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The Effects of Volatile Anesthetics on Calcium Regulation by Malignant Hyperthermia–Susceptible Sarcoplasmic Reticulum

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To clarify the mechanism by which volatile anesthetics initiate malignant hyperthermia (MH), we examined the effect of halothane, isoflurane, and enflurane on Ca^{2+} uptake and release by sarcoplasmic reticulum vesicles isolated from MH-susceptible (MHS) and normal pig muscle. Clinical concentrations of these anesthetics (0.1–0.5 mM) stimulated sarcoplasmic reticulum ATP-dependent Ca^{2+} uptake (maximal at approximately 4 mM), whereas 10–20 times the clinical anesthetic concentration inhibited Ca^{2+} uptake. There was no significant difference between MHS and normal sarcoplasmic reticulum in any aspect of Ca^{2+} uptake. Ca^{2+} release from $^{45}Ca^{2+}$-filled sarcoplasmic reticulum vesicles in a 10^{-8} M Ca^{2+}-containing medium (*p*H 7.0) was significantly stimulated at clinical concentrations of all three volatile anesthetics (anesthetic concentration for the 50% stimulation of Ca^{2+} release = 0.096–0.22 mM); however, the rate constant for Ca^{2+} release from MHS sarcoplasmic reticulum was in all cases significantly greater than that from normal sarcoplasmic reticulum. Furthermore, 0.5 mM halothane had no effect on Ca^{2+} release from normal sarcoplasmic reticulum at *p*H values less than 6.8, although it could still significantly stimulate Ca^{2+} release from MHS sarcoplasmic reticulum even at *p*H 6.4; similar results were obtained for isoflurane and enflurane. These studies thus demonstrate that the interaction of volatile anesthetics with the sarcoplasmic reticulum Ca^{2+}-release channel is altered in MHS porcine muscle such that the channel may be activated even at a Ca^{2+} concentration or *p*H that would be expected to maintain the channel in the closed state. (Key words: Anesthetics, volatile: halothane. Complications: malignant hyperthermia. Ions: calcium. Muscle, skeletal: ryanodine receptor; sarcoplasmic reticulum.).

MALIGNANT HYPERTHERMIA (MH) is an inherited syndrome of humans and pigs in which affected individuals respond to certain anesthetic agents with muscle contracture and a rapid increase in metabolism, resulting in the production of heat, carbon dioxide, and acidosis.[1,2] Many experimental findings in human and porcine MH suggest that the regulation of skeletal muscle intracellular Ca^{2+} concentration is defective[3,4] in the regulation of the sarcoplasmic reticulum Ca^{2+}-release channel.[5–12] Genetic linkage studies now indicate tight linkage between the skeletal muscle Ca^{2+}-release channel ryanodine receptor gene (RYR 1) and the gene for MH in both humans[13,14] and pigs.[15,16] Although a recent study has demonstrated that this gene is not a candidate locus for MH in some human families,[17] all breeds of MH-susceptible (MHS) pigs examined to date have an identical mutation in the ryanodine receptor gene.[16] Thus, a working hypothesis is that a mutation (or set of mutations) may cause abnormal regulation of the sarcoplasmic reticulum Ca^{2+}-release channel in MHS porcine muscle and an as yet undetermined number of MHS human families.

To demonstrate that the sarcoplasmic reticulum Ca^{2+}-release channel defect could indeed be responsible for MH, it is necessary to demonstrate that MH-provoking anesthetics are capable of activating this channel both at clinical anesthetic concentrations and at resting myoplasmic Ca^{2+} concentrations. Previous investigations of the effect of volatile anesthetics on MHS muscle have used either intact muscle fibers (for reviews see refs. 1 and 2) or isolated sarcoplasmic reticulum vesicles enriched in the Ca^{2+}-release apparatus.[18–21] However, the Ca^{2+} dependence of Ca^{2+} release from sarcoplasmic reticulum follows a bell-shaped curve, with an optimal Ca^{2+} for stimulating Ca^{2+} release in the range of 1–10 μM.[22,23] To date, no study examining Ca^{2+} release from isolated sarcoplasmic reticulum vesicles has determined the effects of anesthetics on the rate of Ca^{2+} release at defined extravesicular Ca^{2+} concentrations.

In this study, we examined the effect of three volatile anesthetics on both Ca^{2+} uptake and Ca^{2+} release by normal and MHS porcine muscle sarcoplasmic reticulum vesicles. We demonstrated that at low concentrations of myoplasmic Ca^{2+} (10^{-8} M) or at *p*H less than 6.8, clinical concentrations of these anesthetics induce significant Ca^{2+} release from MHS but not from normal sarcoplasmic reticulum vesicles. In contrast, Ca^{2+} uptake by the sarcoplasmic reticulum vesicles was stimulated by concentrations of anesthetics that induced significant Ca^{2+} release. Our results suggest that the sarcoplasmic reticulum Ca^{2+}-release channel may be a primary site of action of volatile anesthetics in skeletal muscle. Furthermore, a mutation in this channel that modifies the interaction of volatile anesthetics with this protein could explain the ability of these anesthetics to increase myoplasmic Ca^{2+} and trigger the MH response in affected individuals.

Materials and Methods

EXPERIMENTAL ANIMALS

Pigs (29–45 kg) were obtained from the University of Minnesota Experimental Farm, where they were part of a swine genetics herd maintained by Dr. William Rempel

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for studies of the inheritance of halothane sensitivity. All Pietrain (homozygous for the MH-susceptibility gene) and Yorkshire (homozygous for the normal allele) pigs were obtained and halothane-tested for MH susceptibility as described previously.[24] Pigs were brought to the laboratory no sooner that 2 weeks after the halothane challenge test.

Sarcoplasmic Reticulum Isolation and Assay Procedures

Animals were killed by intravenous administration of sodium thiamylal (Biotal) (15 mg/kg) followed by T61 euthanasia solution. This protocol was approved by the Institutional Animal Care Committee of the University of Minnesota. Sarcoplasmic reticulum vesicles were prepared from the longissimus dorsi muscle,[5,6] essentially as described by Meissner *et al.*[22,23] Briefly, the membrane pellet obtained by centrifugation of the muscle homogenate between 2,600 and 10,000 × g was resuspended in 0.6 M KCl buffer, incubated on ice for 1 h, and centrifuged at 100,000 × g. The pellets were resuspended in 0.6 M KCl buffer, incubated on ice for 1 h, and centrifuged at 100,000 × g. The resulting preparation (termed unfractionated sarcoplasmic reticulum) was used for Ca^{2+} uptake measurements. Sarcoplasmic reticulum to be used for Ca^{2+} release measurements was further fractionated on discontinuous sucrose density gradients. The vesicles that banded at the 36.4–40.4% sucrose interface after 5 h centrifugation at 85,000 × g were collected (termed heavy sarcoplasmic reticulum), diluted with H_2O, and centrifuged at 100,000 × g for 30 min. All sarcoplasmic reticulum samples were resuspended in a small volume of 10% sucrose, snap-frozen in liquid N_2, and stored at −70° C. All solutions contained aprotinin 1 μg/ml and leupeptin 1 μg/ml to minimize proteolysis.

Saturated aqueous solutions of anesthetic in Teflon-sealed Reacti-vials (Pierce Chemical Co.) were prepared fresh daily by 2–4-h equilibration at room temperature. Saturated aqueous solutions of anesthetics were taken as 20 mM halothane, 15 mM isoflurane, and 15 mM enflurane.[25] From this vial, anesthetic in the aqueous layer was added directly to the Ca^{2+}-uptake or -release medium contained in another Reacti-vial by injection through the septum (anesthetic contained in a volume < 5% of the reaction volume). The actual concentration of anesthetic in the experimental media was derived from the known volume of the Reacti-vials (1 ml), the liquid volume, the remaining gas volume, and the published values for the liquid/gas partition coefficients for these anesthetics.[21,25] The concentrations of the anesthetics in the liquid phase were determined using the distribution coefficients as described by Nelson and Sweo.[21]

Ca^{2+} uptake by sarcoplasmic reticulum vesicles in the presence or absence of anesthetic was determined in 0.5 ml buffer composed of 120 mM KCl, 50 mM phosphate, 0.1 mM $^{45}CaCl_2$, 5 mM MgATP, and 40 mM histidine (*p*H 7.0) at 25° C. Sarcoplasmic reticulum vesicles (20 μg/ml) were placed on the side of the sealed Reacti-vial, and the anesthetic was added through the septum. Ca^{2+} uptake was initiated 10 s later by vortexing this mixture. Samples were filtered through 0.45-μm Millipore HA membranes and washed with ice-cold buffer, and the $^{45}Ca^{2+}$ remaining on the filter was determined in a liquid scintillation spectrometer.

To determine sarcoplasmic reticulum Ca^{2+} release, vesicles (10–15 mg protein/ml) were first passively loaded with $^{45}Ca^{2+}$ to a level of 20–40 nmol/mg[26] by incubation for 2 h at room temperature in 0.1 M KCl, 10 mM PIPES (adjusted to the desired *p*H with KOH), and 5 mM $CaCl_2$ (containing 0.1 mCi/ml $^{45}CaCl_2$). Ca^{2+}-release media contained 0.2 ml 0.1 M KCl, 10 mM 1,4-piperazinediethane-sulfonic acid (PIPES) buffer, and a $CaCl_2$-ethylene glycol-bis(β-amino-ethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) buffer set to give the desired final Ca^{2+} concentration (total $CaCl_2$ of 5 mM). $^{45}Ca^{2+}$-loaded sarcoplasmic reticulum vesicles were placed on the side of the release medium–containing vial, and the vial was sealed. Various concentrations of volatile anesthetic were added to the medium by injection through the Teflon seal and allowed to equilibrate for 10 s. Ca^{2+} release then was initiated by vortexing. Sarcoplasmic reticulum Ca^{2+} release was stopped at various times (2–5 s using a metronome) by the addition of 0.1 M KCl, 10 mM PIPES, 10 mM EGTA, 10 mM $MgCl_2$, and 10 μM ruthenium red through the Teflon seal. The sarcoplasmic reticulum Ca^{2+} content was then determined after filtering samples through 0.45-μm Millipore filters and washing with ice-cold medium containing the Ca^{2+}-release–inhibiting medium. The fraction of total loaded Ca^{2+} that was not releasable was determined 5 s after mixing $^{45}Ca^{2+}$-loaded sarcoplasmic reticulum into 1 μM Ca^{2+}, 5 mM ATP–containing media (which maximally activates Ca^{2+} release). Sarcoplasmic reticulum Ca^{2+}-release rates were corrected for the nonreleasable content (approximately 20% of the loaded $^{45}Ca^{2+}$).

Statistical Analysis

All experiments were performed on sarcoplasmic reticulum preparations obtained from at least three different MHS or three normal pigs. Sample means were compared by use of Student's *t* test and considered significantly different at $P < 0.05$.

Results

Effect of Volatile Anesthetics on Sarcoplasmic Reticulum Ca^{2+} Uptake

To examine the effects of the volatile anesthetics on Ca^{2+} uptake by sarcoplasmic reticulum vesicles, it was es-

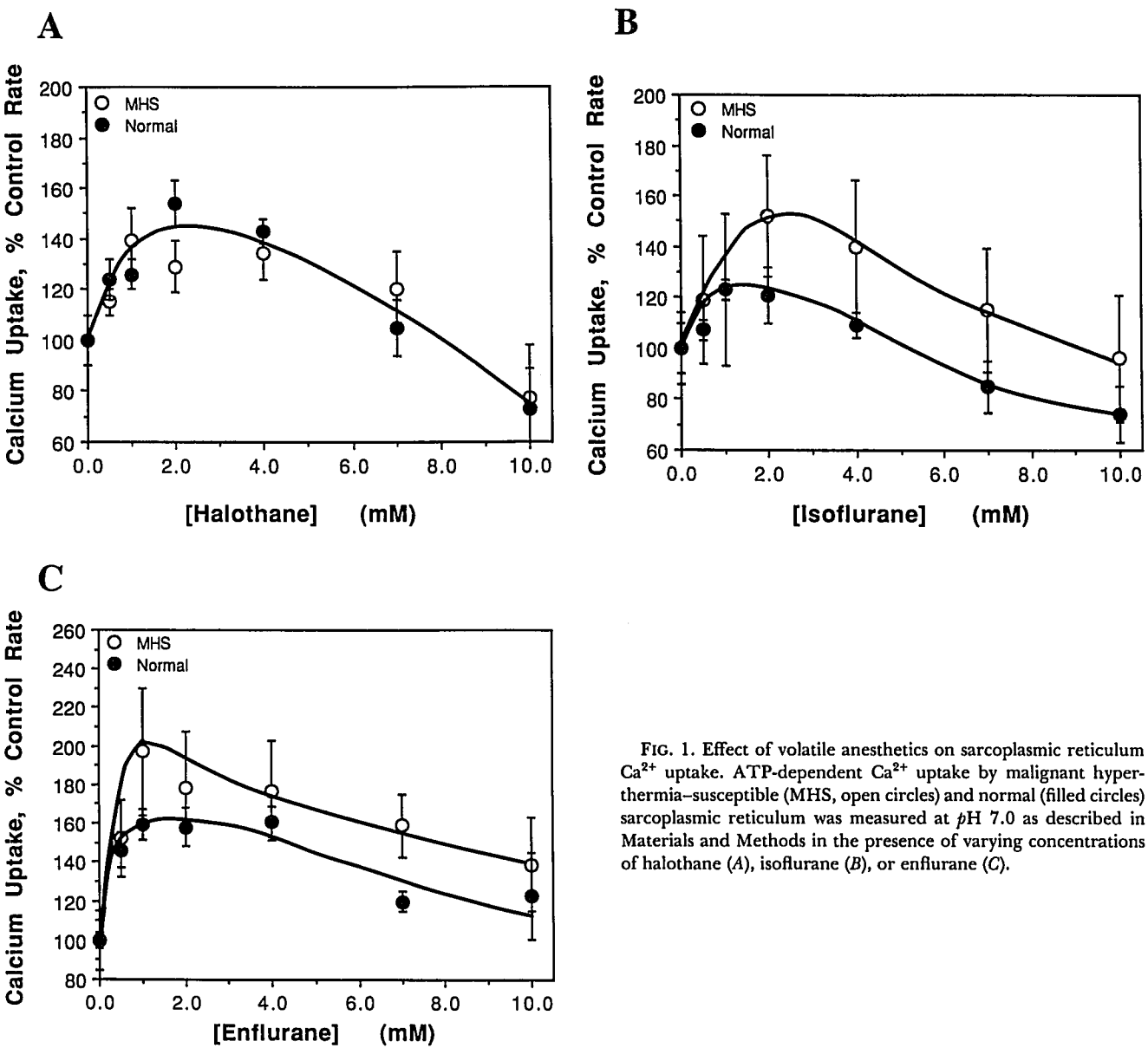

FIG. 1. Effect of volatile anesthetics on sarcoplasmic reticulum Ca^{2+} uptake. ATP-dependent Ca^{2+} uptake by malignant hyperthermia–susceptible (MHS, open circles) and normal (filled circles) sarcoplasmic reticulum was measured at *p*H 7.0 as described in Materials and Methods in the presence of varying concentrations of halothane (*A*), isoflurane (*B*), or enflurane (*C*).

sential to ensure that the anesthetics were incubated with the sarcoplasmic reticulum in a sealed environment. The most reliable way to achieve this was to inject the desired volumes of a saturated aqueous solution of anesthetic into the Ca^{2+}-uptake medium that itself was contained in a 1 ml Reacti-vial sealed with a Teflon gasket. The unfractionated sarcoplasmic reticulum vesicles used in these studies demonstrated Ca^{2+} uptake activities comparable to those previously reported by us for porcine sarcoplasmic reticulum.[26] Ca^{2+} uptake by MHS sarcoplasmic reticulum (0.66 ± 0.18 μmol $Ca^{2+} \cdot$ mg protein$^{-1} \cdot$ min^{-1}) and normal sarcoplasmic reticulum (0.62 ± 0.13 μmol $Ca^{2+} \cdot$ mg protein$^{-1} \cdot$ min^{-1}) did not differ.

When Ca^{2+} uptake by sarcoplasmic reticulum was determined in the presence of halothane, isoflurane, or enflurane, the rate of Ca^{2+} uptake in the presence of 0.5–4 mM anesthetic was greater than that for the controls that had no anesthetic (figs. 1A–1C). As the concentration of anesthetic was increased further, the relative stimulation of Ca^{2+} uptake decreased and eventually reached a level less than (for halothane and isoflurane) or similar to (enflurane) that of the control samples.

When the effect of halothane on MHS and normal sarcoplasmic reticulum Ca^{2+} uptake were compared, it was clear that there was no difference in the sensitivity of these two types of sarcoplasmic reticulum to this anesthetic (fig.

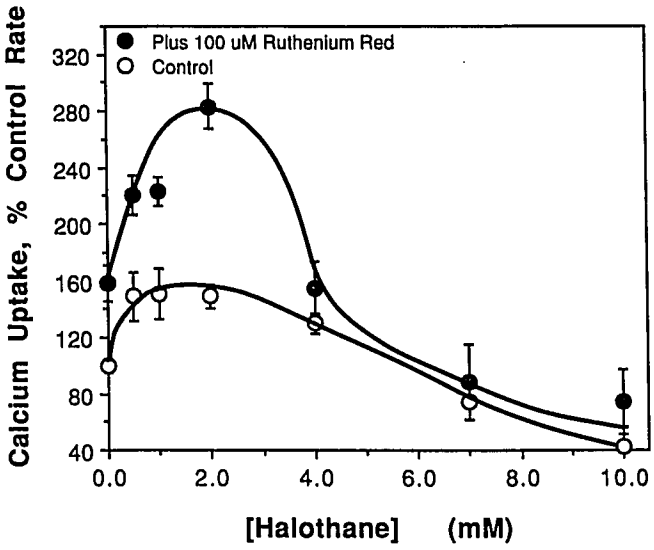

FIG. 2. Effect of ruthenium red on the halothane-induced stimulation of sarcoplasmic reticulum Ca^{2+} uptake. ATP-dependent Ca^{2+} uptake was measured at *p*H 7.0 as described in Materials and Methods in the presence (filled circles) or absence (open circles) of 100 μM ruthenium red.

1A). Although both isoflurane and enflurane appeared to stimulate Ca^{2+} uptake by MHS sarcoplasmic reticulum to a greater extent than that by normal sarcoplasmic reticulum (figs. 1B and 1C), there were no statistically significant differences between MHS and normal sarcoplasmic reticulum ($P > 0.05$) except for 7 mM enflurane. Most importantly, in the clinically relevant range of 0–0.5 mM anesthetic,[25] the percent stimulation of Ca^{2+} uptake by a given anesthetic was identical for MHS and normal sarcoplasmic reticulum.

One concern in the studies described here is that the unfractionated sarcoplasmic reticulum vesicle fraction used still contained a significant proportion of terminal cisternae-derived sarcoplasmic reticulum that is enriched in the Ca^{2+}-release channel. Thus, the Ca^{2+} accumulation measurements made here reflect a balance between $^{45}Ca^{2+}$ uptake by the Ca^{2+} pump and $^{45}Ca^{2+}$ release *via* the Ca^{2+}-release channel. To obviate this problem, the effect of halothane on ATP-dependent $^{45}Ca^{2+}$ uptake was examined in the presence of 100 μM ruthenium red, which completely inhibits the sarcoplasmic reticulum Ca^{2+}-release mechanism (data not shown). In the absence of halothane, ruthenium red significantly stimulated Ca^{2+} uptake by 58% ($P < 0.01$) (fig. 2); identical results were obtained for MHS and normal sarcoplasmic reticulum, so data are pooled in figure 2. As halothane was added, the relative stimulation of Ca^{2+} uptake increased (maximal stimulation of 80% over control values in both the absence and presence of ruthenium red). Halothane stimulated Ca^{2+} uptake in either the presence or absence of ruthenium red, indicating that the effect of volatile anesthetics on Ca^{2+} uptake seen in figure 1 was indeed due to the action of these agents on the ATP-dependent Ca^{2+}-pump mechanism.

Effect of Volatile Anesthetics on Sarcoplasmic Reticulum Ca^{2+} Release

Volatile anesthetics can trigger MH crisis in humans and swine when the muscle is in the relaxed state. Thus, the effect of anesthetics on sarcoplasmic reticulum Ca^{2+} release *in vitro* was examined in a 10^{-8} M ionized Ca^{2+}-containing medium, a Ca^{2+} concentration significantly less than that necessary to activate muscle contraction. Under these conditions, Ca^{2+} release from both normal and MHS sarcoplasmic reticulum was slow enough to be measured accurately at time intervals of 2 s or more (*i.e.*, the rate of Ca^{2+} release was shown to be linear). As with Ca^{2+} uptake, sarcoplasmic reticulum Ca^{2+} release was measured in a sealed environment to ensure minimal loss of the volatile anesthetics. Furthermore, the Ca^{2+}-release protocol used in our study directly measured the release of Ca^{2+} from $^{45}Ca^{2+}$-filled passively loaded vesicles (*i.e.*, vesicles that had not been exposed to Ca^{2+}-precipitating agents) in which MHS and normal sarcoplasmic reticulum contained similar initial Ca^{2+} loads (20–40 nmol/mg).

The rate constant for Ca^{2+} release when MHS sarcoplasmic reticulum vesicles were transferred to 10^{-8} M Ca^{2+} (*p*H 7.0) in the absence of halothane was significantly greater than that of normal sarcoplasmic reticulum under the same conditions (fig. 3). Thus, in contrast to normal sarcoplasmic reticulum, even at this low Ca^{2+} concentration there appeared to be some Ca^{2+}-release channel–mediated Ca^{2+} release occurring from MHS sarcoplasmic reticulum vesicles. The rate constant for Ca^{2+} release from both MHS and normal vesicles under these conditions was stimulated by halothane (fig. 3, open symbols); the rate constant, which was maximal at approximately 0.5 mM halothane, was significantly different from that in the absence of halothane for both types of vesicles ($P < 0.005$). At any given halothane concentration, the rate constant for Ca^{2+} release from MHS sarcoplasmic reticulum was significantly greater than from normal sarcoplasmic reticulum (figs. 3A and 3B).

In contrast, when Ca^{2+}-filled vesicles were transferred to a release-inhibiting medium (containing 10 mM $MgCl_2$, 10 mM EGTA, and 10 μM ruthenium red that inhibit Ca^{2+} release *via* the ryanodine receptor[23]), the stimulation of the rate constant for Ca^{2+} release by halothane was markedly reduced (fig. 3, filled symbols). Although there was not a complete inhibition of Ca^{2+} release by this medium, the maximal stimulation of Ca^{2+} release by halothane from both MHS and normal sarcoplasmic reticulum were now similar. These data also indicate that the Ca^{2+}

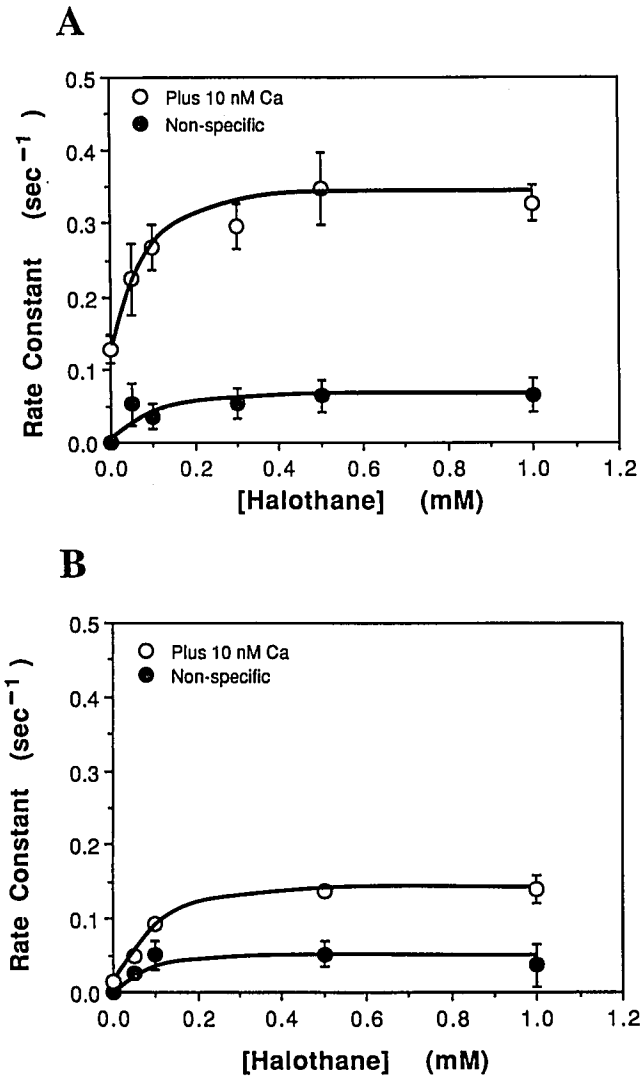

FIG. 3. Effect of halothane on the rate constant of Ca^{2+} release from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and Ca^{2+} release was determined after dilution into various concentrations of halothane in 10^{-8} M Ca^{2+} medium (open circles) or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red release-inhibiting medium (filled circles) as described in Materials and Methods. Points represent the means ± SE of duplicate determinations on three MHS and three normal sarcoplasmic reticulum preparations.

release from MHS vesicles in 10^{-8} M Ca^{2+} in the absence of halothane is predominantly channel-mediated, because it is inhibited by known inhibitors of the sarcoplasmic reticulum Ca^{2+}-release channel.[22,23]

A possible complication with the approach used here is that the time between the addition of halothane to the sealed tube containing the $^{45}Ca^{2+}$-loaded sarcoplasmic reticulum (in a 2-μl volume on the wall of the Reacti-vial vessel) and the initiation of Ca^{2+} release could vary from 5–15 s and may not have been sufficient for equilibration of the anesthetic with the medium. However, when this equilibration time was varied from 5 to 120 s, the stimulation of the rate constant for Ca^{2+} release by 0.5 mM halothane was similar. Thus, halothane concentrations were apparently at equilibrium, and Ca^{2+} release was activated only after dilution of the vesicles into the anesthetic-containing Ca^{2+}-release medium. Although the rapid time scale necessary for these experiments precluded precise confirmation of anesthetic equilibration, anesthetic concentrations reported here assume equilibration with both the aqueous and gas phases in the sealed tube.

Isoflurane and enflurane also enhanced the rate constant for Ca^{2+} release from $^{45}Ca^{2+}$-loaded vesicles upon transfer to 10^{-8} M Ca^{2+}-containing medium; the rate constants were again greater for MHS than for normal sarcoplasmic reticulum (figs. 4 and 5, open symbols). As with halothane, the anesthetic-induced Ca^{2+} release was markedly reduced when vesicles were transferred to the release-inhibiting medium, with the rate constants for Ca^{2+} release from MHS and normal sarcoplasmic reticulum now not significantly different (filled symbols).

The anesthetic concentration for the 50% stimulation of Ca^{2+} release ($C_{0.5}$) could be accurately determined from Hill plots only for MHS sarcoplasmic reticulum; the stimulation of normal sarcoplasmic reticulum Ca^{2+} release by anesthetic was too small to be accurately determined. These $C_{0.5}$ values (0.097 ± 0.065 mM halothane, 0.171 ± 0.031 mM isoflurane, 0.220 ± 0.049 mM enflurane, mean ± SD for three different MHS sarcoplasmic reticulum samples for each anesthetic) are within the range of anesthetic concentrations (0.1–0.5 mM) that induce contractures in MHS muscle bundles[27] and trigger MH crises *in vivo*.[28]

Effect of Dantrolene on Sarcoplasmic Reticulum Ca^{2+} Release

Dantrolene has been proposed to inhibit the increase in sarcoplasmic Ca^{2+} during an MH episode by acting on the sarcoplasmic reticulum Ca^{2+}-release channel.[20,29,30] However, as shown in figure 6A, inclusion of 40 μM dantrolene in the release medium (a concentration that is clinically effective and is close to the maximum solubility of this agent in buffered medium[29]) did not inhibit Ca^{2+} release from MHS sarcoplasmic reticulum in either the presence or absence of halothane. Similar results were obtained when 40 μM dantrolene was also included during the 2-h $^{45}Ca^{2+}$ loading step (data not shown).

The experiment shown in figure 6A was performed at 22° C. However, Ohta *et al.*[31] have reported that dantrolene inhibited sarcoplasmic reticulum Ca^{2+} release in skinned fibers only at 38° C. When our experiment was repeated at 38° C (fig. 6B, adjusting the Ca^{2+} EGTA buffer accordingly), there also was no inhibition of sarcoplasmic reticulum Ca^{2+} release by 40 μM dantrolene.

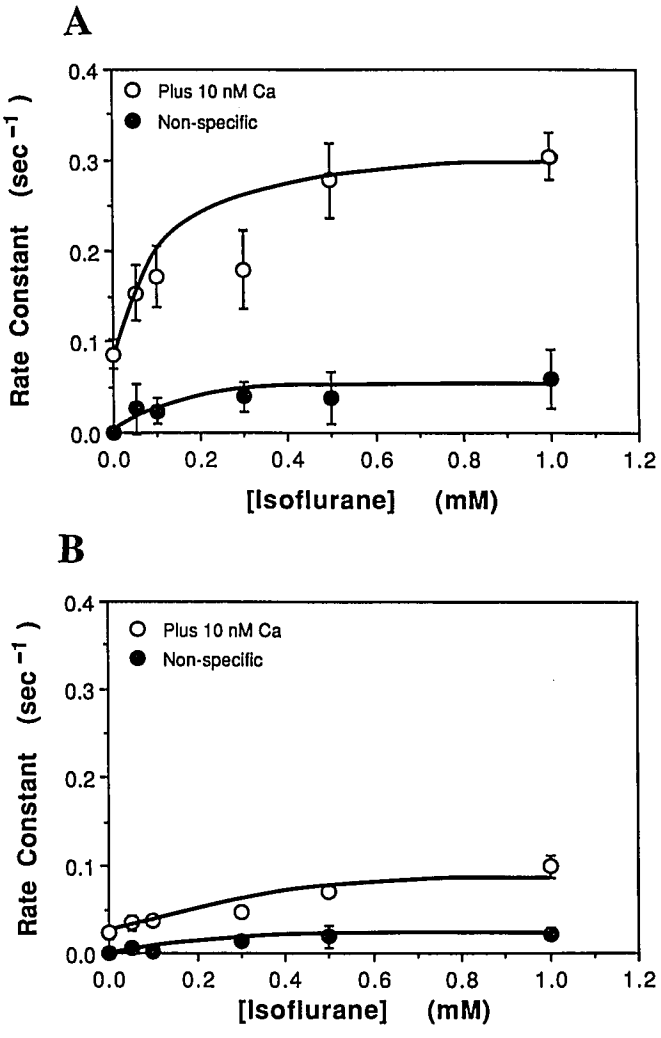

FIG. 4. Effect of isoflurane on the rate constant of Ca^{2+} release from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and Ca^{2+} release was determined in the presence of varying concentrations of isoflurane in 10^{-8} M Ca^{2+} medium (open circles) or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red release-inhibiting medium (filled circles), as described in the legend to figure 3, for three MHS and three normal sarcoplasmic reticulum preparations.

pH Dependence of the Anesthetic-Induced Sarcoplasmic Reticulum Ca^{2+} Release

The rate constant for Ca^{2+} release from MHS sarcoplasmic reticulum, induced by transfer of $^{45}Ca^{2+}$-filled vesicles to 10^{-8} M Ca^{2+} medium, decreased as the *p*H was decreased (fig. 7); under these conditions the rate constant for normal sarcoplasmic reticulum Ca^{2+} release was already very slow at *p*H 7.0, so a further decrease was not easily measurable. Although the anesthetic-induced stimulation of Ca^{2+} release from both normal and MHS sarcoplasmic reticulum decreased with decreasing *p*H, the effect of *p*H on this stimulation was significantly different for these two types of sarcoplasmic reticulum. At *p*H less than 6.8, 0.5 mM halothane had no effect on Ca^{2+} release from normal sarcoplasmic reticulum (fig. 7B). In contrast, 0.5 mM halothane could significantly stimulate the rate constant for Ca^{2+} release from MHS sarcoplasmic reticulum even at *p*H 6.4 (fig. 7A). With isoflurane (fig. 8) and enflurane (fig. 9), the difference in the response of MHS and normal sarcoplasmic reticulum is even more pronounced. Thus, at *p*H less than 7.0, both anesthetics had no effect on the rate constant for Ca^{2+} release from normal sarcoplasmic reticulum, whereas they were able to stim-

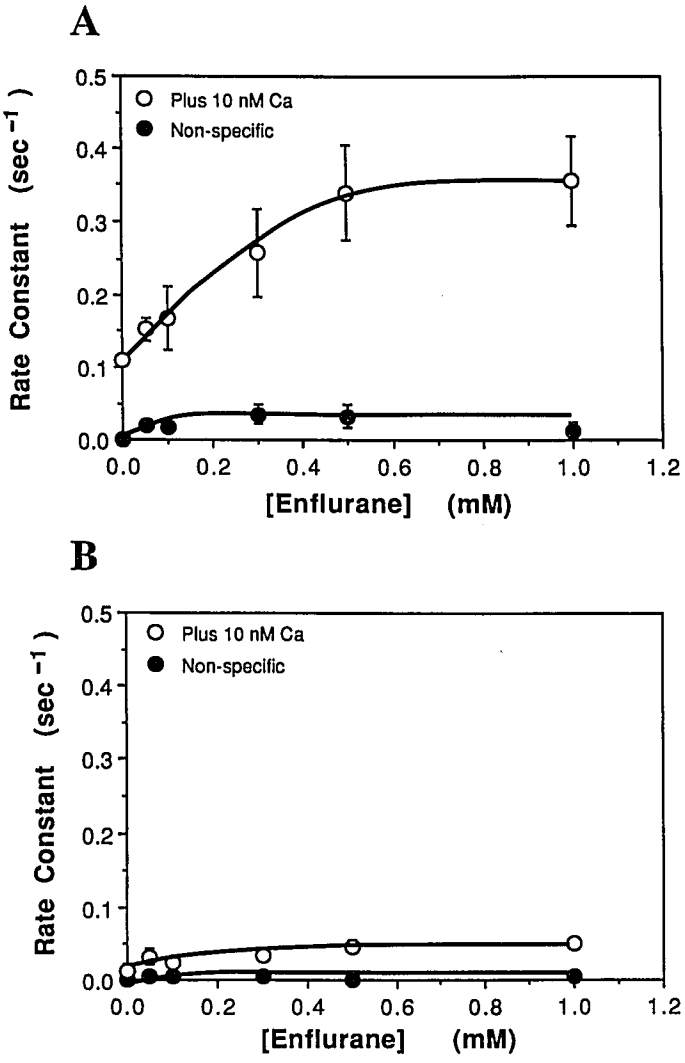

FIG. 5. Effect of enflurane on the rate constant of Ca^{2+} release from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and Ca^{2+} release was determined in the presence of varying concentrations of enflurane in 10^{-8} M Ca^{2+} medium (open circles) or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red release-inhibiting medium (filled circles), as described in the legend to fig. 3, for three MHS and three normal sarcoplasmic reticulum preparations.

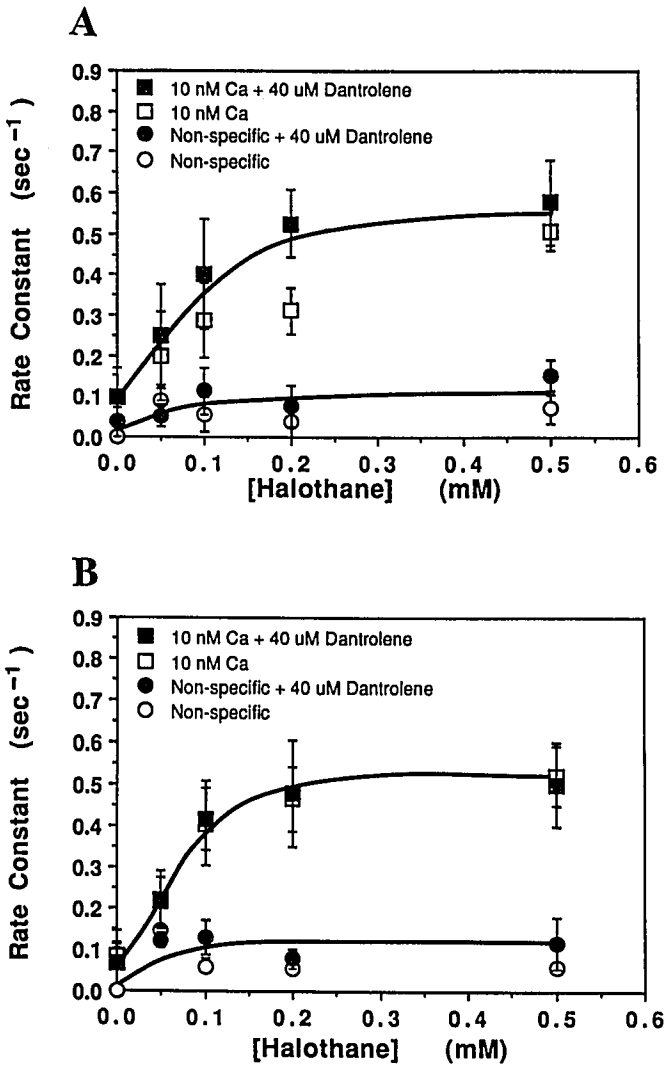

FIG. 6. Effect of dantrolene on the halothane-induced stimulation of Ca^{2+} release from malignant hyperthermia–susceptible (MHS) sarcoplasmic reticulum at 22 and 38° C. MHS sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and the rate constant for Ca^{2+} release was determined after dilution into various concentrations of halothane in 10^{-8} M Ca^{2+} medium (squares) or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red release-inhibiting medium (circles). The release medium included (filled symbols) or omitted (open symbols) 40 μM dantrolene that was added from a 4 mM dantrolene stock solution in dimethyl sulfoxide; an equivalent volume of dimethyl sulfoxide was included in the release media that omitted dantrolene. Experiments were performed at 22° C (*A*) or 38° C (*B*).

ulate significantly the rate constant for Ca^{2+} release from MHS sarcoplasmic reticulum even at *p*H 6.4 (figs. 8 and 9).

The halothane dependence of Ca^{2+} release from normal and MHS sarcoplasmic reticulum also was examined at *p*H 6.8 and *p*H 6.6 (fig. 10). At both *p*H values, as observed previously at *p*H 7.0, the stimulation of the rate constants for Ca^{2+} release by halothane were maximal by 0.3 mM halothane; *i.e.*, there were no significant differences in the halothane sensitivity at these different *p*H values. Similar results were obtained with isoflurane and enflurane (data not shown).

Discussion

Sarcoplasmic Ca^{2+} levels in skeletal muscle are determined primarily by the balance of Ca^{2+} uptake and Ca^{2+} release from the sarcoplasmic reticulum. Therefore, if

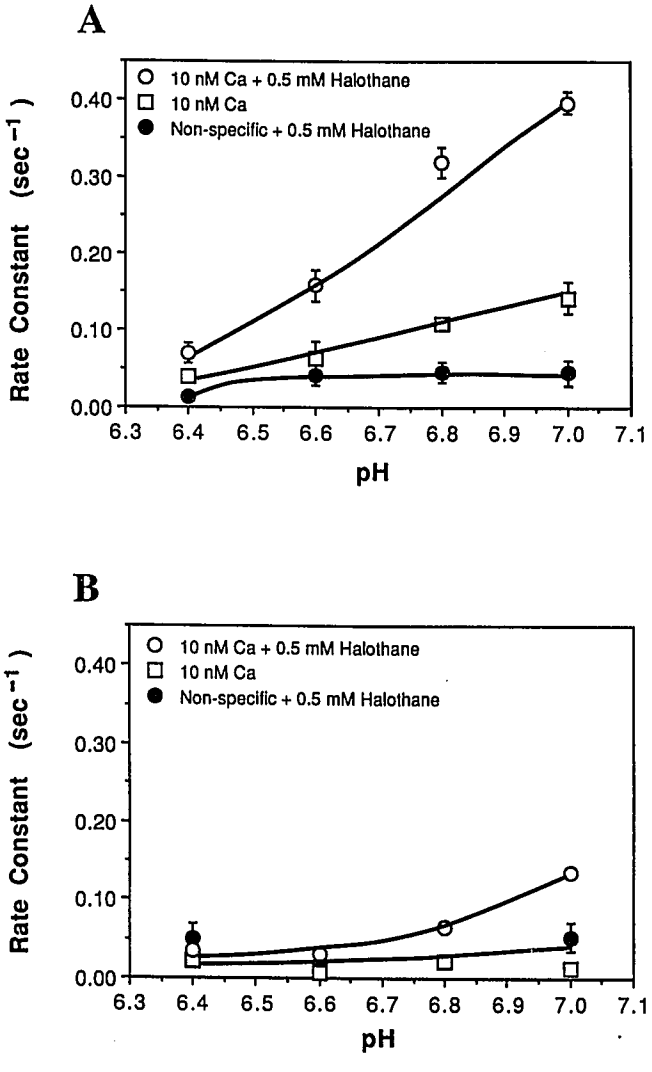

FIG. 7. Effects of *p*H on the halothane-induced release of Ca^{2+} from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and the rate constant for Ca^{2+} release was determined after dilution into 10^{-8} M Ca^{2+} plus 0.5 mM halothane medium (open circles), 10^{-8} M Ca^{2+} medium (open squares), or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red plus 0.5 mM halothane medium (filled circles), as described in Materials and Methods. Points represent the means ±SE of duplicate determinations on three MHS and three normal sarcoplasmic reticulum preparations.

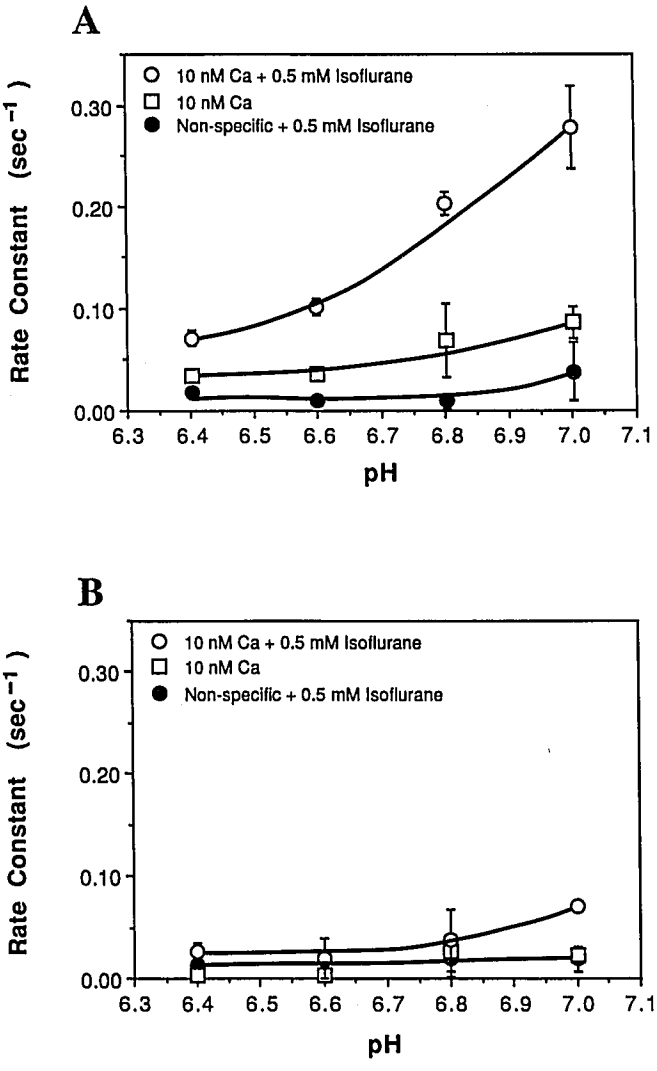

FIG. 8. Effect of *p*H on the isoflurane-induced release of Ca^{2+} from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and the rate constant for Ca^{2+} release was determined after dilution into 10^{-8} M Ca^{2+} plus 0.5 mM isoflurane medium (open circles), 10^{-8} M Ca^{2+} medium alone (open squares), or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red plus 0.5 mM isoflurane medium (filled circles), as described in the legend to figure 7 for three MHS and three normal sarcoplasmic reticulum preparations.

the sarcoplasmic reticulum is the site of the primary defect in MHS muscle, it is likely that MH triggering agents, such as the volatile anesthetics, act on a component in this membrane to produce the rapid elevation of sarcoplasmic Ca^{2+} that is the hallmark of the MH response.[3,4] Thus, it is important to differentiate between the effect of MH-triggering agents on the Ca^{2+} pump from those on the Ca^{2+}-release mechanism when determining the mechanism of initiation of MH. Furthermore, because MH can be triggered in the unconscious patient, it is important to demonstrate that any triggering agent can alter the sarcoplasmic reticulum Ca^{2+} permeability at resting sarcoplasmic Ca^{2+} concentrations.

At concentrations that trigger the MH response, all three volatile anesthetics examined in this study stimulated ATP-dependent Ca^{2+} uptake by sarcoplasmic reticulum vesicles (40–100%, fig. 1). These data are very similar to those reported previously by Nelson and Sweo,[21] who also observed that at 1.4–1.7 MAC (0.8–0.96 mM anesthetic) the effect of enflurane on Ca^{2+} uptake was greater than the halothane effect, which in turn was greater than the isoflurane effect. The mean values for the stimulation of

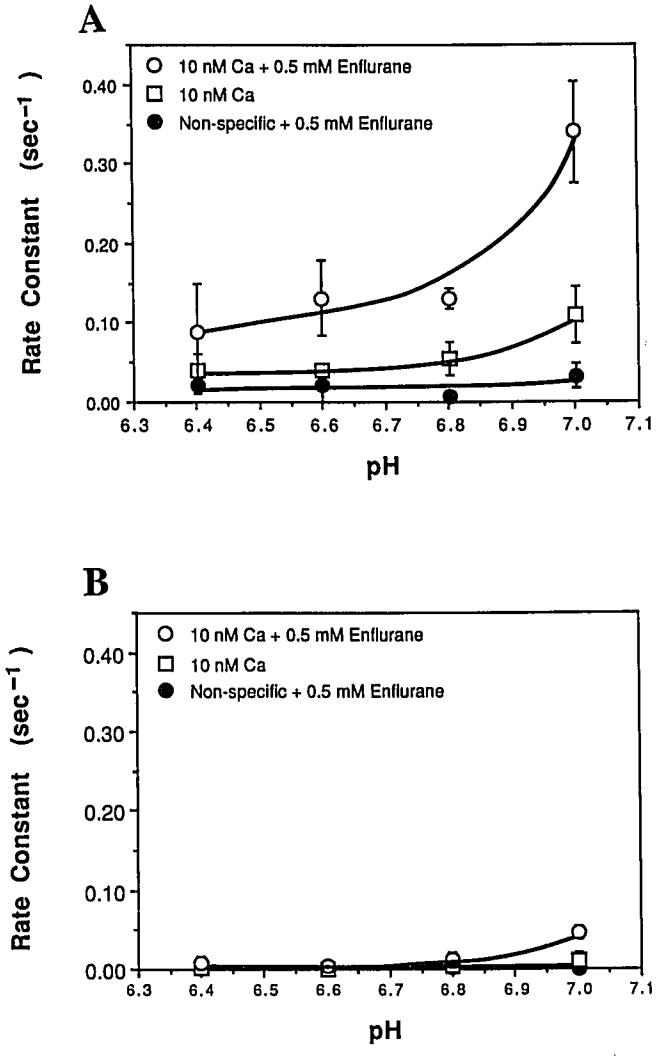

FIG. 9. Effect of *p*H on the enflurane-induced release of Ca^{2+} from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum. MHS (*A*) and normal (*B*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and the rate constant for Ca^{2+} release was determined after dilution into 10^{-8} M Ca^{2+} plus 0.5 mM enflurane medium (open circles), 10^{-8} M Ca^{2+} medium (open squares), or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red plus 0.5 mM enflurane medium (filled circles), as described in the legend to figure 7 for three MHS and three normal sarcoplasmic reticulum preparations.

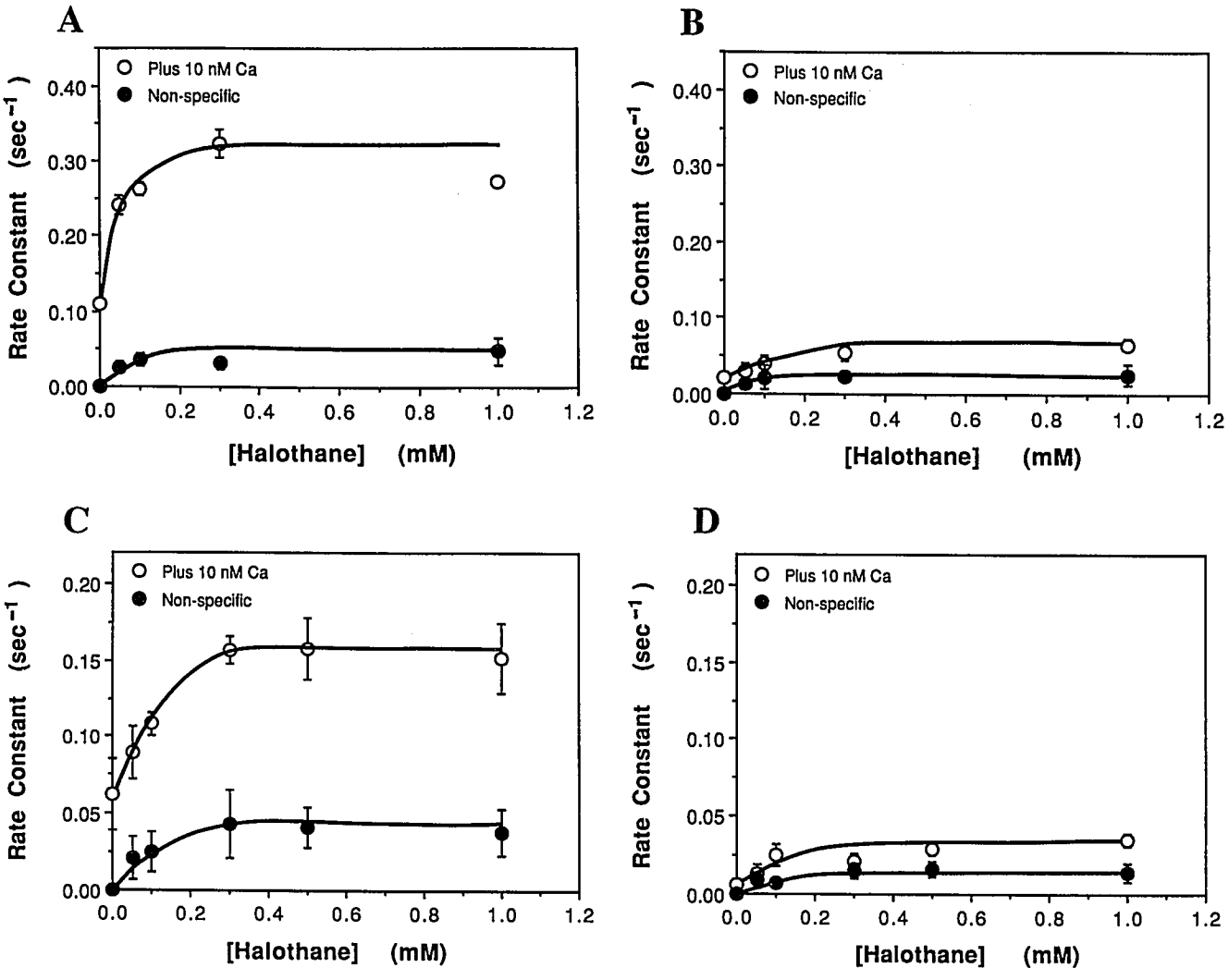

FIG. 10. Effect of halothane on Ca^{2+} release from malignant hyperthermia–susceptible (MHS) and normal sarcoplasmic reticulum at *p*H 6.8 and 6.6. MHS (*A, C*) and normal (*B, D*) sarcoplasmic reticulum were loaded with $^{45}Ca^{2+}$, and the rate constant for Ca^{2+} release was determined after dilution into various concentrations of halothane in 10^{-8} M Ca^{2+} medium (open circles) or 10 mM $MgCl_2$, 10 mM EGTA, 10 μM ruthenium red release-inhibiting medium (filled circles) at either *p*H 6.8 (*A, B*) or *p*H 6.6 (*C, D*) as described in Materials and Methods. Points represent the means ±SE of duplicate determinations on three MHS and three normal sarcoplasmic reticulum preparations.

Ca^{2+} uptake reported in our study at 0.5 and 1 mM anesthetic exhibits a similar order of effectiveness (figs. 1 and 2); however, with the overlap of standard errors there was no significant difference between the three anesthetics. There are a number of previous reports of agents that can stimulate sarcoplasmic reticulum Ca^{2+} uptake (up to 2-fold); these include the volatile anesthetic diethyl ether (5%)[32] and dimethyl sulfoxide (20%).[33] These effects have been explained as due in part to an increase in sarcoplasmic reticulum intravesicular volume and altered lipid–protein interactions that permit faster translocation of Ca^{2+}.[32,34] A similar mechanism of action is likely responsible for the actions of the volatile anesthetics on Ca^{2+} uptake reported in the present study.

Studies that have measured the effects of volatile anesthetic on sarcoplasmic reticulum have reported both stimulation[18,35–37] and inhibition[18,19,38,39] of ATP-dependent Ca^{2+} uptake; in studies with up to 2 mM halothane, there is a trend for this agent to stimulate Ca^{2+} uptake, whereas halothane concentrations greater than 5 mM tend to inhibit Ca^{2+} uptake. This agrees with our results (fig. 3) and those of Nelson and Sweo,[21] although the latter authors proposed that concentrations of anesthetic that stimulated the pump did so by interacting directly with this enzyme, whereas the higher concentrations that inhibited Ca^{2+} uptake had a general perturbation effect on the lipid portion of the sarcoplasmic reticulum membrane. Irrespective of the mechanism responsible for the inhi-

bition of sarcoplasmic reticulum Ca^{2+} uptake by volatile anesthetics, this inhibition occurs only at anesthetic concentrations that are 10–20 times the clinically effective concentrations. Thus, they cannot account for the demonstrated ability of clinical concentrations of these agents to trigger the MH response by producing a sustained elevation of sarcoplasmic Ca^{2+} concentration. Most importantly, our studies demonstrate that the effects of halothane, isoflurane, and enflurane on ATP-dependent Ca^{2+} uptake by MHS and normal sarcoplasmic reticulum are not significantly different. This would support our[26] and others'[37] previous conclusion that there is no defect in the sarcoplasmic reticulum Ca^{2+} pump in MHS muscle.

Although a clinical effect of these anesthetic agents on the sarcoplasmic reticulum Ca^{2+} pump might be to enhance muscle relaxation, our data demonstrates that the opposite is true for the sarcoplasmic reticulum Ca^{2+}-release channel. As reported by Nelson and Sweo,[21] all three volatile anesthetics stimulated Ca^{2+} release from Ca^{2+}-filled sarcoplasmic reticulum vesicles at concentrations that were 10–20 times lower than those required to inhibit ATP-dependent Ca^{2+} uptake. Indeed, the concentrations of anesthetics affecting the sarcoplasmic reticulum Ca^{2+}-release channel are in the clinically relevant range and are below most reported values for the effect of these agents on other membrane channels and pumps. The far lower effective concentration would more logically argue for a direct interaction between the volatile anesthetics and the sarcoplasmic reticulum ryanodine receptor Ca^{2+}-release channel protein and not the Ca^{2+} pump. That the Ca^{2+} release measured in this study is primarily mediated by the 540-kD ryanodine receptor protein is demonstrated by the very low rate of Ca^{2+} release in the presence of the channel inhibitors ruthenium red, Mg^{2+}, and EGTA. Furthermore, anesthetic-induced, Ca^{2+}-channel blocker–inhibitable Ca^{2+} release was not observed in a light sarcoplasmic reticulum fraction lacking the ryanodine receptor protein (data not shown). The anesthetic-induced nonspecific Ca^{2+} release seen in heavy sarcoplasmic reticulum, however, could contribute *in vivo* to triggering a greater Ca^{2+} release *via* the Ca^{2+}-release channel.

A major finding of the present study is that volatile anesthetics stimulated the sarcoplasmic reticulum Ca^{2+}-release channel at a Ca^{2+} concentration (10^{-8} M) that would be expected to maintain this channel in the closed state. Although Ohnishi *et al.*[20] have reported previously (with an indirect measurement of Ca^{2+} release) that halothane is unable to stimulate sarcoplasmic reticulum Ca^{2+} release even in the presence of 10^{-7} M Ca^{2+}, our own previous study that directly examined Ca^{2+} release from $^{45}Ca^{2+}$-filled vesicles demonstrated a significant halothane-stimulation of sarcoplasmic reticulum Ca^{2+} release in 10^{-7} M Ca^{2+} medium.[26] These differences likely reflect differences in the procedures used to measure sarcoplasmic reticulum Ca^{2+} release. Our data also demonstrate that in addition to stimulating sarcoplasmic reticulum Ca^{2+} release in 10^{-8} M Ca^{2+} medium, these volatile anesthetics induce a significantly greater rate of Ca^{2+} release from MHS than from normal sarcoplasmic reticulum. If extrapolated to an *in vivo* situation, this could explain how volatile anesthetics are able to effect the MH-triggering elevation of sarcoplasmic Ca^{2+} concentration in the sedated individual in whom the musculature is in the relaxed state; *i.e.*, myoplasmic Ca^{2+} concentration is $\leq 10^{-7}$ M. Thus, like our several previous studies documenting an enhanced Ca^{2+} release induced by a variety of ligands that are known to regulate this channel protein,[5,6,26,40] the MHS Ca^{2+}-release channel is more sensitive to clinical concentrations of volatile anesthetic (*i.e.*, releases a greater fraction of stored Ca^{2+}) than the normal channel.

That Ca^{2+} release from sarcoplasmic reticulum vesicles and single-channel activity is inhibited as the *p*H is lowered has now been well documented.[22,41] However, our data are the first to demonstrate that a decrease in *p*H not only inhibits the sarcoplasmic reticulum Ca^{2+}-release channel, but it also influences the ability of anesthetics to stimulate this channel. Notably, below *p*H 6.8 in normal sarcoplasmic reticulum, the Ca^{2+}-release channel is essentially completely inhibited in either the presence or absence of a volatile anesthetic. In contrast, the MHS Ca^{2+}-release channel still can be activated by these anesthetics at *p*H values as low as 6.4. This has interesting implications for the etiology of an MH episode: the acidosis that accompanies the MH response *in vivo* may not be sufficient to counteract completely the anesthetic-induced stimulation of Ca^{2+} release *via* the MHS sarcoplasmic reticulum Ca^{2+}-release channel. In contrast, in normal muscle, even if the addition of a volatile anesthetic is able to induce Ca^{2+} release, producing a small elevation in sarcoplasmic Ca^{2+}, the accompanying fall in *p*H (resulting from the Ca^{2+}-dependent activation of glycogenolysis and glycolysis) will in turn inhibit the Ca^{2+}-release channel, allowing the sarcoplasmic reticulum Ca^{2+} pump to return sarcoplasmic Ca^{2+} to resting levels.

Our inability to demonstrate an inhibition of Ca^{2+} release from sarcoplasmic reticulum vesicles by dantrolene leaves unresolved the question of this important therapeutic agent's site of action in skeletal muscle. Other studies have reported that dantrolene either has no effect[11] or inhibits sarcoplasmic reticulum Ca^{2+} release.[20,31,42] In agreement with our results reported here, dantrolene has no effect on the single-channel activity of the sarcoplasmic reticulum Ca^{2+}-release channel in lipid bilayers.§ Interestingly, it has been proposed that this drug may act by inhibiting charge movement by the T tubule voltage sensor.[43,44]

§ Fill M: Personal communication.

In concluding that the increase in sarcoplasmic Ca^{2+} triggered by volatile anesthetics in MH muscle is due to the action of these agents on the sarcoplasmic reticulum Ca^{2+}-release channel, it is necessary to explain how this can be reconciled with the ability of similar concentrations of these same anesthetics to stimulate ATP-dependent sarcoplasmic reticulum Ca^{2+} uptake. The most likely explanation is that in intact muscle, where Ca^{2+} uptake by the sarcoplasmic reticulum is not massively stimulated by oxalate, phosphate, or pyrophosphate (as used in isolated sarcoplasmic reticulum ATP-dependent Ca^{2+} uptake studies[18]), Ca^{2+} release *via* the Ca^{2+}-release channel is much more rapid than Ca^{2+} uptake by the ATP-dependent Ca^{2+} pump. The Ca^{2+} pump would be active, consuming ATP, but as long as the Ca^{2+}-release channel was held in the open state by the volatile anesthetic, myoplasmic Ca^{2+} would remain elevated. This would contribute to the lactic acidosis that accompanies the MH response[1,2] as glycolysis attempted to maintain sarcoplasmic ATP levels in the face of sustained ATP hydrolysis by the sarcoplasmic reticulum ATP-dependent Ca^{2+} pump.

Additional studies are necessary to determine the significance of our observations with isolated vesicles to the clinical effects of halothane, isoflurane, and enflurane *in vivo*. However, our studies clearly document that the interaction of volatile anesthetics with the sarcoplasmic reticulum Ca^{2+}-release channel is altered in MH. Ongoing studies in our laboratory are attempting to define whether this is due to an interaction with the Ca^{2+}-release channel protein itself or rather with components of the lipid bilayer that in turn influence the permeability of this channel protein.

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References

1. Gronert GA: Malignant hyperthermia, Myology. Edited by Engel AG, Banker BQ. Minneapolis, McGraw-Hill Publications, 1986, pp 1763–1784
2. Gronert GA, Mott J, Lee J: Aetiology of malignant hyperthermia. Br J Anaesth 60:253–267, 1988
3. Lopez JR, Allen PD, Alamo L, Jones D, Sreter F: Myoplasmic free [Ca^{2+}] during a malignant hyperthermia episode in swine. Muscle Nerve 11:82–88, 1988
4. Iaizzo 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. Pflugers Arch 411:648–653, 1988
5. Mickelson JR, Gallant EM, Litterer LA, Johnson KM, Rempel WE, Louis CF: Abnormal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. J Biol Chem 263:9310–9315, 1988
6. Mickelson JR, Gallant EM, Rempel WE, Johnson KM, Litterer LA, Jacobson BA, Louis CF: Effects of the halothane-sensitivity gene on sarcoplasmic reticulum function. Am J Physiol 257: C787–794, 1989
7. Fill M, Coronado R, Mickelson JR, Vilven J, Ma J, Jacobson BA, Louis CF: Abnormal ryanodine receptor channels in malignant hyperthermia. Biophys J 50:471–475, 1990
8. Ohnishi ST, Taylor S, Gronert GA: Calcium-induced Ca^{2+} release from sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia. FEBS Lett 161:103–107, 1983
9. Nelson TE: Abnormality in calcium release from skeletal muscle sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia. J Clin Invest 72:862–870, 1983
10. Kim DH, Sreter FA, Ohnishi ST, Ryan JF, Roberts J, Allen PD, Meszaros LG, Antonui B, Ikemoto N: Kinetic studies of Ca^{2+} release from sarcoplasmic reticulum of normal and malignant hyperthermia susceptible pig muscles. Biochim Biophys Acta 775:320–327, 1984
11. 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
12. Fill M, Stefani E, Nelson TE: Abnormal human sarcoplasmic reticulum Ca^{2+} release channels in malignant hyperthermia skeletal muscle. Biophys J 59:1085–1090, 1991
13. MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG: Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature (London) 343:559–561, 1990
14. McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K: Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. Nature (London) 343:562–564, 1990
15. Harbitz I, Chowdhary B, Thomsen PD, Davies W, Kaufmann U, Kran S, Gustavsson I, Christensen K, Hauge JG: Assignment of the porcine calcium release channel gene, a candidate for the malignant hyperthermia locus, to the 6p11-q21 segment of chromosome 6. Genomics 8:243–248, 1990
16. Fujii J, Otsu K, Zorzato F, DeLeon S, Khanna VK, Weiler JE, O'brian PJ, MacLennan DH: Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Nature (London) 253:448–451, 1991
17. Levitt RC, Nouri N, Jedlicka AE, McKusick VA, Marks AR, Shutack JG, Fletcher JE, Rosenberg H, Meyers DA: Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. Genomics 11:543–547, 1991
18. Gronert GA, Heffron JJA, Taylor SR: Skeletal muscle sarcoplasmic reticulum in porcine malignant hyperthermia. Eur J Pharmacol 58:179–187, 1979
19. Blanck TJJ, Gruener R, Suffecool SL, Thompson M: Calcium uptake by isolated sarcoplasmic reticulum: Examination of halothane inhibition, pH dependence, and Ca^{2+} dependence of normal and malignant hyperthermic human muscle. Anesth Analg 60:492–498, 1981
20. Ohnishi ST, Waring AJ, Fang S-RG, Horiuchi K, Flick JL, Sadanaga KK, Ohnishi T: Abnormal membrane properties of the sarcoplasmic reticulum of pigs susceptible to malignant hyperthermia: Modes of action of halothane, caffeine, dantrolene, and two other drugs. Arch Biochem Biophys 247:294–301, 1986
21. Nelson TE, Sweo T: Ca^{2+} uptake and Ca^{2+} release by skeletal muscle sarcoplasmic reticulum: Differing sensitivity to inhalational anesthetics. ANESTHESIOLOGY 69:571–577, 1988
22. Meissner G: Adenine nucleotide stimulation of Ca^{2+}-induced Ca^{2+}

release in sarcoplasmic reticulum. J Biol Chem 259:2365–2374, 1984
23. Meissner G, Darling E, Eveleth J: Kinetics of rapid Ca^{2+} release by sarcoplasmic reticulum: Effects of Ca^{2+}, Mg^{2+}, and adenine nucleotides. Biochemistry 25:236–244, 1986
24. McGrath CJ, Rempel WE, Addis PB, Crimi AJ: Acepromazine and droperidol inhibition of halothane-induced malignant hyperthermia (porcine stress syndrome) in swine. Am J Vet Res 42:195–198, 1981
25. Firestone LL, Miller JC, Miller KW: Tables of physical and pharmacological properties of anesthetics, Molecular and Cellular Mechanisms of Anesthetics. Edited by Rotti SH, Miller KW. New York, Plenum Publishing, 1986, pp 455–470
26. Mickelson JR, Ross JA, Reed BK, Louis CF: Enhanced Ca^{2+}-induced calcium release by isolated sarcoplasmic reticulum vesicles from malignant hyperthermia susceptible pig muscle. Biochim Biophys Acta 862:318–328, 1986
27. Britt BA: Muscle assessment of malignant hyperthermia patients, Malignant Hyperthermia. Edited by Britt BA. Boston, Martinus Nijhoff, 1987, pp 193–267
28. Lerman J, Relton JES: Anesthesia for malignant hyperthermia susceptible patients, Malignant Hyperthermia. Edited by Britt BA, Boston, Martinus Nijhoff Publishing, 1987, pp 369–392
29. Britt BA: Dantrolene: An update, Malignant Hyperthermia. Edited by Britt BA. Boston, Martinus Nijhoff, 1987, pp 325–367
30. Lopez JR, Medina P, Alamo L: Dantrolene sodium is able to reduce the resting ionic $[Ca^{2+}]_i$ in muscle from humans with malignant hyperthermia. Muscle Nerve 10:77–79, 1987
31. Ohta T, Ito S, Ohga A: Inhibitory action of dantrolene on Ca-induced Ca^{2+} release from sarcoplasmic reticulum in guinea pig skeletal muscle. Eur J Pharmacol 178:11–19, 1990
32. Salama G, Inesi A: Mode of action of diethyl ether on ATP dependent Ca^{2+} transport by sarcoplasmic reticulum vesicles. Biochem Pharmacol 32:3465–3477, 1983
33. The R, Hasselbach W: Stimulatory and inhibitory effects of dimethyl sulfoxide and ethylene glycol on ATPase activity and calcium transport of sarcoplasmic reticulum. Eur J Biochem 74: 611–621, 1977
34. Bigelow DJ, Thomas DD: Rotational dynamics of lipid and Ca-ATPase in sarcoplasmic reticulum. J Biol Chem 262:13449–13456, 1987
35. Isaacs H, Heffron JJA: Morphological and biochemical defects in muscles of human carriers of the malignant hyperthermia syndrome. Br J Anaesth 47:475–480, 1975
36. Britt BA, Kalow W, Gordon A, Humphrey JG, Rewcastle NB: Malignant hyperthermia-an investigation of five patients. Can Anaesth Soc J 20:431–467, 1973
37. Nelson TE, Jones EW, Venable JH, Kerr DD: Malignant hyperthermia of Poland China swine: Studies of a myogenic etiology. ANESTHESIOLOGY: 36:52–56, 1972
38. Diamond EM, Berman MC: Effect of halothane on the stability of Ca^{2+} transport activity of isolated fragmented sarcoplasmic reticulum. Biochem Pharmacol 29:375–381, 1980
39. Dhalla NS, Sulakhe PV, Clinch NF, Wode JG, Naimark A: Influence of fluothane on calcium accumulation by the heavy microsomal fraction of human skeletal muscle: Comparison with a patient with malignant hyperthermia. Biochem Med Metab Biol 6:333–343, 1972
40. Mickelson JR, Litterer LA, Jacobson BA, Louis CF: Stimulation and inhibition of [^{3}H]ryanodine binding to sarcoplasmic reticulum from malignant hyperthermia susceptible pigs. Arch Biochem Biophys 278:251–257, 1990
41. Rousseau E, Pinkos J: pH modulates conducting and gating behavior of single calcium release channels. Pflugers Arch 415: 645–647, 1990
42. Van Winkle WB: Calcium release from skeletal muscle sarcoplasmic reticulum: Site of action of dantrolene sodium? Science 193: 1130–1131, 1976
43. Hui CS: Pharmacological studies of charge movement in frog skeletal muscle. J Physiol (Lond) 337:509–529, 1983
44. Morgan KC, Bryant SH: The mechanism of action of dantrolene sodium. J Pharmacol Exp Ther 201:138–147, 1977