Anesthesiology
69:571-577, 1988

Ca^{2+} Uptake and Ca^{2+} Release by Skeletal Muscle Sarcoplasmic Reticulum:

Differing Sensitivity to Inhalational Anesthetics

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The effects of halothane, enflurane, and isoflurane were measured on two different mechanisms of Ca^{2+} regulation by isolated skeletal muscle sarcoplasmic reticulum (SR) membranes. A 100,000-dalton Ca^{2+}–ATPase protein transports Ca^{2+} from outside to inside the SR membrane. At concentration ranges representing anesthetic levels of 0.06 to 2.3 times MAC, halothane, enflurane, and isoflurane each increased rate of Ca^{2+} uptake by SR. Each concentration of isoflurane produced a greater rate of Ca^{2+} uptake, whereas halothane and enflurane produced maximum stimulation of Ca^{2+} uptake at 1 and 1.6 times MAC, respectively. The second Ca^{2+} regulation mechanism studied was a Ca^{2+} release channel in the SR membrane. The release of Ca^{2+} *via* this mechanism requires a critical threshold Ca^{2+} load (nmol Ca^{2+}/mg SR protein) for Ca^{2+}-induced Ca^{2+} release to occur. Each anesthetic tested effectively lowered the critical Ca^{2+} load threshold for Ca^{2+} release, *i.e.*, the Ca^{2+} channel was more readily induced to an open state in the presence of anesthetic. The concentrations of anesthetics having this effect on the putative Ca^{2+} channel were between 0.0026 and 0.078 MAC equivalents for each agent, and these concentrations are much lower than the anesthetic concentrations affecting Ca^{2+} uptake. These data show that in isolated skeletal muscle SR membranes a Ca^{2+} channel release function is altered at anesthetic concentrations far below those that change Ca^{2+} uptake function by a Ca^{2+}–ATPase and below concentrations of the volatile agents producing clinical anesthesia. The Ca^{2+} channel effect may represent protein–anesthetic interaction, whereas the Ca^{2+}–ATPase effect may occur by a generalized SR membrane perturbation by the anesthetics. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Ions, calcium: channels; regulation; transport. Muscle, skeletal: sarcoplasmic reticulum.)

SKELETAL MUSCLE, unlike cardiac muscle, is not dependent upon extracellular Ca^{2+} for contractility because of adequate, stable intracellular stores of Ca^{2+}. These stores of Ca^{2+} in skeletal muscle sarcoplasmic reticulum (SR) reside primarily within the terminal sacs (cisternae) of the SR, and these structures are electromechanically coupled to the transverse tubule aspect of the sarcolemma (fig. 1). The terminal cisternae contain a Ca^{2+} binding protein, calsequestrin, and the Ca^{2+} channels that open to release Ca^{2+} in response to signals coupled by the feet structures[1] to depolarization of the sarcolemma *via* the transverse tubule. The released Ca^{2+} causes activation of contraction when the myoplasmic free Ca^{2+} exceeds 5×10^{-7} M. Recent studies report purification and reconstitution of a 400-K protein from SR that binds ryanodine and is thought to represent a Ca^{2+} channel.[2-4] Structural similarities suggest the origin of this Ca^{2+} channel protein to be the junctional feet attaching the T-tubule to the terminal cisternae. The longitudinal elements of the SR contain very few Ca^{2+} channels but have a high composition of the 100,000-dalton Ca^{2+}–ATPase protein that pumps Ca^{2+} from outside to inside the SR membrane. The Ca^{2+} channels in the SR terminal cisternae open to release Ca^{2+} and initiate contraction, and the Ca^{2+}–ATPase pumps the Ca^{2+} into the SR membrane to promote relaxation of skeletal muscle. These two functions of the SR, *i.e.*, Ca^{2+} release and Ca^{2+} uptake, are important elements in the electromechanical coupling pathway of skeletal muscle. Little is known about the effects of volatile anesthetics on these two functions of the SR and what role, if any, such effects may have on skeletal muscle in clinical anesthesia. Several different nerve and muscle mechanisms on which volatile anesthetics may act are illustrated in figure 1. Utilizing *in vivo* or *in vitro* nerve–muscle preparations, it is difficult to differentiate which of these SR membrane mechanisms the anesthetic agents might be affecting. By utilizing isolated SR membrane vesicles, it becomes possible to study membrane–anesthetic interactions. However, investigations of volatile anesthetics effect on Ca^{2+} uptake and release by isolated SR membranes have been confounded by variations in methodology. The impact of these variations in methodology on SR membrane studies has been discussed.[5,6] Variations in the manner in which volatile anesthetics have been applied in SR studies is equally concerning. These varying methodologies may explain why, for example, halothane has been reported to depress,[7-10] stimulate,[10-13] or have no effect[14] on Ca^{2+} uptake by isolated SR membranes. In the present study we have isolated SR membrane vesicles in which two different Ca^{2+} regulatory functions, *i.e.*, Ca^{2+} uptake and Ca^{2+} release, can be studied. The effects of halothane, enflurane, and isoflurane on these Ca^{2+} regulatory functions of the SR have been measured.

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Received from the Department of Anesthesiology, University of Texas Health Science Center, Houston, Texas. Accepted for publication May 5, 1988. Supported in part by NIH Grant No. GM23875 and Medical Student Research Training Program Grant No. 5T35-HL-07615.
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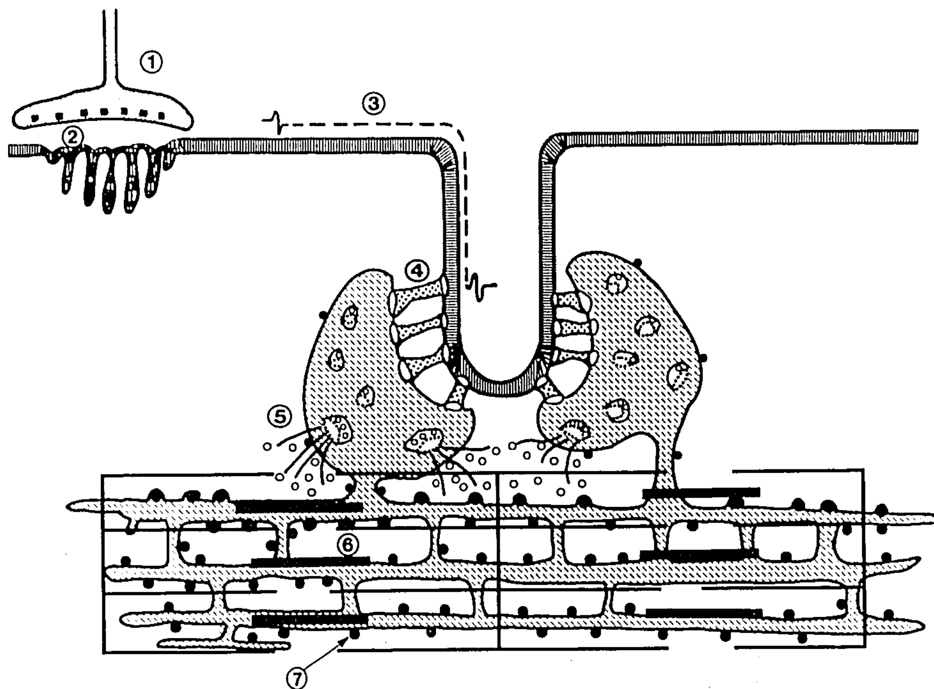

FIG. 1. Illustration of structures in skeletal muscle electromechanical coupling where anesthetics may act. (1) Nerve ending where acetylcholine is stored and released; (2) the muscle endplate containing acetylcholine receptors; (3) the propagated muscle action potential; (4) feet structures between transverse tubule; and (5) terminal cisternae of SR that contain Ca^{2+} release channels; (6) the thick and thin contractile elements where Ca^{2+} activates contraction; and (7) the Ca^{2+}–ATPase protein that pumps Ca^{2+} back inside the longitudinal elements of SR. The present study measured effects of volatile anesthetics on Ca^{2+} release channels (5) and the Ca^{2+} pump protein (7) in isolated SR membrane vesicles.

Methods

Two separate SR membrane vesicle fractions were isolated as follows. White skeletal muscle obtained from the back and hind limbs of rabbits was sliced into small pieces after placing the excised muscle on an ice block. The sliced muscle was then suspended in 5 volumes of 20 mM histidine, *p*H 6.8, and homogenized for 20 s at 3-min intervals, and the 20-s homogenization was repeated six times. During homogenization *p*H was checked and when necessary adjusted to 6.8 by addition of 0.1 M NaOH. The homogenate was centrifuged at 8,700 × *g* for 15 min to spin down and remove myofibrillar material. The supernatant was filtered through filter paper (Whatman reeve angel grade 802, Whatman Inc., Clifton, NJ) and then adjusted to 0.6 M KCl to solubilize actomyosin complex. The supernatant was then centrifuged at 17,700 × *g* for 45 min, and the resultant pellet was suspended in 20 mM histidine (*p*H 6.8), 150 mM KCl, this suspension was centrifuged at 48,000 × *g*, and the pellet was finally suspended at 20–30 mg/ml in 20 mM histidine, 150 mM KCl, and 0.3 M trehalose. This fraction (8,700–17,000 × *g*) is referred to as a heavy SR (HSR) fraction. The supernatant from the 17,000 × *g* spin was then centrifuged at 48,000 × *g* for 60 min, the pellet was suspended in 20 mM histidine, 150 mM KCl, and this suspension was centrifuged for 30 min at 48,000 × *g*. The pellet was finally suspended in 20 mM histidine, 150 mM KCl, 0.3 M trehalose, and referred to as the light SR (LSR) fraction. The homogenization and centrifugation procedures were performed at 4° C. A Beckman (Spinco Div., Palo Alto, CA) JA-21 refrigerated centrifuge with JA-10 and JA-20 rotors was used. The SR samples were stored at −85° C until used for these studies. Protein concentration was determined by the Lowry method, and Ca^{2+} uptake and release are expressed per milligram of SR protein.

The Ca^{2+} uptake and Ca^{2+} release properties of the SR were measured in a 3-ml cuvette with absorbance changes measured by a Hewlett-Packard Model 8451 diode array spectrophotometer (Hewlett-Packard, Scientific Instrument Div., Palo Alto, CA). Contents of the cuvette were continuously mixed by a magnetic stir bar and maintained at 30° C by a circulating water bath. Changes in Ca^{2+} concentration outside the SR membrane were quantitated by dual wavelength spectrophotometry (absorbance 650–absorbance 700 nm) using the Ca^{2+} indicator dye arsenazo III. The rate of inward Ca^{2+} transport was determined in a 1 ml volume containing MgATP, 1 mM; histidine, 20 mM (*p*H 6.8); KCl, 150 mM; NaN_3, 5 mM; arsenazo III, 16 μM; creatine-phosphate, 5 mM; creatine-phosphokinase, 5 μg/ml; K-oxalate, 5 mM; and SR protein, 50 μg/ml. The inward Ca^{2+} transport was initiated by the addition of 75 μM Ca^{2+} to the cuvette contents described above. Rate of Ca^{2+} uptake was determined from the slope of the absorbance change *versus* time recorded plot of the reaction.

Ca^{2+}-induced Ca^{2+} release from the HSR was measured in a 1-ml cuvette volume containing the same substances and concentrations as described for the Ca^{2+} uptake except that K-oxalate was omitted and the SR concentration

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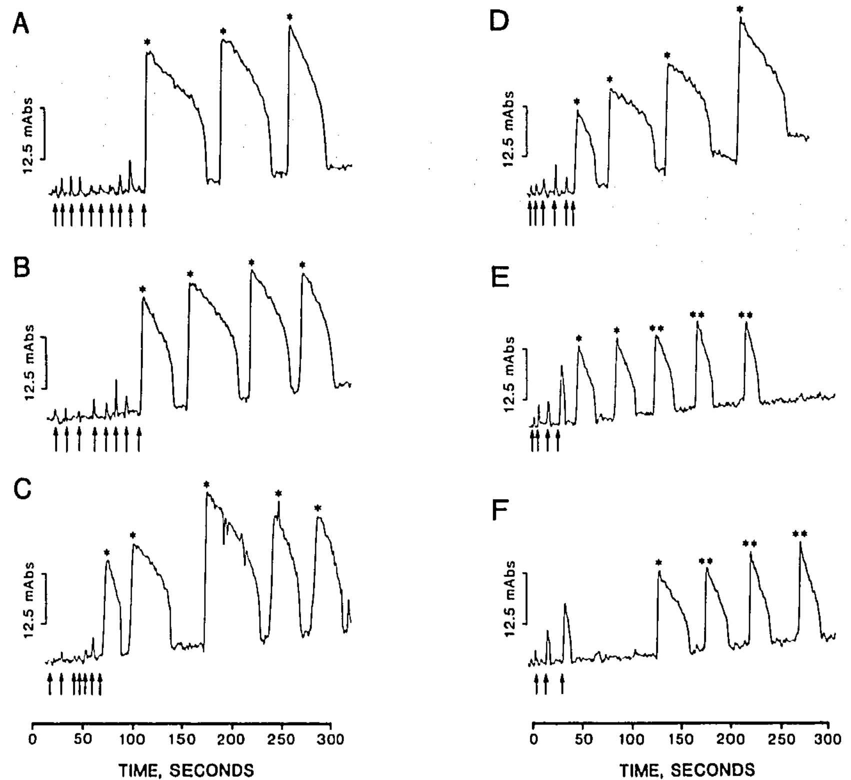

FIG. 2. Effect of varying concentrations of halothane on the threshold Ca^{2+} load for Ca^{2+}-induced Ca^{2+} release. Arrows (↑) indicate addition of 5 nmol Ca^{2+}. *Ca^{2+}-induced Ca^{2+} release. **Spontaneous Ca^{2+} release, *i.e.*, no Ca^{2+} added. Added Ca^{2+} and any subsequent Ca^{2+} released by SR was then actively transported into membrane, reversing the Ca^{2+} signal. Threshold values, nmol Ca^{2+}/mg SR protein are as follows: *A.* control = 50; *B.* .004 MAC = 40; *C.* .0056 MAC = 35; *D.* .011 MAC = 30; *E.* .022 MAC = 20; *F.* .045 MAC = 15.

was 1 mg/ml. The SR was loaded with Ca^{2+} by sequentially adding 5 nmol Ca^{2+} boluses. Each 5 nmol Ca^{2+} bolus was added after the previous Ca^{2+} bolus had been completely taken up by the SR. These 5 nmol Ca^{2+} additions continued until finally the addition of Ca^{2+} initiated reversible Ca^{2+}-induced Ca^{2+} release from the SR. The total cumulative Ca^{2+} added to initiate Ca^{2+}-induced Ca^{2+} release is referred to as the Ca^{2+} threshold. This procedure is illustrated by the data in figure 2.

Volatile anesthetics were equilibrated inside the cuvettes in the following manner. The cuvette was sealed by a teflon septum-lined screw cap that allowed the anesthetics to be injected into the sealed cuvettes. Each anesthetic was prepared in stock solutions in ethanol such that a constant volume of 0.5 μl added to the cuvette produced the required concentration of anesthetic. After adding anesthetic agent a 2-min equilibration period was allowed before initiating experiments by addition of Ca^{2+}. Under the conditions of measurement in the present study the cuvette had a liquid volume of 1.0 ml and a gas phase volume of 3.58 ml. A liquid/gas partition coefficient of 0.96 was determined in the system for halothane by sampling the gas and liquid phases and determining the concentration of halothane in each by gas chromatography. Values for the liquid/gas partition coefficients for enflurane and isoflurane of 0.95 and 0.74, respectively, were extrapolated to 30° C from other published values.[15] The anesthetic concentrations are expressed as a percent of the MAC producing anesthesia in 50% of treated rabbits. The relationships between liquid and gas concentrations of the anesthetics in the cuvette system and their MAC values are described (table 1). The MAC values reported in this study relate to the anesthetic concentration in the gas phase of the cuvette. The effect of each anesthetic concentration, added in 50 μl ethanol, was compared to an equal volume of ethanol without anesthetic. The ethanol control produced 0.05% ethanol in final concentration.

Three different rabbits were used to isolate three different LSR and HSR fractions. Two controls were determined immediately before duplicate determinations at each anesthetic concentration for each of the three membrane preparations. The data were statistically analyzed first by analysis of variance, which revealed a significant (P = 0.0013) interaction between anesthetic agent and the concentration of anesthetic. This indicated that these factors are not acting independently of each other and that the main effects, *i.e.*, anesthetic agents and concentration, cannot be considered. Instead, simple effects, *i.e.*, anesthetic agent *versus* anesthetic agent at each concentration, were tested by the least significant difference method at the 5% level of significance.

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TABLE 1. Distribution of Volatile Anesthetics in a Closed Cuvette System* and the Relationship to MAC Values for Rabbit

Anesthetic	Total Amount Added (μM)	Coefficient of Distribution 30° C	Liquid Concentration (μM)	Gas Concentration (μM)	MAC Value (gas)	MAC Vol%
Halothane	2.55	0.96	540	563	1.0	1.4
Enflurane	5.10	0.95	1070	1126	1.0	2.9
Isoflurane	3.65	0.74	625	845	1.0	2.1

* The closed cuvette system contained 1.0 ml of aqueous volume and 3.58 ml of gas volume. Example for halothane:

$$\text{Coefficient distribution} = 0.96 = \frac{(2.55 - X)/1 \text{ ml aqueous}}{X/3.58 \text{ ml gas}}$$

where X = moles anesthetic in gas phase at equilibrium.

Results

Anesthetic Agent Effects on Ca^{2+} Uptake

The rate of Ca^{2+} uptake for the controls averaged 23.7 ± 1.8 nmol $Ca^{2+}/mg \cdot s^{-1}$ for the light SR fractions and 15.6 ± 1.6 nmol $Ca^{2+}/mg \cdot s^{-1}$ for the heavy SR fractions. At anesthetic concentrations ranging from 0.06 to 2.3 × MAC (*i.e.*, 6–230% MAC), each of the agents tested increased the Ca^{2+} uptake rate above that for the controls (fig. 3). The effects of these anesthetic agents on Ca^{2+} uptake by LSR and HSR fractions were qualitatively and quantitatively similar (fig. 3). Halothane increased the rate of Ca^{2+} uptake maximally at approximately 1.1 MAC for HSR and LSR. At a halothane concentration equivalent to 2.3 × MAC, rate of Ca^{2+} uptake was below peak halothane effect but above control levels (fig. 3). The effects of enflurane on Ca^{2+} uptake by LSR and HSR fractions were qualitatively similar to halothane effects. At 1.6 × MAC enflurane produced rates of Ca^{2+} uptake approximately 1.8 times greater than control rates for both LSR and HSR fractions (fig. 3). Increasing enflurane concentration from 1.6 to 2.1 × MAC reduced the extent of Ca^{2+} uptake rate activation of both LSR and HSR fractions (fig. 3). For each increase in isoflurane concentration from 0.06 to 1.9 × MAC, there was an increase in the rate of Ca^{2+} uptake by LSR and HSR fractions (fig. 3). Unlike halothane and enflurane, there was no decrease in activation of Ca^{2+} uptake rate at the higher concentrations of isoflurane tested. Over clinically relevant concentrations of these anesthetic agents, the effect of each is to increase the rate of Ca^{2+} uptake above the rates observed in the absence of anesthetic. Statistically significant differences ($P < 0.05$) were observed for the Ca^{2+} uptake activation in HSR fractions as follows: at 1.4–1.7 MAC enflurane effect > halothane effect and > isoflurane effect; at 1.9–2.2 MAC enflurane and isoflurane effects > halothane effect.

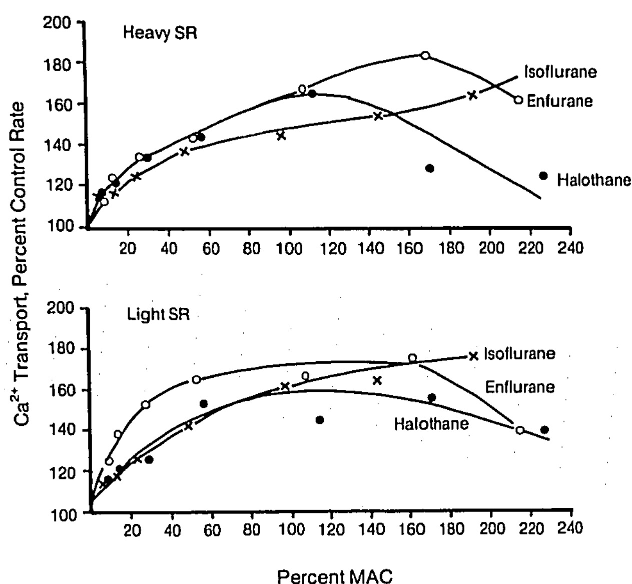

FIG. 3. Effect of volatile anesthetics on rate of Ca^{2+} uptake in heavy and light fractions of isolated skeletal muscle SR vesicles. Ca^{2+} uptake was measured spectrophotometrically after the SR was equilibrated in a closed cuvette at one of the anesthetic concentrations shown. MAC is percent of ED_{50} anesthetic dose.

Effects on Ca^{2+}-Induced Ca^{2+} Release

The phenomenon of Ca^{2+}-induced Ca^{2+} release from skeletal SR membranes is demonstrated by the experimental results presented (fig. 2). In the presence of MgATP when Ca^{2+} is added to the outside of the SR membrane, the Ca^{2+} pump protein transports Ca^{2+} to the inside. In our experiments relatively small, 5 nmol amounts of Ca^{2+} are added each time, and this is initially rapidly removed by the SR. As more and more 5 nmol Ca^{2+} additions are made and removed by the SR, a critical Ca^{2+} load is achieved inside the SR. When this critical Ca^{2+} load is obtained, then the next addition of Ca^{2+} causes a release of Ca^{2+} from the SR. This critical Ca^{2+} load is referred to as the threshold for Ca^{2+}-induced Ca^{2+} release. The Ca^{2+}-induced Ca^{2+} release is a reversible process, and the SR can reuptake the released Ca^{2+} (fig. 2). It is proposed that this Ca^{2+} release process is due to Ca^{2+} channels in the SR membrane that open and release Ca^{2+} under specific conditions. Also, it is necessary for

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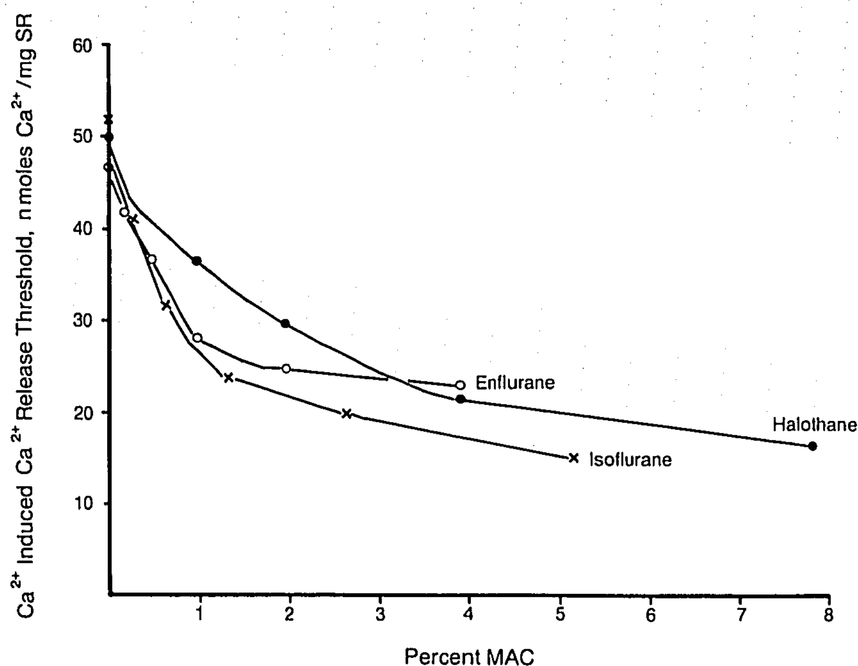

FIG. 4. Effect of volatile anesthetics on threshold for Ca^{2+} release from isolated skeletal muscle SR membrane vesicles. The threshold Ca^{2+} load necessary for Ca^{2+}-induced Ca^{2+} release to occur is reduced by each of the anesthetic agents tested.

these channels to close in order for the SR Ca^{2+} pump to be able to completely reuptake the released Ca^{2+}.

Each volatile anesthetic tested had a similar effect on the Ca^{2+}-induced Ca^{2+} release process, *viz.*, each tended to lower the threshold Ca^{2+} at which Ca^{2+} release occurred (fig. 4). It is notable that the concentration range for anesthetic agent effects on the Ca^{2+} release function, *i.e.*, 0.0025–0.08 MAC, is 1–2 orders of magnitude below the anesthetic agent concentration range affecting Ca^{2+} uptake, *i.e.*, 0.10–2.3 MAC. Halothane had a half-maximal effect on Ca^{2+} release at a concentration equivalent to 0.015 MAC, whereas enflurane and isoflurane had half-maximal effects at 0.005 and 0.006 MAC, respectively. Higher concentrations of anesthetic agents could not be tested because they prevented achieving the minimal Ca^{2+} load in the SR, a necessity for Ca^{2+}-induced Ca^{2+} release to occur. As supported by the experiments described below, this failure of SR to load Ca^{2+} in the presence of high concentrations of anesthetics is not a consequence of an inhibition of Ca^{2+} uptake but more likely is related to the opening of Ca^{2+} channels.

The effect of these volatile anesthetics to cause Ca^{2+} release to occur at lower Ca^{2+} thresholds appears to be related to Ca^{2+} channel events. Ruthenium red, an agent that blocks Ca^{2+} channels but does not affect the Ca^{2+} uptake system,[16] blocks the effect of these anesthetic agents on Ca^{2+} release. This blocking effect of ruthenium red is illustrated for halothane (fig. 5) but was also observed for enflurane and isoflurane (data not shown). Similar conclusions were made in a previous study for halothane effects on isolated SR membranes.[6] We have previously demonstrated that this same Ca^{2+} release channel is ryanodine-sensitive.[17]

Discussion

The SR performs major functions in regulating the free Ca^{2+} concentration in the myoplasm and by so doing it controls contractility. A 100,000-dalton Ca^{2+}–ATPase pump protein utilizes MgATP to translocate Ca^{2+} from myoplasm to the inside of the SR membrane. This process is essential for normal skeletal muscle relaxation to occur. Release of Ca^{2+} from the SR storage sites occurs through Ca^{2+} channels that open in response to the muscle action potential. These Ca^{2+} channel mechanisms probably represent the primary source of Ca^{2+} for initiating contraction. Obviously, any effect of potent inhalation anesthetics such as halothane, enflurane, or isoflurane on these SR membrane functions could alter contractility. Isolated SR membranes have been studied in many different ways, and there is considerable knowledge about structure–function relationships. Most studies have attempted to determine the effect of anesthetics on the Ca^{2+} uptake function of SR because this was the earliest and best understood property of the membrane. In the absence of oxalate or other Ca^{2+} precipitating anions, Ca^{2+} uptake occurs until the intravesicular concentration reaches a level that inhibits the Ca^{2+} pump. Under these conditions of study only nmol amounts of Ca^{2+} are taken up by 1 mg of SR protein and halothane, 18–118 mM (25–250 × MAC), with decreased rate and amount of Ca^{2+} binding.[10] These same concentrations of halothane also de-

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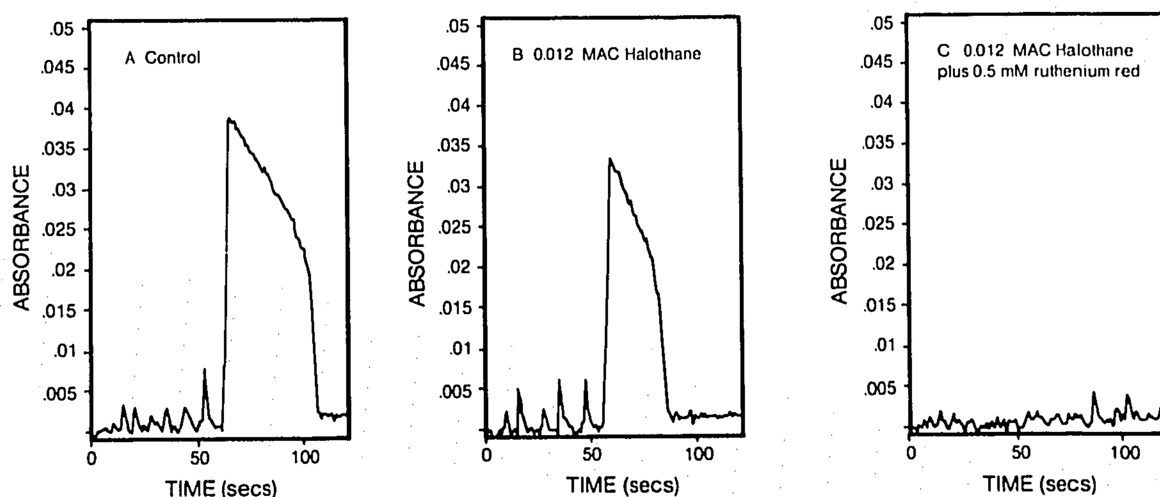

FIG. 5. The effect of ruthenium red on halothane effect to lower Ca^{2+} threshold for Ca^{2+}-induced Ca^{2+} release from a rabbit heavy SR fraction. *A.* Control and a total of 8 × 5 nmol Ca^{2+} = 40 nmol Ca^{2+}/mg SR for threshold. *B.* In presence of 0.015 MAC halothane threshold is reduced to 30 nmol Ca^{2+}/mg SR. *C.* 0.5 mM ruthenium red blocks the effect of 0.015 MAC halothane and no Ca^{2+} release occurred after adding 85 nmol Ca^{2+}/mg SR.

creased rate and amount of Ca^{2+} uptake in the presence of oxalate.[10] Oxalate forms insoluble Ca^{2+}–oxalate and prevents the intravesicular Ca^{2+} from reaching a concentration that inhibits the pump. Under these experimental conditions μmol Ca^{2+}/mg SR membrane are accumulated. Other studies that measured halothane effects in the presence of oxalate reported stimulation of Ca^{2+} uptake rate and total amount[10–13] or inhibition.[7–10] Although methodology varies considerably among these studies, there is a trend for halothane to stimulate Ca^{2+} uptake at concentrations of 3–4 × MAC or below, whereas halothane concentrations greater than 10 × MAC tend to inhibit Ca^{2+} uptake. It may be that halothane concentrations below 3–4 × MAC are acting directly on the 100,000-dalton Ca^{2+}–ATPase transport protein and/or its immediate membrane domain, whereas halothane concentrations above 10 × MAC have general perturbation effects on the lipid moiety of the SR membrane.

Relative to these previous studies on the effect of halothane on Ca^{2+} uptake function in the SR, our studies provide new insights. Our studies are limited to varying anesthetic concentrations and this has shown in the case of halothane that its stimulatory effects on Ca^{2+} uptake are limited to an upper concentration of 3 × MAC and with greater concentrations activation decreases. If we had tested higher concentrations of halothane, we may have observed inhibition of Ca^{2+} uptake as reported by others.[8,10] The range of anesthetic-related enflurane concentrations that stimulated Ca^{2+} uptake was double that for halothane and that for isoflurane was greater than enflurane. If these Ca^{2+} uptake-inactivating effects of the anesthetics at higher concentrations are a consequence of generalized membrane effects, then it would appear that the efficacy rating would be halothane > enflurane > isoflurane. Because these inactivating effects occur at anesthetic concentrations outside the clinical range, they may have no relation to the anesthetic properties of these drugs.

In addition to studying the effects of these inhalation anesthetics on the Ca^{2+}–ATPase pump protein, we investigated the effects on a separate function, the Ca^{2+} release channel of the SR. By investigating two different functions that are anatomically separated in the SR membrane, it may be possible to determine if a generalized membrane effect is produced by the anesthetics or if the anesthetic is acting directly on the protein.

We observed that the volatile anesthetic agent effects on the Ca^{2+}–ATPase pump protein of the SR membrane occurred at concentrations 10 times greater than those concentrations affecting the Ca^{2+} channel. In relation to anesthetic potency, the ED_{50} effects on Ca^{2+} uptake occurred within the useful clinical concentration range for each of the agents tested. If this *in vitro* effect of inhalational anesthetics to increase Ca^{2+} uptake into the storage membranes is also occurring during *in vivo* clinical anesthesia, then the result would be to augment skeletal muscle relaxation. This is consistent with the function of this SR Ca^{2+} pump to remove Ca^{2+} from the myoplasm and produce muscle relaxation.

Although the clinical effects of these anesthetic agents on the Ca^{2+}–ATPase pump might be to enhance muscle relaxation, it appears that the opposite is true for the anesthetic effects on the Ca^{2+} channel. The Ca^{2+} channel evaluated in this study is a receptor-operated Ca^{2+} channel and Ca^{2+} is the agonist that causes the channel to open and release Ca^{2+}. Under our experimental conditions the

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channel can be opened by Ca^{2+} only after a critical Ca^{2+} load has been taken up by the SR membrane vesicle. The effect of the anesthetics used in this study was to lower the threshold amount of Ca^{2+} load necessary for Ca^{2+} to open the channel. Thus, the critical balance for Ca^{2+} channel opening events between Ca^{2+} concentration inside the membrane and the Ca^{2+} agonist concentration outside the membrane is altered by the presence of the volatile anesthetics. The sensitivity of this Ca^{2+} channel to these agents is 10 times greater than that for the Ca^{2+} uptake system. In relation to skeletal muscle relaxation, the net effect of these agents on the Ca^{2+}-induced Ca^{2+} release channel is to release Ca^{2+} from the SR storage site, increase myoplasmic Ca^{2+}, and produce contracture. Whether these effects have any relation to those occurring during clinical anesthesia is unknown. There is considerable disagreement as to whether the Ca^{2+}-induced Ca^{2+} release channel plays any role in normal excitation–contraction coupling.[18] There is a good evidence that this mechanism for Ca^{2+} release is pathogenic for anesthetic-induced malignant hyperthermia.[19–22]

Additional studies are necessary to determine what significance, if any, these observations on an isolated membrane system have to the clinical effects of halothane, enflurane, and isoflurane. The findings from this study are of considerable interest because they demonstrate similarities and dissimilarities in the dose effect of these agents on two different functional mechanisms in the same membrane. It is of particular interest to note the concentration of anesthetics affecting the Ca^{2+} channel properties of the SR because these are far below clinical MAC values and below most reported values that have biologic effect.

Finally, we have demonstrated a closed cuvette with liquid/gas phases and the methods by which we relate anesthetic effect to its gas phase concentration and to clinical MAC values. Several past studies with volatile anesthetics are flawed because the concentration of anesthetic was not maintained at a constant concentration.

The laboratory technical support provided by Pat Turk and D. LaLonde and typing of the manuscript by Margie Choate are gratefully acknowledged.

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