedures were performed while the animals were mechanically ventilated and anesthetized with thiamylal (Bio-tal, Biocentric 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 MgCl_2 , 2.35 mM CaCl_2 , 1.0 mM NaH_2PO_4 , 12.0 mM NaHCO_3 , 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% O_2 and 5% CO_2 . All experiments were conducted at 37°C. Halothane was applied to the bathing solution via a fluothane vaporizer (Fluotec, Fraser, Sweetman Inc., Buffalo, NY). The concentration of halothane in the bathing solution was determined as previously described.¹⁹ Samples of the bathing solution were taken throughout each experiment. The number of aliquots which were taken (ranging between 5 and 20) depended on the duration of each experiment. Halothane concentration was expressed as partial pressure in the bathing solution. When dantrolene (Dantrium, Eaton Labs, Norwich, NY) was used, it was added to the bathing solution (10 μM) 30 min prior to the addition of halothane by using sonication and exhaustive stirring. We only investigated the electrical properties of the fibers on the surface (first layer) of a bundle. Membrane potentials were recorded with glass microelectrodes which had tip diameters of 0.5 μm , resistances of <15 M Ω , tip poten-

tials of <5 mV, and were filled with 3.0 M KCl. The microelectrodes were shielded and capacity compensated. Action potentials were initiated by brief pulses (0.1–0.5 msec) of depolarizing current injected via an intracellular microelectrode. A second microelectrode was used to record the action potentials. These two microelectrodes were separated by a distance of >500 μm . Each action potential was stored using a Nicolet 4094 storage oscilloscope (Madison, WI). Subsequently, the maximum rate of rise, peak potential, maximum rate of fall, and the time required for 90% repolarization of each response was determined. Force was registered simultaneously. Current-voltage relationships were determined with the three-microelectrode technique previously described.¹² The electrical properties of a muscle were not investigated during progressive contracture or relaxation.

Scheffe's multiple contrast (nonparametric) test was used to determine statistical differences within the data.

RESULTS

Whenever MHS muscles were exposed to halothane at bath concentrations $\geq 0.8\%$, 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 ($n = 308$; $\bar{X} \pm \text{SD}$) 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-

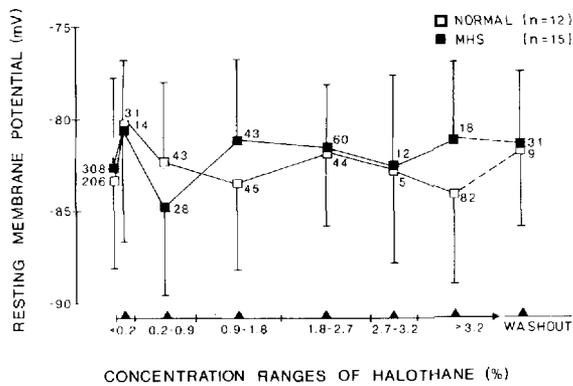


FIGURE 1. No effects of halothane on resting membrane potentials in either MHS or normal muscle. The mean resting membrane potentials ($\bar{X} \pm SD$) were plotted at zero halothane and at six graded halothane concentrations (bath partial pressure, %). The mean values before, during, and after halothane 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.

tween 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 ± 5.2 mV ($n = 9$) in the presence of dantrolene alone, -81.3 ± 2.6 mV ($n = 34$) in the presence of dantrolene and $1.2-3.2\%$ halothane, and -80.8 ± 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

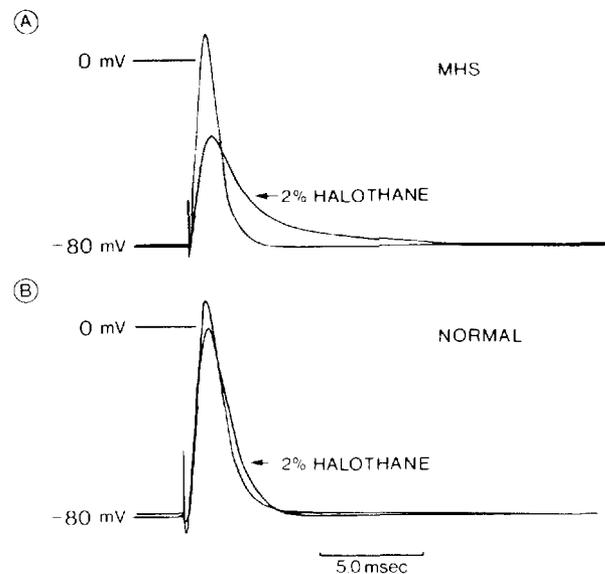


FIGURE 2. The effects of halothane on action potentials elicited in MHS (A) and normal (B) intercostal muscle fibers. At low concentrations of halothane, the conformation of the action potentials of MHS fibers was more affected than those of normal fibers. Action potentials of normal fibers were not 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.

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

Table 1. Effects of halothane on action potentials.

Animal type	Resting potential (mV)	Maximum of rise (V/sec)	Peak potentials (mV)	Maximum rate of repolarization (V/sec)
Control conditions				
Normal	-79.4 ± 4.0 (46)	372.9 ± 171.2 (30)	13.5 ± 15.5 (46)	103.4 ± 28.0 (29)
MHS	-81.0 ± 4.1 (107)	237.2 ± 101.8 (12)	10.0 ± 15.8 (107)	106.6 ± 36.8 (90)
Halothane 1.75–2.7%				
Normal	-81.8 ± 4.0 (47)	387.1 ± 196.9 (47)	13.5 ± 16.7 (47)	80.9 ± 28.6 (47)
MHS	-81.5 ± 3.4 (47)	171.4 ± 111.1 (37)	-2.1 ± 19.3 (47)	48.1 ± 19.2 (39)
Halothane 2.7–3.2%				
Normal	-82.8 ± 5.0 (12)	473.4 ± 221.8 (12)	11.0 ± 15.1 (12)	79.0 ± 38.1 (10)
MHS	-82.7 ± 4.8 (21)	106.4 ± 23.8 (15)	-15.6 ± 8.2 (21)	31.2 ± 9.7 (21)
Halothane 1.75–2.7% + $10 \mu M$ Dantrolene				
MHS	-79.2 ± 2.2 (23)	282.8 ± 94.9 (23)	12.07 ± 7.6 (23)	60.0 ± 18.7 (15)
Halothane 2.5–3.2% + $10 \mu M$ Dantrolene				
MHS	-81.8 ± 3.5 (12)	248.9 ± 123.5 (12)	2.7 ± 8.8 (12)	62.2 ± 22.5 (12)

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 mean 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 $10 \mu\text{M}$ 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 ($P < 0.01$).

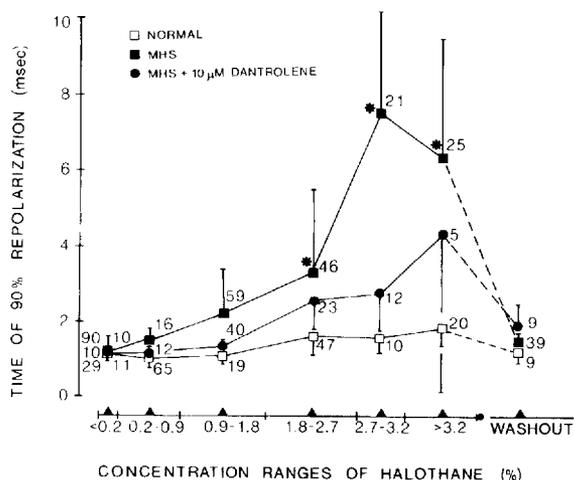


FIGURE 3. The 90% repolarization times of action potentials elicited in normal and MHS muscle fibers as a function of the halothane concentration. The mean repolarization time (msec, $\bar{X} \pm \text{SD}$) was plotted at zero halothane, at six graded halothane concentrations (both partial pressure, %), and after halothane washout. The solid squares refer to the MHS muscle fibers, open squares to the normal fibers, and solid circles to the effects of $10 \mu\text{M}$ dantrolene on the 90% repolarization time in MHS fibers. The 90% repolarization times were significantly different between normal and MHS action potentials at all halothane concentrations above 1.75% ($P < 0.01$). Dantrolene shortened the repolarization time of action potentials elicited in MHS fibers during halothane exposure. The numbers next to the symbols indicate how many responses were evaluated.

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 $122.0 \pm 29.0 \mu\text{S}/\text{cm}^2$ ($n = 22$) for MHS fibers and $117 \pm 47.0 \mu\text{S}/\text{cm}^2$ ($n = 50$) for normal fibers in normal solution, and $99.0 \pm 26.0 \mu\text{S}/\text{cm}^2$ ($n = 12$) for MHS fibers and $94.0 \pm 37.0 \mu\text{S}/\text{cm}^2$ ($n = 12$) 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_+ conductance. Halothane (0.7–2.8%) had no effect on the current-voltage relationship of normal fibers, but decreased the slope mea-

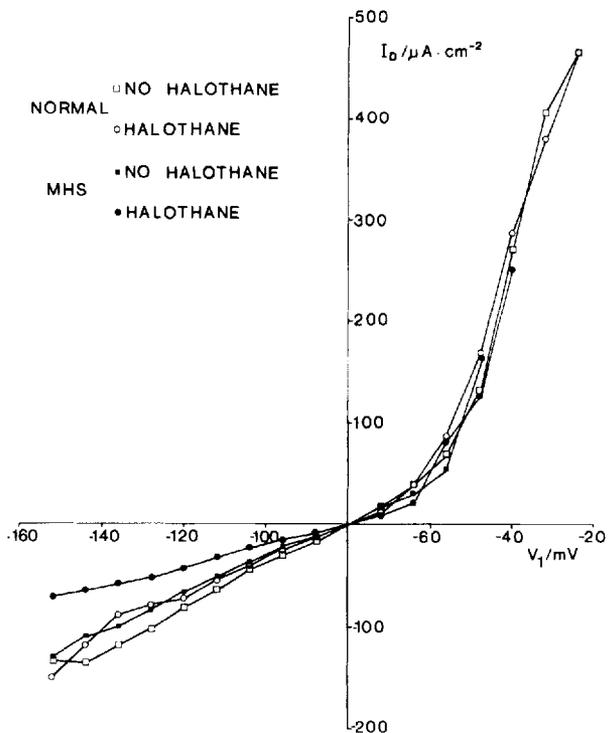


FIGURE 4. Current-voltage relationships of normal and MHS muscle fibers with and without halothane (0.7–2.8%). There was no difference between the current-voltage relationships for normal fibers without halothane, normal fibers with halothane, or MHS fibers without halothane. Only the current-voltage relationship of MHS fibers in halothane, at potentials more negative than -80 mV (anomalous rectification), was altered. Plotted points represent mean values.

sured from MHS fibers at potentials more negative than -80 mV. In the presence and absence of halothane the currents recorded from MHS fibers were statistically different only at -152 mV ($P < 0.01$). When halothane was present in the bath, the membrane potentials of MHS fibers could not be controlled by the voltage clamp unit in the potential range more positive than -50 mV, whereas in normal fibers depolarization was possible to -20 mV. Large movement artefacts occurred in MHS muscle fibers at potentials more positive than -50 mV.

DISCUSSION

Skeletal muscles of a human or swine susceptible to malignant hyperthermia may go into contractures upon exposure to various anesthetic agents.⁸ It has been suggested that this anesthetic-induced reaction involves an initial depolarization of the surface membrane.^{5,7,8} 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^{5,7}, but is consistent with previous reports by Bradley et al.,¹ Lopez et al.,¹³ and more recent studies by Gallant.^{3a} 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.² 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.⁴ 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 Ca^{2+} 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.^{11,18} 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.⁵ 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 Ca^{2+} 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.⁴ 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.¹⁷

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

1. Bradley WG, Ward M, Murchison D, Hall L, Woolf N: Clinical, electrophysiological and pathological studies on malignant hyperpyrexia. *Proc R Soc Med (Lond)* 66:67-68, 1973.
2. Bryant SH, Anderson IL: Mechanical activation and electrophysiological properties of intercostal muscle fibers from malignant-hyperthermia-susceptible (MHS) pigs. *Neurosci Abstr* 3:213, 1977.
3. Endo M, Yagi S, Ishizuka T, Horiuti K, Koga Y, Amaha K: Changes in the Ca-induced Ca release mechanism in the sarcoplasmic reticulum of the muscle from a patient with malignant hyperthermia. *Biomed Res* 4:83-89, 1983.
- 3a. Gallant EM: Porcine malignant hyperthermia: no role for plasmalemmal depolarization. *Muscle Nerve* 11:785-786, 1988.
4. Gallant EM, Fletcher TF, Goettl VM, Rempel WE: Porcine malignant hyperthermia: cell injury enhances halothane sensitivity of biopsies. *Muscle Nerve* 9:174-184, 1986.
5. Gallant EM, Godt RE, Gronert GA: Role of plasma membrane defect of skeletal muscle in malignant hyperthermia. *Muscle Nerve* 2:491-494, 1979.
6. Gallant EM, Godt RE, Gronert GA: Mechanical properties of normal and malignant hyperthermia susceptible porcine muscle: effects of halothane and other drugs. *J Pharmacol Exp Ther* 213:91-96, 1980.
7. Gallant EM, Gronert GA, Taylor SR: Cellular membrane potentials and contractile threshold in mammalian skeletal muscle susceptible to malignant hyperthermia. *Neurosci Lett* 28:181-186, 1982.
8. Gronert GA: Malignant hyperthermia, in Engel AG, Banker BQ (eds): *Myology*. New York, McGraw-Hill, 1986, pp. 1763-1784.
9. Gronert GA, Heffron JJA, Taylor SR: Skeletal muscle sarcoplasmic reticulum in porcine malignant hyperthermia. *Europ J Pharmacol* 58:179-187, 1979.
10. Iuzzo PA, Klein W, Lehmann-Horn F: Fura-2 detected myoplasmic calcium and its correlation with contracture force in skeletal muscle from normal and malignant hyperthermia susceptible pigs. *Pfluegers Arch* 411:648-653, 1988.
11. Kurihara S, Konishi M, Miyagishima T, Sakai T: Effects of enflurane on excitation-contraction coupling in frog skeletal muscle fibres. *Pfluegers Arch* 402:345-352, 1984.
12. Lehmann-Horn F, Rüdell R, Dengler R, Lorkovic H, Haass A, Ricker R: Membrane defects in paramyotonia congenita with and without myotonia in a warm environment. *Muscle Nerve* 4:396-406, 1981.
13. Lopez JR, Alamo L, Jones D, Allen P, Papp I, Gergely J, Sreter F: Dantrolene reverses the syndrome of malignant hyperthermia by reducing the level of intracellular Ca^{2+} . *Biophys J* 47:313a, 1985.
14. McGrath CJ, Lee JC, Rempel WE: Halothane testing for malignant hyperthermia in swine: dose-response effects. *Am J Vet Res* 45:1734-1736, 1984.
15. Nelson TE: Dantrolene does not block calcium pulse-induced calcium release from a putative calcium channel in sarcoplasmic reticulum from malignant hyperthermia and normal pig muscle. *FEBS Lett* 167:123-126, 1984.
16. Ohnishi ST, Taylor SR, Gronert G: Calcium-induced Ca^{2+} release from sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia. *FEBS Lett* 161:103-107, 1983.
17. Sandow A, Taylor SR, Preiser H: Role of the action potential in excitation-contraction coupling. *Fed Proc* 24:1116-1123, 1965.
18. Urban BW: Modifications of excitable membranes by volatile and gaseous anesthetics, in Covino BG, Fozzard HA, Rehder K, Strichartz G (eds.): *Effects of Anesthesia*. Bethesda, MD, American Physiological Society, 1985, pp. 13-28.
19. Van Dyke RA, Wood CL: Binding of radioactivity from ^{14}C -labeled halothane in isolated perfused rat livers. *Anesthesiology* 38:328-332, 1973.