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Effects of Several Volatile Anesthetics on the Ca^{2+}-Related Functions of Skinned Skeletal Muscle Fibers from the Guinea Pig

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ABSTRACT

The effects of various volatile anesthetics on intramuscular Ca^{2+}-related functions were studied with the skinned fiber technique in guinea pig skeletal muscles. All the volatile anesthetics tested significantly enhanced Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR) at clinical concentrations with negligible effects both on Ca^{2+} sensitivity of the contractile system and on Ca^{2+} uptake by the SR. A comparison was made of the enhancing effect of several volatile anesthetics on CICR at clinical concentrations. Halothane was the most potent, followed by methoxyflurane, isoflurane, enflurane, sevoflurane and diethyl ether. If CICR plays an important role in triggering MH, this order of volatile anesthetics on their enhancing effect on CICR, also corresponds to their potency in triggering MH.

Key words: *Calcium induced calcium release (CICR)*
Skinned fiber
Volatile anesthetics

Most volatile anesthetics have been reported to trigger malignant hyperthermia (MH)[5,13] in man and swine[1,2,4,15,16,23,25]. Although the exact pathogenesis of MH has not been clearly elucidated, it is generally accepted that MH is a disorder of Ca^{2+} regulation in skeletal muscle[12].

The specific *in vitro* nature of skeletal muscles resected from MH patients and animals is their higher sensitivity to the contracture inducing action of caffeine and halothane[17]. Both caffeine and halothane activate the Ca^{2+}-induced Ca^{2+} release (CICR)[8,11] mechanism of the sarcoplasmic reticulum (SR) causing contracture[9]. In the light of this information, abnormal CICR mechanism of the SR may be responsible for this characteristic and for triggering the disease[10].

According to some reports[3,14,24], volatile anesthetics, including halothane, augmented *in vitro* muscle contractures in normal animal and human muscles. Although this effect is seemingly associated with the triggering of MH[24], the real active site has not been clearly demonstrated among the various intracellular processes affecting Ca^{2+}-induced contracture responses.

Unlike the ordinary muscle contracture test, the skinned fiber technique[19] enables one to examine independently the following Ca^{2+} related functions in skeletal muscle; 1) Ca^{2+} uptake by the SR, 2) Ca^{2+} release from the SR, and 3) Ca^{2+} sensitivity of the contractile system.

In the present study, the effects of various volatile anesthetics on these Ca^{2+}-related functions are examined in the skinned skeletal muscle fibers of guinea pigs, and their role in MH triggering is discussed.

MATERIALS AND METHODS

The experimental method (skinned fiber preparation, experimental setup and solutions) was the same as that described by Ohta et al[22] except for the materials. Skinned fibers were obtained from extensor digitorum longus (EDL) muscles of male guinea pigs (body weight; 200—250g).

Volatile anesthetics were dissolved in the solutions in test tubes with glass plugs to obtain a concentrated solution and were diluted to desired concentrations immediately prior to the experiment.

The anesthetics used were commercially available diethyl ether, halothane, enflurane, methoxyflurane, isoflurane and sevoflurane, and were

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confirmed not to contain any contaminant gas-chromatographically.

As the experiments were conducted in open system, the agents in the well escaped by volatilization. The changes in the concentrations over a period of time was measured by gas chromatography.

The following three Ca^{2+}-related functions of skinned fibers were examined in the presence and absence of the volatile anesthetics, and the results were compared between the two.

(1)Effect on the Ca^{2+} sensitivity of the contractile system

(2)Effect on the Ca^{2+} uptake by the SR

(3)Effect on CICR from the SR

The experimental procedures to determine the respective factors were conducted in essentially the same way as described by Ohta et al[22].

In the course of CICR experiment, two additional experiments were conducted. Volatile anesthetics and procaine were dissolved in the solutions and their combined effects were examined.

The effects of various volatile anesthetics were compared in experiments on identical fibers and the potency order of their effect on CICR was determined.

Statistical significance was examined by the Student *t* test.

RESULTS

1. Concentrations of volatile anesthetics during experiments

All volatile anesthetics took a single exponential time course of decline as they evaporated from the open experimental well after being poured from the test tubes (data not shown). Over 90% of the original concentration was guaranteed when the exposure time of the agent to the open air was less than 3 minutes.

2. Effect on the contractile system

Figure 1 shows that sevoflurane did not affect the Ca^{2+} sensitivity of the contractile system. The same result was obtained in all other anesthetics (data not shown).

3. Effect on the Ca^{2+} uptake by the SR

As shown in Fig. 2, 500μM of sevoflurane did not influence the time course of Ca^{2+} uptake by the SR at pCa 7.2. The initial rates of Ca^{2+} uptake at any pCa were not affected by the agent (Fig. 3). Similarly, none of the volatile anesthetics tested depressed or stimulated Ca^{2+} uptake by the SR in the skinned fibers (data not shown).

4. Effect on CICR from the SR

At all the four pCa tested, the rates of Ca^{2+} release from the SR were significantly increased by 500μM of sevoflurane, while in Ca^{2+} free solution remained unchanged (Fig. 4). This enhancement by sevoflurane was nullified by 5mM procaine as shown in Fig. 5.

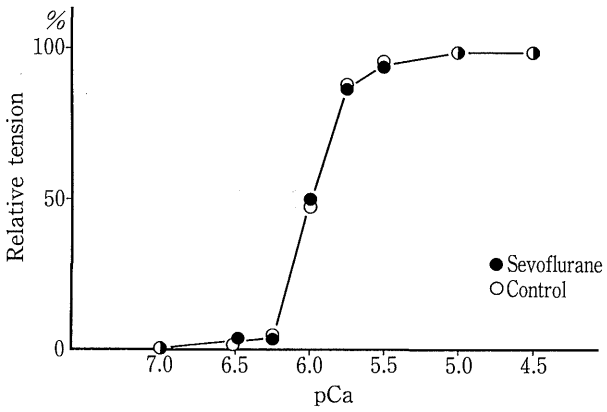

Fig. 1. Negligible effects of 500μM sevoflurane on Ca^{2+} sensitivity of the contractile system of skinned fibers. The figure shows one typical result from several experiments.

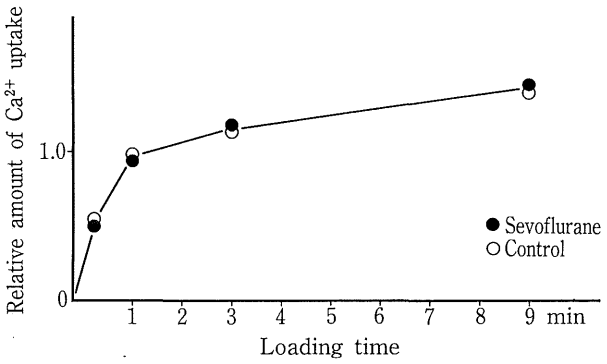

Fig. 2. No remarkable effect of 500μM sevoflurane on the amount of Ca^{2+} taken up by the sarcoplasmic reticulum (SR) at pCa 7.2 in the skinned fibers. Relative amount of Ca^{2+} was expressed compared to that loaded in the SR with pCa 6.7 for 2 minutes. The figure shows one typical result from several identical experiments.

Enhancement of CICR by sevoflurane increased in a dose-dependent manner up to the concentration of 1.5 mM at pCa 6.0 where no effect was observed on Ca^{2+} release (leakage) rate in the solutions containing no Ca^{2+} (Fig. 6). Over 5 mM of sevoflurane destroyed the SR and abolished its Ca^{2+} accumulating activity irreversibly.

All anesthetics tested significantly enhanced CICR at clinical concentrations. When the degree of enhancement of CICR at pCa 6.0 at their clinical concentrations is compared (Fig. 7), halothane was greatest, followed by methoxyflurane, isoflurane, enflurane, sevoflurane and diethyl ether.

DISCUSSION

Tension production in skeletal muscle *in vivo* and also *in vitro* is the result of reaction of the contractile proteins with Ca^{2+}, and myoplasmic Ca^{2+} concentration is controlled by the function of Ca^{2+} pump and Ca^{2+} channel on the SR. Intramuscular

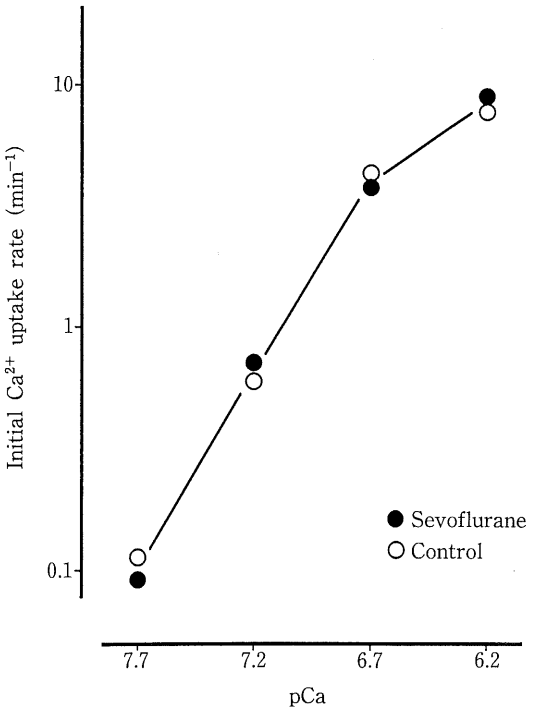

Fig. 3. No remarkable effect of 500μM sevoflurane on the initial rates of Ca^{2+} uptake at various pCa. The rates of uptake are plotted in an arbitrary unit. The figure shows one typical result from several identical experiments.

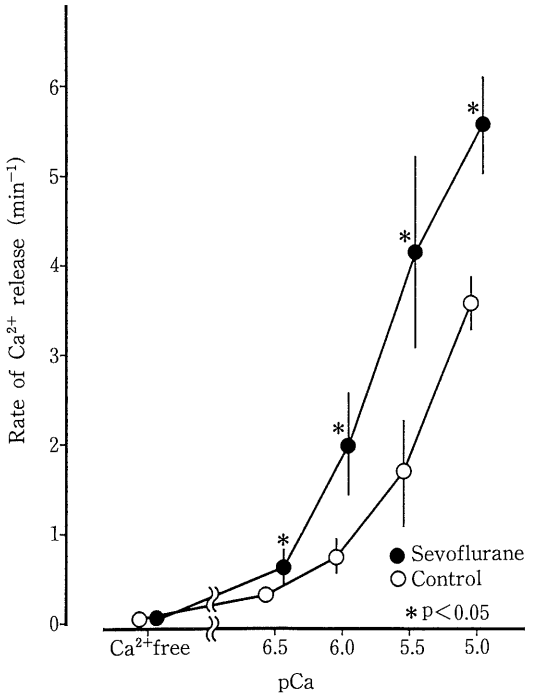

Fig. 4. Effect of 500μM sevoflurane on Ca^{2+} release rates from the sarcoplasmic reticulum (SR) in the skinned fibers. No effect on Ca^{2+}-independent Ca^{2+} leakage (Ca free) and an enhancing effect on Ca^{2+}-induced Ca^{2+} release (CICR) were seen. Mean ± S.D. (n=3). *p<0.05.

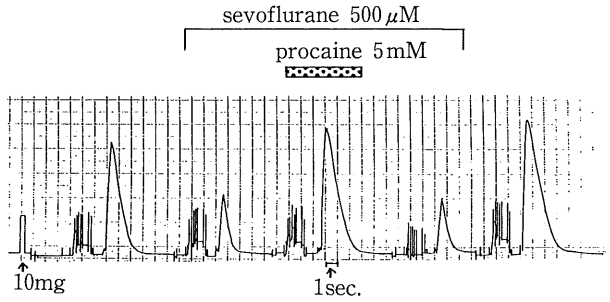

Fig. 5. Tension traces of an experiment showing an enhancing effect of sevoflurane on Ca^{2+} release at pCa 6.0 (second and fourth runs) compared with controls (first and last runs), and inhibition of the effect by 5mM procaine (middle run).

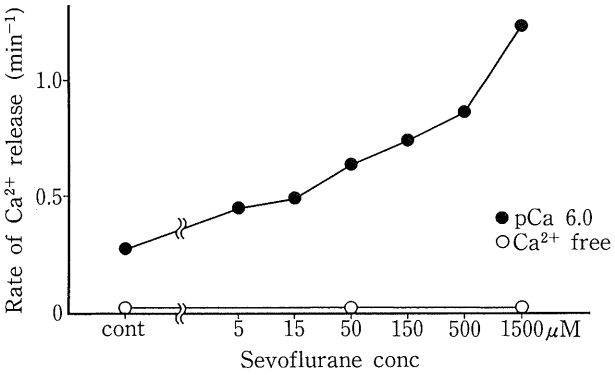

Fig. 6. Dose dependence of sevoflurane's effect on Ca^{2+} release rates from the sarcoplasmic reticulum. There was no effect on Ca^{2+}-independent Ca^{2+} leakage (open circles), and dose-dependent augmentation of the enhancing effect on CICR at pCa 6.0 (closed circles) was seen as the concentration rose.

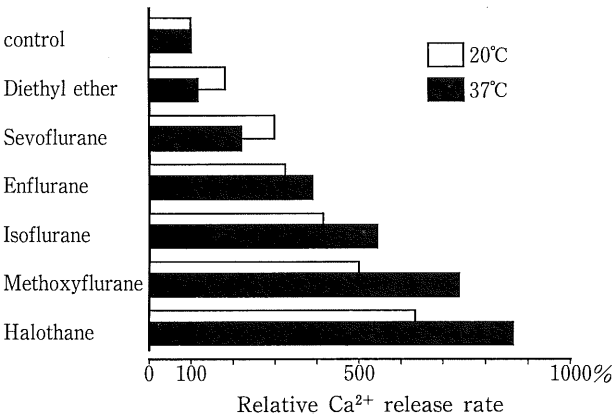

Fig. 7. Comparison of the enhancing effects of several volatile anesthetics on CICR rates at pCa 6.0, expressed as how many times each agent magnified the rate of CICR compared to the rate in the absence of the agent, at pCa 6.0. The concentration of diethyl ether was 10mM and the others were 500μM. The figure shows one typical result of several identical experiments.

pCa are known to move from 6.5 to 5.0 when skeletal muscle contracts[6].

Three possible mechanisms can be considered to explain the known phenomenon that volatile anesthetics augment muscle contracture *in vitro*[3,14,24], 1) inhibition of Ca^{2+} pump on the SR, 2) activation of Ca^{2+} release channel on the SR, and 3) raised Ca^{2+} sensitivity of the contractile system.

Using the skinned fiber technique, the present study demonstrated that all anesthetics tested facilitated the Ca^{2+} channel opening by Ca^{2+} (CICR) on the SR without any accompanying change in the Ca^{2+} pump of the SR and in Ca^{2+} sensitivity of the contractile system.

Nelson et al[6] recently reported the enhancing effect of halothane, enflurane and isoflurane on both Ca^{2+} uptake and Ca^{2+} release of SR using isolated SR membrane vesicles method. Their observation does not agree with our finding that volatile anesthetics did not facilitate Ca^{2+} uptake by SR. If volatile anesthetics enhance Ca^{2+} uptake by the SR, it should lower myoplasmic Ca^{2+} concentration and lead to relaxation of the muscle strips. That is not case. This contradiction is most likely explained by the difference in the experimental method used by the two groups. Nelson's group used the isolated SR vesicle method, while our group used the skinned fiber method. Their method for preparing SR vesicle employs a fragmentation procedure which may destroy the physiological structure of SR membrane. On the other hand, our method, in which only sarcoplasmic membrane is removed by saponin, clearly preserves the physiological structure of SR. Thus, it is not reasonable to compare their results to ours because of the difference in the physiological condition of the SR.

The order of various volatile anesthetics in CICR enhancement employed in skinned fibers is similar to that based on their augmentation of muscle contracture observed in whole muscle[24]. If this muscle contracture by anesthetics is related to the *in vivo* MH triggering mechanism in skeletal muscle, the potency of CICR enhancement may also correspond to the capability of MH triggering.

This study further supports the CICR hypothesis of MH triggering[10,18,21]. According to this hypothesis, an imbalance of Ca^{2+} uptake and Ca^{2+} release is evoked by a trouble on the side of the release, and this is caused by a reaction of genetically defective Ca^{2+} channel on the SR to volatile anesthetics. The most important piece of evidence for this hypothesis is given by Ohta et al[22]: dantrolene, an effective therapeutic agent against MH, directly inhibits CICR besides being an excitation-contraction (EC) uncoupler to reduce Ca^{2+} in myoplasm[7].

In skeletal muscles, it seems that CICR does not play a primary physiological role in raising Ca^{2+} [9], and an entirely different Ca^{2+} release mechanism is used in physiological EC coupling, although details are not completely known. Thus, there are at least two mechanisms for SR Ca^{2+} channel opening, although recent studies have raised the possibility that these two functionally distinct Ca^{2+} release mechanisms are two different functional modes of one and the same Ca^{2+} channel. If MH is triggered by the effects of volatile anesthetics on CICR, MH in the patients having apparently normal physiological Ca^{2+} release mechanisms may be a disorder of Ca^{2+} channel on the SR in which a hidden, physiologically unused mechanism comes into action triggered by volatile anesthetics.

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