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The Effects of Extracellular Magnesium on Myoplasmic $[Ca^{2+}]$ in Malignant Hyperthermia Susceptible Swine

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It is now well established that the pathophysiology of the malignant hyperthermia (MH) syndrome is related to a malfunction of intracellular calcium homeostasis. Magnesium plays important roles in the basic contractile properties of muscle, and many of its actions are antagonistic to those of calcium. The aim of this study was to determine the effectiveness of magnesium sulphate to prevent the MH episode in susceptible animals and correlate this with its effects on the intracellular free calcium ($[Ca^{2+}]_i$). The experiments were carried out using six control (Yorkshire) and ten MH-susceptible crossbred swine (Poland China × Pietrain). After determination of resting concentrations of $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$, each animal was given either two iv bolus doses of 50 mg/kg or one iv bolus of 100 mg/kg of $MgSO_4$. The resting $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ were determined by means of ion-selective microelectrodes. The resting $[Ca^{2+}]_i$ in normal muscle fibers was 0.11 ± 0.01 μM (mean ± SEM), whereas in the MH muscles the resting $[Ca^{2+}]_i$ was 0.36 ± 0.01 μM. In neither group was the resting $[Ca^{2+}]_i$ modified by $MgSO_4$. This cumulative dose of $MgSO_4$ (100 mg/kg) was not able to prevent the induction of an MH episode by 2% halothane. Although $MgSO_4$ did not directly decrease $[Ca^{2+}]_i$, it did attenuate the increase in $[Ca^{2+}]_i$ associated with the syndrome from 7.29 ± 0.43 μM in untreated animals to 0.84 ± 0.03 μM in $MgSO_4$ pretreated swine. In addition, the limb rigidity that accompanies this increase in calcium was prevented by $MgSO_4$ pretreatment. Baseline measurements of $[Mg^{2+}]_i$ were not different in control and MH-susceptible muscles. Administration of $MgSO_4$ (100 mg/kg) increased $[Mg^{2+}]_i$ 1.8-fold ($P < 0.001$). These results indicate that $MgSO_4$ by itself was ineffective both in decreasing resting $[Ca^{2+}]_i$ and in preventing the changes associated with the MH episode. However, $MgSO_4$ was able to quantitatively reduce the increase in $[Ca^{2+}]_i$, prevent the limb rigidity, and reduce the increment in body temperature usually associated with the clinical syndrome. (Key words: Malignant hyperthermia: calcium ions; skeletal muscle. Measurement technique: ion-selective microelectrode.)

MALIGNANT HYPERTHERMIA (MH) is a myopathic syndrome triggered when susceptible patients or animals are exposed to volatile anesthetics and/or muscle relaxants that depolarize the post-synaptic membrane.[1] It is now well established that the pathophysiology of MH syndrome is related to a malfunction of the intracellular calcium homeostasis.[1–4] An abnormally high resting free intracellular calcium concentration, ($[Ca^{2+}]_i$) has been found in susceptible swine[3] and patients.[4] In addition, there is a considerable increase in $[Ca^{2+}]_i$ that occurs during an MH episode.[5]

It is known that Mg^{2+} in skeletal muscle binds to contractile proteins and may thus affect tension development.[6] Furthermore, there is evidence that Mg^{2+} may influence the Ca^{2+} release from the sarcoplasmic reticulum and may also affect the activity of the ATP-dependent calcium transport mechanism across the sarcoplasmic reticulum membrane.[7–9]

Several possible approaches to prevent or to treat MH syndrome have previously been suggested.[1,2,10] Magnesium sulphate has been tried for treatment of MH episode in swine.[10,11] The rationale for its use as a therapeutic agent in MH episode has been based on its ability to compete with calcium in many of its actions.[7] Hall *et al.*[10] reported that magnesium sulphate in near-toxic doses was successful in preventing MH in susceptible swine. Flewellen and Nelson[11] have reported that $MgSO_4$ was able to attenuate but not prevent the MH episode in pretreated MH-susceptible swine.

The aim of this study was to explore the effect of $MgSO_4$ on changes in the intracellular free calcium and magnesium concentrations in MH-susceptible swine and correlate the possible modifications on $[Ca^{2+}]_i$ induced by changes in $[Mg^{2+}]_i$.

Materials and Methods

The experiments were carried out in six control Yorkshire swine and in ten MH-susceptible crossbred swine (Poland China × Pietrain) on two occasions 3 weeks apart in random order for measurements of $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$. Each animal was placed into only one treatment group and served as its own untreated control. Six control Yorkshire swine and six MH-susceptible crossbred swine (Poland China × Pietrain) were used for measurements of serum $[Mg^{2+}]$. The protocols used in the present study were approved by the institutional committee for the protection of animals at both institutions. The average weights of the control animals at the time of the study was 31.6 ± 1.8 kg and the MH group was 33.1 ± 3.1 kg. Susceptibility to MH was confirmed by a challenge with halothane as previously described.[12] Tachycardia (heart

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rate > 130 beats per min) limb rigidity, and an increase in the body temperature were taken as indications of susceptibility to MH.

Anesthesia was induced with intravenous (iv) sodium thiopental (10–15 mg/kg) and maintained with fentanyl (iv) (0.025 mg/kg) and a mixture of N_2O/O_2 (66:34%). Spontaneous movements were prevented with pancuronium iv (0.15 mg/kg) to perform the electrophysiologic measurements. Additional iv doses of 0.025 mg/kg of fentanyl and 0.06 mg/kg of pancuronium were given every 15–30 min as needed. *In vitro* experiments were performed on intact muscle bundles, which demonstrated that sodium thiopental, fentanyl, and pancuronium in concentrations similar to those expected *in vivo* confirm that these drugs did not alter $[Ca^{2+}]_i$ in control or MH-susceptible muscle fibers. Three control or five MH swine were pretreated with 100 mg/kg of $MgSO_4$ iv in two divided doses of 50 mg/kg, whereas a similar group received a single dose of 100 mg/kg (three control and five MH) immediately before exposure to halothane. The $MgSO_4$ bolus was administered over 45 s to 1 min. The administration of the second dose of $MgSO_4$ was performed not later than 10 min after the first dose. We did not use higher concentrations of $MgSO_4$ (200 mg/kg) because of difficulty with cardiovascular side effects in pilot studies. Dantrolene 1 or 2 mg/kg was used to reverse the MH episode.[13] Fluids and all drugs used were administered *via* an ear vein catheter. Rectal temperature was monitored continuously using YSI temperature probe. The experimental protocol followed in this study is shown in figure 1 where letters indicate the stages of the experiments during which the *in vivo* measurements of $[Ca^{2+}]_i$ or $[Mg^{2+}]_i$ were carried out.

Ion-selective Microelectrodes

Glass microelectrodes with a tip outer diameter of 0.4 μm or less were pulled from glass capillaries with filaments (WPI-1B150F-6). Before pulling the capillaries were cleaned with HCl and distilled water and dried overnight at room temperature. The pulled microelectrodes were heated to 250° C and then silanized by exposure to tri-n-butylchlorosilane vapor for 30 min. The microelectrodes tips were filled with the neutral ligand by application of a small drop of the resin at the back of the electrodes.

Ca^{2+}-selective Microelectrodes

Silanized tip microelectrodes were first back filled with the liquid sensor based on the neutral synthetic ion ligand ETH 1001 (Fluka)[14] and then the remaining part of the barrel was similarly back filled with pCa7 solution.[15]

Although all microelectrodes were prepared in a similar manner, the performance of the ion-selective microelectrodes can show significant variations; therefore, every electrode was calibrated individually before and after five measurements in a series of solutions of different pCa (pCa3–pCa9) which had similar composition to those used by Tsien and Rink with the addition of $MgCl_2$ 1.5 mM, and Na 8 mM.[16] The microelectrode properties remained fairly constant, and recalibration was thus not necessary after each impalement.

The Ca^{2+} microelectrode potential was measured by means of a high input impedance electrometer differential amplifier (FD 223 WP Instruments, Inc., New Haven, Connecticut). The potential measured in the calibration solutions was plotted against pCa, and only those microelectrodes that showed a linear response between pCa3 and pCa7 (30.5 mV per decade $[Ca^{2+}]$ at 37° C) were used (fig. 2A). Data were discarded if the calibration curve before and after the experiment did not meet these criteria. We tested the performance of these ion-selective microelectrodes *in vitro* to ensure that their output was not interfered with by any of the drugs used or by changes in free $[Mg^{2+}]$ (0–4 mM).

Mg^{2+}-selective Microelectrodes

The microelectrode tips were back filled with the neutral ligand ETH 1117 (Fluka).[17] The shank and shoulder of the microelectrodes were filled with 10 mM $MgCl_2$. The effect of Ca^{2+} in the range between pCa7 and pCa3 and *p*H between 6.1 and 7.1 on the response of the Mg^{2+} microelectrodes was negligible, results that agree with those previously reported by Hess *et al.*[18] The response of the Mg^{2+}-selective microelectrode shows a significant interference from K^+ and to a lesser extent from Na^+ at the concentration in which both cations have been found in skeletal muscle. Therefore, they were calibrated in solutions containing known free Mg^{2+} and concentrations of $[Na^+]_i$ and $[K^+]_i$ that were measured directly in a previous study using a the same preparation and experimental conditions.[19]

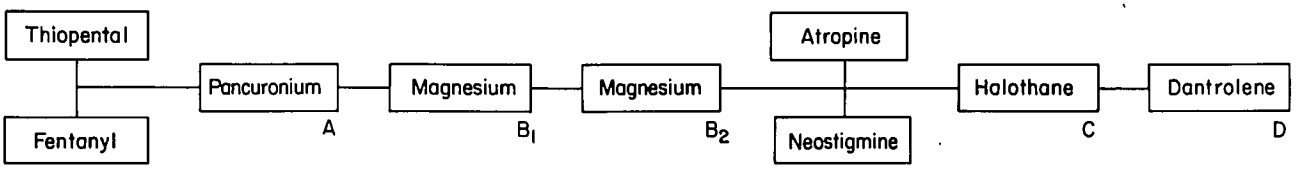

FIG. 1. The experimental protocol followed. Letters indicate the stages when determinations of $[Ca^{2+}]_i$ or $[Mg^{2+}]_i$ were performed.

The Mg^{2+}-selective microelectrode potential was measured in a identical manner as described for the Ca^{2+} microelectrode. In pure $MgCl_2$ the response followed the Nernst predictions, but when the cation composition of the calibration solution mimicked the intracellular cation composition, the response of the Mg^{2+}-selective microelectrode was reduced from 30.5 mV to 18–26 mV from 1 to 10 mM Mg^{2+} (fig. 2B). The response of the Mg^{2+}-selective microelectrodes in these calibrating solutions indicates that they can adequately measure free $[Mg^{2+}]$ from 0.5 to 10 mM, which covers the physiologic range.

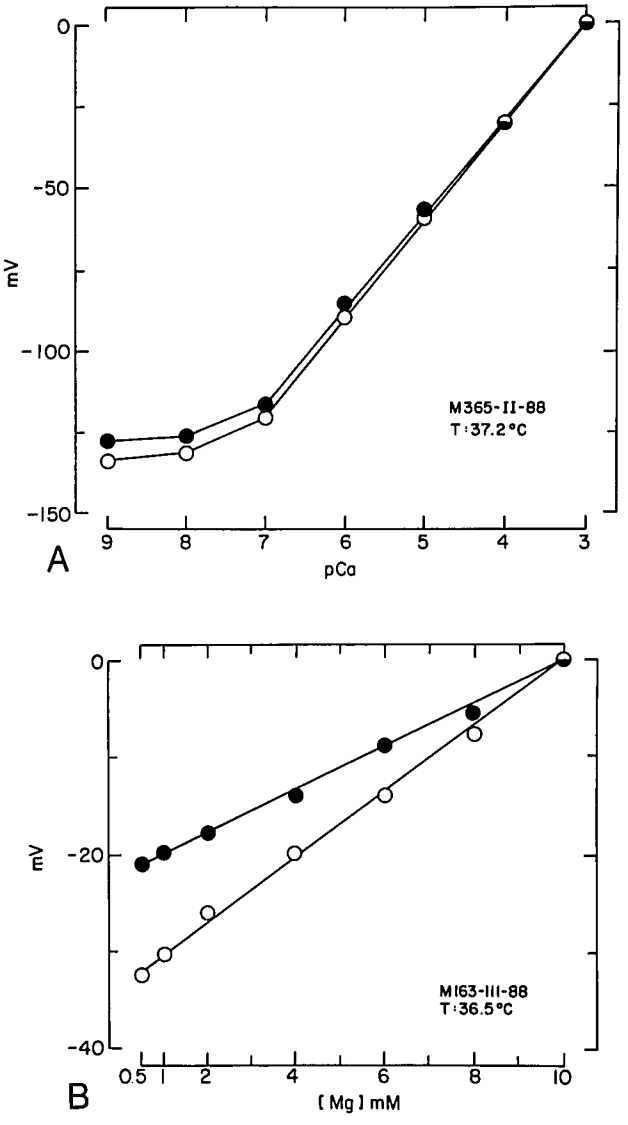

FIG. 2. *A.* Calibration curve of a Ca^{2+}-selective microelectrode, with a tip external diameter approximately 0.4 μm obtained during the exposure of the microelectrode tip to solutions of different pCa. The two curves represent measurements before (○) and after (●) calcium determinations. *B.* Calibration curve of a Mg^{2+}-selective microelectrode in the presence of different concentrations of MgCl alone (○) and in combination with Na^+ (8 mM), K^+ 110 mM, and Ca^{2+} 10 μM (●).

Recording Procedure

After induction of anesthesia a 5-cm incision was made over the peroneus longus muscle of the right hind leg. After the muscle was identified its surface was freed of connective tissue, and then the superficial muscle fibers were exposed and kept covered with warm mammalian Ringer's solution (37° C).

The 3 M KCl and the Ca^{2+}- or Mg^{2+}-selective microelectrodes were placed into the pool of Ringer's solution made around the incision and then set to zero with respect to a Ag/AgCl pellet also inserted into the pool. A muscle fiber was impaled with conventional 3 M KCl microelectrode (8–10 MΩ tip resistance) to measure the resting membrane potential (V_m), and an adjacent fiber was impaled with the calcium- or magnesium-selective microelectrodes to measure the calcium potential VCa_E or magnesium potential VMg_E, which represents the potential recorded by the calcium or magnesium microelectrode and corresponds to the sum of the resting membrane potential and the Ca^{2+} or Mg^{2+} specific potential. After both microelectrodes reached a stable value, V_m was subtracted from VCa_E or VMg_E using a high impedence electrometer to obtain VCa or VMg, which are both equivalent to the intracellular free $[Ca^{2+}]$ or $[Mg^{2+}]$. The determinations of $[Ca^{2+}]$ or $[Mg^{2+}]$ were carried out between 5 and 20 min after the bolus of $MgSO_4$ was injected because in this interval the $[Mg^{2+}]$ in plasma reached a near steady state. Flexibly mounted microelectrodes were used to achieve a more stable intracellular recording. Signals were displayed on digital voltmeters (Simpson M-465) and recorded on a two-channel recorder (Linear M 005).

Criteria for Collecting Experimental Data

Five impalements with both ion-selective electrodes were performed in different fibers for the resting control measurements. Two to three measurements were done after the administration of Mg^{2+}. After the administration of halothane in susceptible animals it was possible to make only 1–2 measurements because the clinical condition of the animal made it impossible to delay dantrolene administration for more measurements. Two to three measurements were attempted after the administration of dantrolene. However, data were discarded if: 1) the microelectrode calibration curve performed before and after each experiment differed by no more than 4 mV (8%); 2) the membrane potential of the muscle fibers was less than −80 mV; or 3) membrane potential shift by more than 4 mV during the Ca^{2+} or Mg^{2+} measurements.

Determination of Total [Mg] in Plasma

The total Mg concentration was determined in preliminary studies using atomic absortion (Varian Pechtron 320). Samples of blood were taken every 2 min for 50 min after either a single or double bolus of $MgSO_4$ was injected.

Studies were carried out both at the Boston Biomedical Research Institute, Muscle Department, Boston, Massachusetts and at Centro de Biofisica y Bioquimica, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela, using littermates and their progeny.

Results are presented as mean ± SEM. Student's *t* test for paired and unpaired data was used for comparison between two groups. Significance was accepted at the $P < 0.05$ level.

Results

Measurements of Resting $[Ca^{2+}]_I$

Although there was no significant difference in the resting membrane potential between these two groups, the resting myoplasmic free calcium concentration was 3.2 times greater in MH-susceptible muscle fiber than in controls (table 1). The mean intracellular free $[Ca^{2+}]_i$ in controls was 0.11 ± 0.01 μM (range 0.09–0.13 μM n = 12) while it was 0.36 ± 0.01 μM (range 0.29–0.42 μM n = 10) in the MH-susceptible swine ($P < 0.001$).

Table 1. Effects of $MgSO_4$, Halothane, and Dantrolene on V_m and $[Ca^{2+}]_i$

	Resting Membrane Potential (−mV)	$[Ca^{2+}]_i$ (μM)	N
Control	−85 ± 0.8	0.11 ± 0.01	12
MHS	−83 ± 0.9	0.36 ± 0.01	10
Control after $MgSO_4$ treatment	−85 ± 0.6	0.12 ± 0.01	16
MHS after $MgSO_4$ treatment	−83 ± 1.0	0.34 ± 0.01	12
MHS nonpretreated with $MgSO_4$ exposed to halothane	−85 ± 1.1	7.29 ± 0.43	10
MHS pretreated with $MgSO_4$ exposed to halothane	−82 ± 1.0	0.84 ± 0.03	7
MHS nonpretreated with $MgSO_4$ and treated with dantrolene (1 mg/kg) during an MH episode	−85 ± 1.0	1.62 ± 0.36	10
MHS nonpretreated with $MgSO_4$ and treated with dantrolene (2 mg/kg) during an MH episode	−85 ± 0.9	0.15 ± 0.01	12
MHS pretreated with $MgSO_4$ and treated with dantrolene (1 mg/kg) during an MH episode	−84 ± 1.0	0.12 ± 0.01	10

Values are mean ± SEM; n = number of measurements. The $MgSO_4$ dose was 100 mg/kg in all cases.
MHS = malignant hyperthermia susceptible.

Plasma [Mg] in Control and Susceptible Animals

The resting total [Mg] in plasma from MH susceptible swine was 0.76 ± 0.06 mM (n = 10). After the injection of a bolus of 50 mg/kg $MgSO_4$ the plasma [Mg] reached a peak value of 1.38 ± 0.03 mM (n = 10) at about 10 min. Between 5 and 20 min the [Mg] declined exponentially to 1.14 ± 0.01 mM (n = 10). The injection of 100 mg/kg induced an increment in plasma [Mg] reaching a new value of 1.68 ± 0.05 mM (n = 10). This was not significantly different than when a second dose of 50 mg was given 15 min after the first, 1.76 ± 0.042 (n = 10). This [Mg] also declined exponentially to 1.43 ± 0.03 mM (n = 10) between 5 and 20 min. Identical results were observed in the control animals: resting, 0.75 ± 0.03 mM; 50 mg/kg $MgSO_4$, 1.37 ± 0.05; and 100 mg/kg $MgSO_4$, 1.69 ± 0.07.

$[Mg^{2+}]_I$ in Control and Susceptible MH Muscle Fibers

Figure 3 shows simultaneous measurements of resting membrane potential and intracellular free magnesium concentration from a control (A) and from an MH-susceptible muscle fiber (B) 15 min after the $MgSO_4$ bolus was injected. The mean resting intracellular free $[Mg^{2+}]$ in the normals was 1.66 ± 0.15 mM (range 1.05–2.40, n = 12) and 1.50 ± 0.24 mM (range 0.98–2.31, n = 13) in MH susceptibles. The administration of 50 mg/kg $MgSO_4$ did not induce detectable changes in $[Mg^{2+}]_i$ in either group despite the fact that we could detect change in plasma [Mg] by 49%. However, when 100 mg/kg (total dose) was administered, a significant increment in $[Mg^{2+}]_i$ in both groups of muscle fibers could be observed (table 2). Figure 3*C* shows a typical experiment in which the resting $[Mg^{2+}]_i$ was measured in MH muscle fibers after the administration of 50 mg/kg of $MgSO_4$, and figure 3*D* shows the results after the second dose of 50 mg/kg (total dose 100 mg/kg). The mean resting $[Mg^{2+}]_i$ in MH muscle fibers was 1.77 ± 0.18 mM (range 0.86–2.44, n = 18) after the administration of 50 mg/kg $MgSO_4$ and 2.86 ± 0.46 mM (range 1.84–3.25 mM, n = 10) after a total dose of 100 mg/kg $MgSO_4$ ($P < 0.001$). Similar results were obtained in muscle fibers from control swine when they were pretreated with 50 or 100 mg/kg (table 2). Neither exposure to halothane nor the clinical effects of the MH syndrome had any effect on $[Mg^{2+}]_i$.

Effects of Magnesium on Resting $[Ca^{2+}]_I$ before and during the MH Episode

Figure 4 shows the effect of $MgSO_4$ on the resting membrane potential and myoplasmic free calcium concentration in a MH muscle fiber. It can be observed that the administration of $MgSO_4$ (100 mg/kg) did not induce

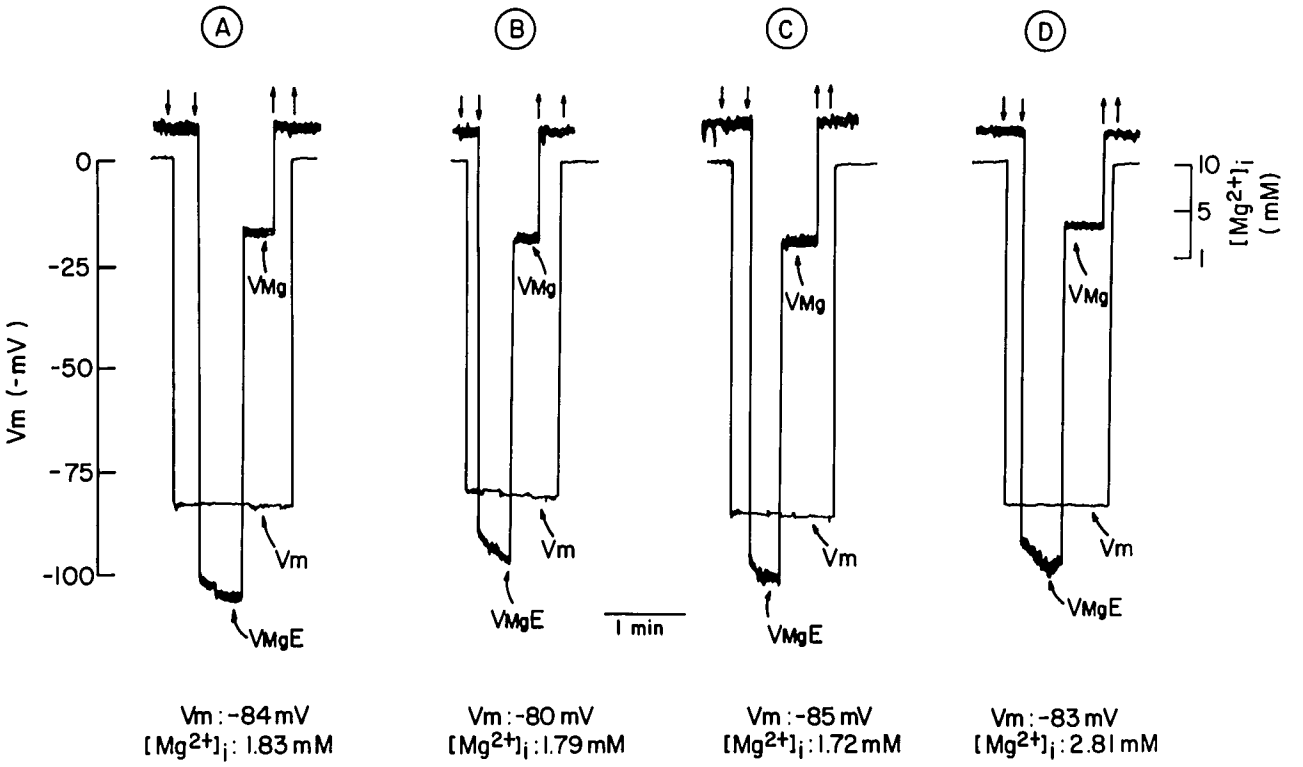

FIG. 3. Recordings of the simultaneous measurements of resting membrane potential (V_m) and intracellular free magnesium concentration (VMg) from (*A*) control muscle; (*B*) MH-susceptible muscle; (*C*) after administration of 50 mg/kg of $MgSO_4$; and (*D*) after administration of 100 mg/kg $MgSO_4$ (cumulative dose). Records in figures 2*B*, 2*C*, and 2*D* were obtained from the same MH muscle. At the bottom of the figure are the actual values for V_m and $[Mg^{2+}]_i$ expressed in millivolts and millimolars, respectively.

any detectable change in the resting membrane potential or myoplasmic free calcium concentration. The mean resting $[Ca^{2+}]_i$ after $MgSO_4$ administration was 0.12 ± 0.01 μM (range 0.08–0.14 μM, n = 16) in control muscle fibers and 0.34 ± 0.01 μM (range 0.26–0.40 μM, n = 12) in MH-susceptible fibers. These were not different from the mean $[Ca^{2+}]_i$ values found before $MgSO_4$ administration in these same swine (table 1).

TABLE 2. Effects of $MgSO_4$ on V_m, $[Mg^{2+}]_i$ in Control, and MHS Swine

	Resting Membrane Potential (−mV)	$[Mg^{2+}]_i$ (mM)	n
Control	−86 ± 0.8	1.66 ± 0.15	12
MHS	−84 ± 1.0	1.50 ± 0.24	13
Control after $MgSO_4$ 50 mg/kg	−84 ± 0.8	1.59 ± 0.30	12
MHS after $MgSO_4$ 50 mg/kg	−83 ± 1.0	1.77 ± 0.18	18
Control after $MgSO_4$ 100 mg/kg	−85 ± 0.9	2.68 ± 0.56	16
MHS after $MgSO_4$ 100 mg/kg	−86 ± 1.0	2.86 ± 0.46	10

Values are mean ± SEM; n = number of measurements.

The inhalation of halothane (2%) after reversal of the pancuronium effect with neostigmine in MH-susceptible animals, whether or not they were $MgSO_4$-pretreated, triggered the clinical episode, which was associated with an increase in $[Ca^{2+}]_i$ without a change in resting V_m.

The mean resting $[Ca^{2+}]_i$ during MH episode in those swine pretreated with $MgSO_4$ was 0.84 ± 0.03 μM (range 0.70–0.96 μM, n = 7) (table 1; fig. 4*C*). It is important to note that we did not observe limb rigidity during the MH episode as well as the marked increase in body temperature (only 0.5–1° C), which are usually a prominent characteristic of the MH syndrome in these swine. Mg^{2+} did not reduce the tachycardia associated with the clinical syndrome. The lack of limb rigidity might be related to the fact that the $[Ca^{2+}]_i$ did not reach the mechanical threshold (1 μM). The administration of 1 mg/kg dantrolene resolved the clinical manifestation and the increase in $[Ca^{2+}]_i$ (table 1; fig. 4*D*) and decreased the mean $[Ca^{2+}]_i$ to 0.12 ± 0.11 μM (range 0.08–0.13 μM, n = 10). The dose of dantrolene required to reverse the MH episode was less in those animals pretreated with $MgSO_4$ than in those without pretreatment (1 *vs.* 2.0 mg/kg).

Figure 5 illustrates an experiment showing the simultaneous measurements of resting membrane potential and

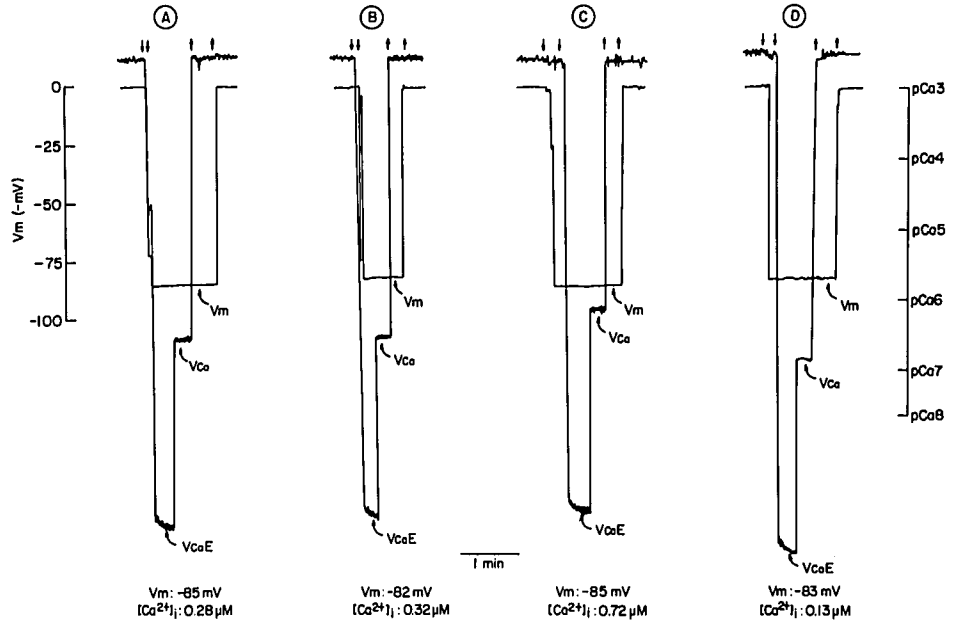

FIG. 4. Recordings of the simultaneous measurements of resting membrane potential (V_m) and $[Ca^{2+}]_i$ in MH-susceptible muscle (*A*) before $MgSO_4$; (*B*) after 100 mg/kg of $MgSO_4$; (*C*) after exposure to halothane 2%; and (*D*) after treatment of dantrolene 1 mg/kg. At the bottom of the figure are the actual values for V_m and $[Ca^{2+}]_i$ expressed in millivolts and micromolars, respectively.

$[Ca^{2+}]_i$ before and during a MH episode triggered by halothane 2% in a susceptible swine in which there was no $MgSO_4$ pretreatment. The exposure of untreated susceptible swine to halothane (2%) after reversal of the pancuronium effect triggered the MH episode with all of its manifestations including limb rigidity. The mean $[Ca^{2+}]_i$ during the MH episode rose to 7.29 ± 0.43 μM (range 5.2–8.3 μM, n = 10) (table 1). Figure 5*A* shows the values of V_m (−83 mV) and $[Ca^{2+}]_i$ (0.35 μM) before the exposure to halothane. Figure 5*B* shows both values after halothane exposure V_m: −90 mV and $[Ca^{2+}]_i$ 7.11 μM. The administration of dantrolene (1.0 mg/kg) only partially antagonized the clinical manifestations of MH and reduced $[Ca^{2+}]_i$ (1.25 μM) (fig. 5*C*). The administration of a second dose of dantrolene (total dose 2.0 mg/kg) reversed all the clinical symptoms and brought the $[Ca^{2+}]_i$ to normal levels (0.12 μM) (fig. 5*D*). The mean resting $[Ca^{2+}]_i$ after 1 mg/kg dantrolene was 1.62 ± 0.36 μM (range 0.98–

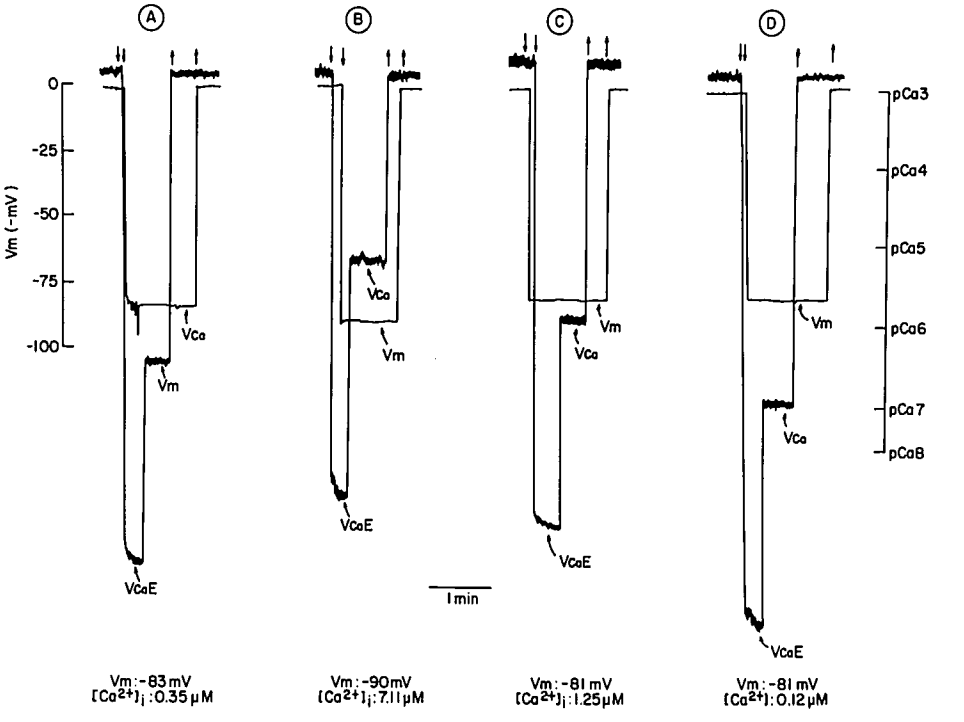

FIG. 5. Recordings of the simultaneous measurements of resting membrane potential (V_m) and $[Ca^{2+}]_i$ in MH-susceptible muscle (*A*) before exposure to halothane 2%; (*B*) after exposure to halothane 2%; (*C*) after treatment of 1 mg/kg dantrolene; and (*D*) after treatment with dantrolene 2 mg/kg (cumulative dose). At the bottom of the figure are the actual values for V_m and $[Ca^{2+}]_i$ expressed in millivolts and micromolars, respectively.

2.3 μM, n = 10) and after 2 mg/kg was 0.15 ± 0.01 μM (range 0.11–0.19 μM, n = 12) (table 1).

Discussion

In the present study we found that $MgSO_4$ with its associated increase in $[Mg^{2+}]_i$ neither altered resting $[Ca^{2+}]_i$ nor prevented the increase in $[Ca^{2+}]_i$ associated with the MH syndrome. However, it was able to attenuate the increment in $[Ca^{2+}]_i$ when the syndrome was triggered and prevented the limb rigidity usually observed during the MH episode. These results are in agreement with a previous report of a reduction in the clinical manifestations of the MH syndrome with $MgSO_4$ pretreatment.[10,11]

The fact that $[Ca^{2+}]_i$ did not reach the mechanical threshold (1 μM) during the MH episode in $MgSO_4$-pretreated animals may explain the lack of the limb rigidity during the episode itself. This attenuation in the increment of $[Ca^{2+}]_i$ and, therefore, the lack of limb rigidity and marked increase in body temperature may be related to a Mg^{2+} effect on Ca^{2+} release and reuptake in skeletal muscle by the sarcoplasmic reticulum (SR).[20–23] The fact that the $[Mg^{2+}]_i$ changes from 1.69 mM to 2.15 mM after $MgSO_4$ treatment in the MH-susceptible muscle fibers suggests that the modification of MH episode and the lower increment of $[Ca^{2+}]_i$ are associated with the increase in intracellular Mg^{2+}. For example, the rapid Ca^{2+} release from the SR induced by halothane[24] and by calcium[23] is inhibited by Mg^{2+}. Both of these Mg^{2+} effects on the SR might explain why the increase in $[Ca^{2+}]_i$ after halothane exposure in $MgSO_4$-pretreated swine was only 12% of the increase seen in similar swine that did not receive $MgSO_4$. Another possible explanation of the Mg^{2+} effects is that this divalent cation is changed the threshold for mechanical activation. These effects have been related to changes in surface potential.[25,26] Divalent cations are assumed to affect the surface potential either by binding to the fixed charges or by increasing the electric field within the membrane.[27,28] However, we believe that the observed effect induced by Mg^{2+} was not related with its action on membrane surface potential because the increment in Mg^{2+} plasma concentration was small (from 0.76 before to 1.43 mM after 100 mg $MgSO_4$) to account for the effect on changes for potential observed previously in which changes from 1.5 to 5 mM were necessary to detect changes in 3–4 mV in the contraction threshold.[27]

The efficacy of dantrolene therapy for the MH episode in swine has been abundantly demonstrated.[29–31] These results confirm our previous finding that the therapeutic effectiveness of dantrolene during an MH episode is associated with the ability of this muscle relaxant to decrease the $[Ca^{2+}]_i$.[5] The dantrolene dose required to completely resolve the MH crisis and reduce $[Ca^{2+}]_i$ was less in swine pretreated with $MgSO_4$ than in those that were not pretreated. This fact might be related to the combined effects of both drugs on the calcium release and uptake by the SR.

The salutary effect of dantrolene and the ineffectiveness of magnesium in completely resolving the clinical MH syndrome and the reduction of $[Ca^{2+}]_i$ might be related to differences in their mode of action on the SR. Dantrolene is a direct muscle relaxant that has no effect on neuromuscular transmission or on the electrical activity or the inward spread of activation in skeletal muscle.[32,33] Dantrolene inhibits Ca^{2+} release associated with muscle activation as well as steady state Ca^{2+} efflux from SR (passive Ca^{2+} release).[34–38] In addition, it blocks the conductance of calcium channels in purified SR membranes studied with patch-clamp.[39] Therefore, dantrolene should be able to modify resting $[Ca^{2+}]_i$, and we have shown that this does happen.[13] Magnesium, however, has been shown to increase SR Ca^{2+} uptake and inhibit active release of SR calcium without any effect on the steady state efflux of Ca^{2+} from SR.[20–23,40] Because magnesium does not modify the passive release of calcium from the SR, it cannot change the resting $[Ca^{2+}]_i$, but it can modify the amount of calcium released from the SR during the MH episode and therefore attenuate the limb rigidity that is characteristic of the syndrome.

Magnesium plays an important role in influencing a large number of muscle functions, such as tension development and excitation–contraction coupling as well as being a cofactor of numerous intracellular enzymes.[41,42] The mean $[Mg^{2+}]_i$ measured in control fibers was not different than in MH-susceptible fibers. The mean value for $[Mg^{2+}]_i$ obtained in the present work is qualitatively in agreement with previous determinations[18,43–46] in which it was found that $[Mg^{2+}]_i$ is in the low millimolar range.

Although Ca^{2+}-induced Ca^{2+} release is not probably the primary mechanism by which contraction is initiated in skeletal muscle,[47,48] Endo[49] suggested that this mechanism is the causative functional lesion in MH-susceptible muscle. It has been demonstrated that the phenomenon of Ca^{2+}-induced release of Ca^{2+} is seen in skinned skeletal muscle fiber preparations but only at low Mg^{2+} concentrations (<0.3 mM).[50–52] The resting $[Mg^{2+}]_i$ in the MH muscle fiber of 1.66 mM was higher than that required to inhibit Ca^{2+}-induced release of Ca^{2+} in their skinned fiber preparations, and increasing this to 2.66 mM after $MgSO_4$ pretreatment did not prevent the onset of the MH syndrome. Because of the magnitude of the $[Mg^{2+}]_i$ measured here, these data essentially preclude the possibility for a role for Ca^{2+}-induced release of Ca^{2+} in the pathophysiology of MH.

In the present study we have confirmed our previous findings that the resting $[Ca^{2+}]_i$ in muscle fibers from control swine is in the order of 0.10 μM, a value that is in good agreement with those reported by others who investigated other excitable cells.[15,16] We again confirm our

previous finding that $[Ca^{2+}]_i$ is higher in MH-susceptible than in control swine.[3,5] In addition, we confirm that there is an increase in $[Ca^{2+}]_i$ during the MH episode that is not associated with any detectable change in resting membrane potential.[5] These findings suggest that the calcium release process during an MH episode is not membrane potential-dependent; therefore, depolarization is not necessary in the development of an MH episode.

The data on resting $[Ca^{2+}]_i$ in MH susceptible muscle are in disagreement with the recent report by Iaizzo *et al.*,[54] who did not find significant differences in $[Ca^{2+}]_i$ between control and MH muscle fibers using Fura-2. These authors also suggested that our higher $[Ca^{2+}]_i$ in MH subjects is related to the invasive nature of the method. Ion-selective microelectrodes allow continuous direct measurements of $[Ca^{2+}]_i$ in muscle cells during resting steady state conditions. The linear response of the Ca^{2+} microelectrodes in the measured range of pCa3–pCa7 demonstrates no limitation of calcium measurement based on the microelectrode's response. That plasma membrane damage is responsible for the increased resting $[Ca^{2+}]_i$ seen in MH-susceptible muscle can be ruled out from the results obtained in humans[4,55] and MH-susceptible swine[56,57] *in vitro*, in which both microelectrodes were placed in the same muscle fiber. Cell damage, if any, would have been reflected by a sustained depolarization. In those studies we obtained quantitatively similar results to the data reported here and found no evidence of membrane damage. Our measurements in controls agree with all previous estimates, and there is no reason for a systematic error only in MH-susceptible fibers. This discrepancy is probably due to difficulties involved in accurately estimating $[Ca^{2+}]_i$ using Fura-2 as a calcium indicator. Up to 60–65% of Fura-2 in the cell can be bound and is not free to interact with calcium, and the presence of a large fraction of bound Fura-2 raises considerable uncertainty about the calibration of the dye signals in terms of absolute levels of $[Ca^{2+}]_i$.[58,59] Fura-2 is a high affinity calcium buffer and can, itself, directly lower absolute and measured $[Ca^{2+}]_i$.[58,60]

We have found similar results to those that were reported by Iaizzo *et al.*[54] using the photoprotein aequorin.[61] In isolated muscle fibers heavily microinjected with aequorin, we could not detect differences between the resting light signal recorded from control and MHS muscle.[61] As with the Fura-2 experiments done by Iaizzo *et al.*,[54] we believe that problems relating to the lack of sufficient sensitivity for measuring resting $[Ca^{2+}]_i$ prevented the detection of the difference.[62]

We believe that our measurements of $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ are indeed representative of the concentrations of these two cations present in the intracellular medium on the basis of the following: 1) the absence of evidence suggesting Ca^{2+} or Mg^{2+} gradients in the intracellular space under resting conditions, and 2) the small dispersion around our mean value despite the fact that such measurements were carried out at different points along the muscle fiber length or in different fibers.

In conclusion, magnesium sulphate does not modify the resting free myoplasmic calcium concentration, and it fails to prevent the MH episode in MH-susceptible swine. However, it modifies the clinical manifestation of MH and the increment in $[Ca^{2+}]_i$ associated with the syndrome.

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