Halothane, Enflurane, and Isoflurane Stimulate Calcium Leakage from Rabbit Sarcoplasmic Reticulum

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The sarcoplasmic reticulum (SR) controls uptake and release of Ca2+ in muscle. Little information is available regarding the effect of volatile anesthetics on Ca2+ release from SR isolated from normal skeletal muscle, even though an abnormality of Ca2+ handling is implicated in malignant hyperthermia. In this study we used a Ca2+ electrode to monitor continuously the release of Ca2+ from SR and the effect of volatile anesthetics on this process. We found that halothane, enflurane, and isoflurane at 0.6, 0.7, and 0.8 vol%, respectively, each increased the velocity of Ca2+ leakage by at least 150% when compared to control. Ruthenium red, a blocker of the SR Ca2+-release channel, was shown to have no effect on the velocity of Ca2+ leakage. Halothane and isoflurane both shortened the time at which Ca2+ leakage began (T) in a dose-dependent fashion. Halothane at 4.8 vol% decreased T from 293 ± 21 s to 149 ± 20 s. Isoflurane (4.8 vol%) decreased T to 203 ± 16 s, and enflurane at 5 vol% had little effect, decreasing T to 259 ± 19 s. We noted a marked stimulation in the ATPase activity of the SR by all three volatile anesthetics. Halothane at 0.63 vol%, enflurane at 0.42 vol%, and isoflurane at 0.62 vol% each increased ATPase activity by at least 300%. We conclude that the stimulation of the velocity of Ca2+ leakage by the volatile anesthetics is related to the more rapid depletion of ATP, but that the shortening of the onset of Ca2+ leakage is a dependent phenomenon with a markedly different dose dependence. We also noted an enflurane-induced decrease in the Michaelis-Menten constant (Km) of the Ca2+ pump for ATP from 3.27 ± 0.78 mm to 0.280 ± 0.079 mm. Our data show that the volatile anesthetics increased the permeability of isolated SR to Ca2+. Although the permeability of the SR in vivo is uncertain, it is possible in vivo, in the presence of volatile anesthetics, that SR might be leaky Ca2+ into the cytoplasm. In malignant hyperthermic muscle, increased permeability of the SR caused by volatile anesthetics might contribute to the triggering of malignant hyperthermia. (Key words: Anesthetics, volatile; halothane; enflurane; isoflurane. Ions: calcium. Skeletal muscle; sarcoplasmic reticulum.)

The sarcoplasmic reticulum (SR) is an internal membrane system in the skeletal muscle cell that controls the availability of ionized calcium (Ca2+) for contraction. This organelle has been implicated as the site of abnormality in skeletal muscle from malignant hyperthermic patients and swine. Because the volatile anesthetics are primary triggers of malignant hyperthermia, it is important to examine their action on SR from normal muscle in order to understand pathologic effects in SR derived from malignant hyperthermic muscle.

Several investigators have independently found an abnormality of Ca2+ release by the SR isolated from malignant hyperthermic pig muscle. In a recent paper, Nelson and Sweo demonstrated the effect of the volatile anesthetics halothane, enflurane, and isoflurane on the Ca2+ uptake and release mechanisms of SR prepared from rabbit white skeletal muscle. Previously, Su and Bell, using skinned fiber preparations, demonstrated that halothane, enflurane, and isoflurane all increase the release of Ca2+ from the SR. SR can be separated by homogenization and centrifugation into heavy and light fractions, each of which has been found to have a predominant uptake or release function, respectively. Nelson and Sweo observed stimulation of Ca2+ uptake by all three volatile anesthetics at a concentration of 1.1 MAC; the uptake studies were performed on a light fraction of SR. They examined Ca2+ release in a heavy fraction of SR and noted that all three volatile anesthetics markedly decreased the Ca2+-induced Ca2+-release threshold at very low concentrations; the anesthetic effect was saturated at only 2% of MAC. The results of Nelson and Sweo and Su and Bell focus on anesthetic effects on heavy SR, where the major Ca2+ release path exists.

The purpose of the present study was to determine whether another site in the SR might also be modified by the volatile anesthetics halothane, enflurane, and isoflurane, resulting in leakage or release of Ca2+ that could lead to an increase in myoplasmic Ca2+. We therefore examined the effect of the volatile anesthetics on Ca2+ leakage from an SR preparation that sediments between 15,000 x g and 40,000 x g. This is similar to the light fraction, in which Nelson and Sweo examined the uptake...
characteristics and which is often referred to as longitudinal SR. The preparation we used has been used extensively for Ca\(^{2+}\) uptake studies, but until recently, little information was available dealing with its Ca\(^{2+}\) leakage properties. In this paper we present data characterizing the modulation of the onset and rate of spontaneous Ca\(^{2+}\) leakage from light SR by the volatile anesthetics. We confirmed the existence of Ca\(^{2+}\) leakage from the light fraction of SR as previously demonstrated by Inesi and de Meis.

**Materials and Methods**

**Sarcoplasmic Reticulum Preparation**

With approval of the Johns Hopkins University Animal Care and Use Committees, SR was isolated from rabbit skeletal muscle by a slight modification of the method of Eletr and Inesi. White New Zealand rabbits were anesthetized with intravenous sodium pentobarbital (30 mg/kg), and 150–200 g of paravertebral white skeletal muscles were excised from the back, weighed, and suspended in 5 times the volume of 10 mM histidine, 10% sucrose, and 0.1 mM EDTA at pH 7.0 and 4°C. The muscles were homogenized for 15 s every 5 min for a total of 45 min. The homogenate was centrifuged in at 15,000 × g and 4°C for 20 min. The supernatant was filtered through two layers of cheesecloth and centrifuged at 40,000 × g and 4°C for 90 min. Pellets were resuspended in approximately 30 ml of 0.6 M KCl, 10 mM histidine (pH 7.0, 4°C). The suspension was then centrifuged for 90 min at 40,000 × g and 4°C. The final pellets of SR vesicles were resuspended in 6–8 ml 10 mM histidine and 30% sucrose (pH 7.0, 4°C), stored in air-tight vials, and frozen in liquid nitrogen. Total protein content was determined spectrophotometrically using the Coomassie binding technique and bovine serum albumin as the standard.

**Calcium Measurement**

Extravesicular pH and Ca\(^{2+}\) were continuously measured with electrodes (pH: Orion; Ca\(^{2+}\): model 011-020-115, Iontics, Inc. with an Orion Ionometer model 399A) in a 2.0-ml reaction solution inside an air-filled 41-ml gas-tight, water-jacketed glass chamber with temperature and pH maintained at 37°C and 7.0 respectively. The Ionometer was modified with an antilog circuit to give a linear response to additions of equivalent amounts of Ca\(^{2+}\). The 2.0 ml reaction solution was magnetically stirred at low speed and contained 50 mM 4-(2-hydroxyethyl)-1-piperazine sulfonic acid (HEPES), 100 mM KCl, and 0.5 mM MgCl\(_2\). The 0.01 mM ethylene glycol bis(β-amino ethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 0.5 mg/ml SR (1.0 mg total), 10.0 μM CaCl\(_2\) (four additions of 2.5 μM for calibration steps of the Ca\(^{2+}\) electrode), and 0.5 mM ATP were sequentially injected into the reaction medium via Hamilton syringes with 4-inch needles through air-tight Teflon/silicone caps. Repeated trials were measured for the amount of Ca\(^{2+}\) uptake, amount of Ca\(^{2+}\) leakage, time in seconds (T) between the end of uptake and the onset of Ca\(^{2+}\) leakage (measured at 5% of the total Ca\(^{2+}\) released), and the maximal rate of Ca\(^{2+}\) release. After the maximal rate of Ca\(^{2+}\) release was observed, 20 μM of a Ca\(^{2+}\) ionophore (A23187 dissolved in ethanol) was added to determine the total amount of "releasable" Ca\(^{2+}\) from the SR.

The reaction chamber and electrodes were thoroughly rinsed three times with distilled, deionized water between trials. After control measurements without volatile anesthetics, repeated trials were studied with additions of halothane (0.62%, 2.22%, and 4.62%), enflurane (0.71%, 1.22%, 2.22%, and 4.33%), and isoflurane (0.81%, 1.30%, 2.28%, and 4.33%). Every experimental determination of T and leakage velocity at a given anesthetic concentration was preceded by a control determination. Thymol-free halothane was obtained from Halocarbon Laboratories, and isoflurane and enflurane were purchased from Anaquest. The halothane syringe was filled in a dark, nitrogen environment to avoid oxidation. The liquid halothane, enflurane, and isoflurane were injected via a gas-tight Hamilton syringe into the 2.0 ml reaction solution through the gas-tight Teflon/silicone cap. After 5 min of equilibration of the buffer, SR, Ca\(^{2+}\), and ATP were sequentially added and measurements made.

**ATPase Activity**

Total ATPase activity was measured in the same medium as used for Ca\(^{2+}\) uptake and leakage experiments. Experiments were performed at 37°C, pH 7.0 in 50 mM HEPES, 100 mM KCl, 0.01 mM EGTA, 0.005 mM CaCl\(_2\), 0.5 mM ATP, and 0.5 mg/ml SR. The reaction was initiated by the addition of SR and terminated at variable times by the addition of an equal volume of 10% trichloroacetic acid. Released phosphate was measured by the method of Huxtable and Bressler. Experiments were performed in 31-ml sealed vials, and equilibrated anesthetic concentration was achieved by the addition of liquid anesthetic to buffer, followed by vigorous mixing.

**45Ca\(^{2+}\) Uptake Measurements**

Radioactive 45Ca\(^{2+}\) uptake into SR vesicles was measured by a modification of the Millipore filtration technique as previously described. The reaction was performed in 31-ml glass vials. The reaction medium consisted of radiolabeled Ca\(^{2+}\) (45CaCl\(_2\), 100,000 cpm/sample), 100 μg SR, 5 mM MgCl\(_2\), 0.1 mM CaCl\(_2\), 5 mM...
K⁺ oxalate, 50 mM HEPES, 100 mM KCl, and varying concentrations of ATP. Anesthetic was added in liquid form to the reaction medium. The reaction was terminated at 30 s by rapid filtration, and the amount of accumulated Ca²⁺ was quantitated by liquid scintillation counting.

**MEASUREMENTS OF VOLATILE ANESTHETICS**

After 5 min of equilibration, a needle was inserted through the Teflon cap and a 1-ml gas-tight Hamilton syringe was used to remove 1 ml gas. The syringe needle was then immersed into 1 ml heptane in a 2-ml nitrogen-flashed bottle; the gas in the syringe was emptied into the heptane; and the bottle was rapidly capped. After mixing the contents of the bottle with a mechanical agitator (Vortex) for 5 s, the heptane was analyzed for anesthetic gases using gas chromatography.¹⁴

**SODIUM DODECYL SULFATE ELECTROPHORESIS**

SR samples were electrophoresed on a Pharmacia Phastgel system using 8—25% gradient sodium dodecyl sulfate gels. Samples were dissolved in 2.5% sodium dodecyl sulfate and 5% β mercaptoethanol prior to application on the gel. Samples were electrophoresed at 250 V and 10 mA and gels stained with Coomassie blue. The electrophoresis demonstrated a pattern consistent with predominantly light SR with little contamination by heavy SR and no evidence of a 450-kD protein, suggesting the absence of the high-conductance Ca²⁺-release channel of SR⁹ (fig. 1).

**STATISTICS**

The effect of anesthetic on T was evaluated by two-way analysis of variance (ANOVA). The statistical significance of the effect of anesthetic on the Michaelis-Menten constant (Kₘ) of the Ca²⁺ pump for ATP was determined by performing an F test of the variances of the Kₘ estimates. The effect of ruthenium red on leakage velocity was compared to control measurements by a paired t test.

**Results**

Figure 2 demonstrates a typical trace from one experiment. Ca²⁺ was added in four equal amounts to use as an internal calibration of the electrode response. The addition of 0.5 mM ATP to the reaction mixture resulted in three distinct phases of the Ca²⁺ trace: 1) a rapid decrease indicative of accumulation of Ca²⁺ by the SR, 2) a plateau indicative of no net change in Ca²⁺; and 3) a progressive increase in the Ca²⁺ signal indicative of Ca²⁺ leakage from the SR. Addition of A23187, the Ca²⁺ ionophore, resulted in the rapid increase in the Ca²⁺ signal due to total release of Ca²⁺ from the SR. The plateau achieved after A23187 addition was taken as the maximal Ca²⁺ that could be released. Approximately 20% of the luminal Ca²⁺ was retained after spontaneous leakage and could be released only with A23187. In order to standardize the time to onset of release it was measured at 5% of the signal at the plateau phase. The uptake and
release curve was the same whether the reaction was started with ATP or SR.

We observed a relatively slow rate of leakage of Ca²⁺ from light SR vesicles (fig. 2). The amount of Ca²⁺ that leaked out was approximately equal to the amount taken up (fig. 3A). T decreased with increased Ca²⁺ concentration and increased with increased ATP concentration (figs. 3B and 3C). The velocity of leak varied with increased Ca²⁺ concentration in a manner similar to that previously reported⁹ (fig. 3D) and increased with increased ATP concentration. This leakage of Ca²⁺ appeared unrelated to the rapid rate of Ca²⁺ release through the high-conductance Ca²⁺-release channel in heavy SR because it was much slower and was insensitive to ruthenium red (fig. 4).¹⁵,¹⁶ At 20 μM ruthenium red, there was no significant change in leakage velocity (control velocity 0.926 ± 0.510 nmol/s vs. velocity with ruthenium red 0.792 ± 0.355 nmol/s) and, as shown in figure 4, no concentration-dependent effect of ruthenium red. We examined the effect of the volatile anesthetics on this phenomenon to determine whether there was a site in the SR other than the high-conductance channel in heavy SR where the volatile anesthetics might act, leading to an increase in myoplasmic Ca²⁺. The response of the Ca²⁺ electrode was too slow to monitor small variations in the rate of Ca²⁺ uptake under the conditions of the present experiments; however, in our system, total uptake of Ca²⁺ was unaffected by exposure of SR to volatile anesthetics.

**ANESTHETIC EFFECTS**

The total amount of Ca²⁺ accumulated and released did not vary with any concentration of halothane, enflurane, or isoflurane. The velocity of efflux was markedly increased relative to control (figs. 1 and 5) but over the concentration range examined did not demonstrate a concentration dependence. Halothane, isoflurane, and enflurane all stimulated the rate of efflux by 150–200%, even at the lowest concentration studied. The onset of release was markedly altered, particularly by halothane (figs. 2B and 6). The time to onset of efflux decreased by approximately 45% as halothane concentration was increased. This inverse relationship of T with halothane concentration was statistically significant at the P < 0.0001 level (ANOVA). Similarly, isoflurane decreased the onset of Ca²⁺ leakage (P < 0.0001), but enflurane had no effect on T when SR was preincubated for only 5 min.

Experiments performed in the presence of an ATP-regenerating system resulted in marked prolongation of T; similarly, T was prolonged with increasing ATP concentration (fig. 3C).

**ATPase Activity**

ATPase activity was measured in order to determine whether the decrease in T was due to the depletion of ATP. As seen in figure 7, all three volatile anesthetics stimulated ATPase activity, resulting in a more rapid depletion of ATP than in control conditions. In the presence of anesthetic, essentially all the ATP was hydrolyzed within 2 min, whereas in the control situation ATP depletion did not occur until approximately 3 min or later. The rate of ATPase activity as a function of anesthetic concentration demonstrated a lack of concentration dependence (fig. 8) similar to that of the stimulation of leakage velocity by volatile anesthetics. At the lowest concentrations of anesthetic used, ATPase activity was stimulated 400, 300, and 400% respectively by 0.63% halothane, 0.62% enflurane, and 0.42% isoflurane.

**Ca²⁺ UPTAKE**

Figure 9 demonstrates the effect of the volatile anesthetics on the ATP dependence of Ca²⁺ uptake. The effect of enflurane appears to be greater than that of either halothane or isoflurane in decreasing the requirement of the Ca²⁺ pump for ATP. Enflurane at 1.7 vol% decreased the concentration of ATP required to obtain the maximal velocity of Ca²⁺ uptake. The Kₘ and maximal velocity of
the reaction (V\text{max}) were obtained by nonlinear regression fit of the data to the Michaelis-Menten equation. Enflurane 1.7 vol% caused a statistically significant decrease in the K\text{m} of the Ca\textsuperscript{2+} pump for ATP, from 3.27 ± 0.78 M to 0.200 ± 0.079 M (P < 0.05), whereas the V\text{max} remained essentially unchanged, 2.382 ± 315 nmoles \cdot mg\textsuperscript{-1} \cdot min\textsuperscript{-1} versus 1,879 ± 203 nmoles \cdot mg\textsuperscript{-1} \cdot min\textsuperscript{-1}. Halothane and isoflurane had no statistically significant effect on the K\text{m}.

**Discussion**

The movement of Ca\textsuperscript{2+} out of light SR vesicles that we observed occurred more slowly and appeared to be dissimilar to the rapid Ca\textsuperscript{2+} release from heavy SR that others have noted\textsuperscript{15,16}, this points to an alternate path of Ca\textsuperscript{2+} efflux from light SR that might be related to "Ca\textsuperscript{2+} slippage through the ATPase channel into the external medium."\textsuperscript{16} This is supported by the electrophoresis of the SR, which demonstrated the presence of the Ca\textsuperscript{2+}-pump protein but the absence of the Ca\textsuperscript{2+}-release channel protein. In the experiments described, the amount of Ca\textsuperscript{2+} accumulated and the amount leaked out were comparable, suggesting that the same vesicle population that accumulated Ca\textsuperscript{2+} had also leaked Ca\textsuperscript{2+}. The modest rate of Ca\textsuperscript{2+} leakage (up to 200 nmoles \cdot mg\textsuperscript{-1} \cdot min\textsuperscript{-1}) observed in our experiments might be an underestimate of maximal activity because of our experimental conditions. The rate of leakage is dependent on the Ca\textsuperscript{2+} load in the SR, as shown in figure 3D. The majority of our experiments were performed at low Ca\textsuperscript{2+} load, where the rate of leakage was low. Furthermore, the rate of leakage was measured with a background of Ca\textsuperscript{2+} accumulation until all ATP was hydrolyzed.

We demonstrated that the volatile anesthetics halothane, enflurane, and isoflurane alter the ability of light skeletal SR vesicles to retain Ca\textsuperscript{2+}. The SR was isolated by homogenization and differential centrifugation, yielding a membrane vesicle population that was able to accumulate Ca\textsuperscript{2+} via a Ca\textsuperscript{2+} pump that requires ATP. These vesicles, however, under our experimental conditions, leaked Ca\textsuperscript{2+}, and this leakage became apparent as the ATP that energizes the Ca\textsuperscript{2+} pump decreased. As indicated in

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**Fig. 3.** Characteristics of Ca\textsuperscript{2+} uptake and release as monitored with a Ca\textsuperscript{2+} electrode. Experiments were performed in a 41-ml air-filled gastight chamber, 2.0 ml of reaction solution, pH 7.0, 37°C. The reaction solution contained 50 mM Hepes, 0.5 mM MgCl\textsubscript{2}, 0.01 mM EGTA, 0.5 mg/ml sarcoplasmic reticulum, 0.01 mM CaCl\textsubscript{2}, and 0.5 mM ATP. A: A measure of the total Ca\textsuperscript{2+} accumulated and Ca\textsuperscript{2+} that leaked out as a function of CaCl\textsubscript{2} concentration. B: The onset time of Ca\textsuperscript{2+} leakage as a function of CaCl\textsubscript{2} concentration. C: The onset time of Ca\textsuperscript{2+} leakage as a function of ATP concentration. D: The velocity of Ca\textsuperscript{2+} leakage as a function of CaCl\textsubscript{2} concentration.
Fig. 4. Relative velocity of Ca\(^{2+}\) leakage as a function of ruthenium red concentration. Experimental conditions are as described in figure 3. The y-axis demonstrates the ratio of the velocity of leakage in the presence of a given concentration of ruthenium red (Ve) to the control value determined just prior to each experimental value (Vc).

Figures 7 and 8, the volatile anesthetics stimulate the rate of ATP hydrolysis, which appears to be related to the increase in the rate of Ca\(^{2+}\) leakage. These observations, along with the prolongation of T with increasing concentration of ATP, suggest a relationship between leakage and ATP depletion. However, that T does not change during enflurane exposure despite the stimulation of ATPase activity by enflurane suggests the involvement of another mechanism leading to Ca\(^{2+}\) leakage. The onset of Ca\(^{2+}\) leakage appears to be dissociated from the rate of ATP hydrolysis in the presence of enflurane, which stimulates ATPase activity and does not decrease T. Furthermore, the concentration dependence of the decrease in T by halothane and isoflurane is much higher than the concentration at which the stimulation of ATPase activity and the velocity of Ca\(^{2+}\) leakage occur. These distinctions suggest that two separate mechanisms may be involved in the decrease in T and the stimulation of the velocity of leakage.

Fig. 5. Velocity of Ca\(^{2+}\) leakage as a function of anesthetic concentration. Velocity was determined from the slope of leakage curves such as in figure 1. Velocity is given as percent of control in the absence of anesthetic. Experimental conditions are as described in Materials and Methods. At each concentration of each anesthetic, the velocity exceeded the control measurements. The values are the means plus the standard deviations.
As discussed above, halothane and isoflurane decreased T. This effect of halothane and isoflurane appears to be unrelated to a stimulation of the rate of ATP hydrolysis, because enfurane increased ATPase activity but had no effect on T. However, we examined the ATP dependence of Ca\(^{2+}\) uptake using \(^{45}\)Ca\(^{2+}\) as a probe. We found that enfurane decreased the \(K_m\) for ATP. In other words, relative to control and halothane and isoflurane, the Ca\(^{2+}\) pump exposed to enfurane can accumulate Ca\(^{2+}\) at lower ATP concentrations. This observation might explain the lack of effect of enfurane on T. Even though all three anesthetics stimulate ATPase activity, resulting in more rapid ATP consumption, in the presence of enfurane the Ca\(^{2+}\) pump can accumulate Ca\(^{2+}\) at far lower ATP levels, preventing the onset of obvious Ca\(^{2+}\) leak (phase 3).

Each phase of the three phases of the uptake and release curve (figure 2) can be approximated by the same two equations:

1. \(2\text{Ca}_0^{2+} + \text{ATP} \rightarrow 2\text{Ca}_1^{2+} + \text{ADP} + P_i\) (enzymatic reaction)

2. \(\text{Ca}_2^{2+} \rightarrow \text{Ca}_0^{2+}\) (diffusion out of vesicle)

where \(\text{Ca}_0^{2+}\) is Ca\(^{2+}\) outside the SR vesicle, and \(\text{Ca}_1^{2+}\) is Ca\(^{2+}\) inside the SR vesicle. Phase 1, the uptake phase, is mainly described by equation 1, where ATP concentration starts at 0.5 mM and gradually declines as ATP is hydrolyzed. During this phase, \(\text{Ca}_2^{2+}\) is initially very low, so equation 2 contributes little to phase 1. During phase 2, the rate of Ca\(^{2+}\) moving in equals the rate of Ca\(^{2+}\) moving out, and both equation 1 and equation 2 are important in the description of this phase. We demonstrated that all three volatile anesthetics stimulate ATPase activity. Therefore, ATP levels will decline more rapidly, and the importance of equation 1 during phase 2 will be of shorter duration; i.e., T will be smaller, and phase 3 will begin sooner. We also noted that in the presence of enfurane, the Ca\(^{2+}\) pump can accumulate more Ca\(^{2+}\) at lower ATP than either control or halothane- or isoflurane-exposed SR. This suggests that equation 1 will contribute to phase 2.
2 for a longer period of time, despite the decline in ATP, resulting in less modification in T by enflurane, as we found. In the final phase, the rate of Ca\(^{2+}\) leakage is predominantly dependent on equation 2 because ATP is depleted, and Ca\(^{2+}\) is initially low. Therefore the rate of leakage is due to the concentration gradient between inside and outside, and if the conductance of the diffusion pathway is unchanged, the velocity with anesthetic should be no different than the velocity of the control. Because we observed a marked stimulation of the rate of leakage in the presence of all three anesthetics, we conclude that the anesthetics were increasing the permeability of the SR to Ca\(^{2+}\).

It is important to consider whether the effect of the volatile anesthetics to enhance Ca\(^{2+}\) leakage would have any important pharmacologic consequences in vivo. In the normally functioning muscle, in which an ATP-regenerating system exists and ~1 mM P\(_{i}\) is present, the existence of low-level Ca\(^{2+}\) leakage in a resting muscle is probably of little consequence. In fact, a low-level Ca\(^{2+}\) leakage pathway has yet to be demonstrated in vivo and is an important area of future investigation to determine the likelihood of the following pathologic mechanism. In situations of metabolic distress, such as ischemia, malignant hyperthermia, and perhaps heat stroke, where ATP levels are decreasing, leakage of Ca\(^{2+}\) might be of sufficient quantity to raise myoplasmic Ca\(^{2+}\) concentration, leading to further contractile dysfunction by stimulation of Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the heavy SR. The delay in onset of malignant hyperthermia that is sometimes noted might be explained by invoking the low-level spontaneous Ca\(^{2+}\) leakage mechanism. If spontaneous but low-level Ca\(^{2+}\) leakage is stimulated by the volatile anesthetics, it might take a period of time before the resting myoplasmic Ca\(^{2+}\) reached a sufficiently high concentration to stimulate continually the abnormally sensitized Ca\(^{2+}\)-induced Ca\(^{2+}\) release found in malignant hyperthermic muscle.

In summary, we demonstrated the leakage of Ca\(^{2+}\) from a standard light SR preparation; the leakage could be continuously monitored by a Ca\(^{2+}\) electrode. We also noted that halothane, isoflurane, and enflurane increased the permeability of the SR to Ca\(^{2+}\) and stimulated the ATPase activity of the SR, whereas enflurane alone altered the ATP requirement of the SR Ca\(^{2+}\) pump.

References

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