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Differential Effects of Sevoflurane, Isoflurane, and Halothane on Ca^{2+} Release from the Sarcoplasmic Reticulum of Skeletal Muscle

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Background: **Although malignant hyperthermia after application of sevoflurane has been reported, little is known about its action on intracellular calcium homeostasis of skeletal muscle. The authors compared the effect of sevoflurane with that of isoflurane and halothane on Ca^{2+} release of mammalian sarcoplasmic reticulum and applied a novel method to quantify Ca^{2+} turnover in permeabilized skeletal muscle fibers.**

Methods: **Liquid sevoflurane, isoflurane, and halothane at 0.6 mM, 3.5 mM, and 7.6 mM were diluted either in weakly calcium buffered solutions with no added Ca^{2+} (to monitor Ca^{2+} release) or in strongly Ca^{2+} buffered solutions with $[Ca^{2+}]$ values between 3 nM and 24.9 μM for $[Ca^{2+}]$–force relations. Measurements were taken on single saponin skinned muscle fiber preparations of BALB/c mice. Individual $[Ca^{2+}]$–force relations were characterized by the Ca^{2+} concentration at half-maximal force that indicates the sensitivity of the contractile proteins and by the steepness. Each force transient was transformed directly into a Ca^{2+} transient with respect to the individual $[Ca^{2+}]$–force relation of the fiber.**

Results: **At 0.6 mM, single force transients induced by sevoflurane were lower compared with equimolar concentrations of isoflurane and halothane ($P < 0.05$). Similarly, calculated peak Ca^{2+} transients of sevoflurane were lower than those induced by equimolar halothane ($P < 0.05$). The Ca^{2+} concentrations at half maximal force were decreased after the addition of sevoflurane, isoflurane, and halothane in a concentration-dependent manner ($P < 0.05$).**

Conclusion: **Whereas sevoflurane, isoflurane, and halothane similarly increase the Ca^{2+} sensitivity of the contractile apparatus in skeletal muscle fibers, 0.6 mM sevoflurane induces smaller Ca^{2+} releases from the sarcoplasmic reticulum than does equimolar halothane. (Key words: Ca^{2+}-release channel; Ca^{2+} sensitivity; fast twitch muscle; skinned fibers.)**

VOLATILE anesthetics induce Ca^{2+} release from the sarcoplasmic reticulum (SR). This effect has been shown in functionally skinned skeletal muscle fibers[1-3] and in skinned cardiac muscle[4-8] with classical volatile anesthetics such as halothane, isoflurane, and enflurane. So far, little information exists about the effect on the SR of sevoflurane. This could be of clinical relevance because the Ca^{2+} release channel has been associated with malignant hyperthermia (reviewed in MacLennan and Phillips[9] and MacLennan and Chen[10]), and already case reports about malignant hyperthermia after administration of sevoflurane have been published.[11-13]

Force measurements are used as an indirect tool in skinned skeletal muscle fibers to measure calcium release from the SR. However, the extent of force induced by the Ca^{2+} release may be influenced by the Ca^{2+} sensitivity of the regulatory proteins (troponin-tropomyosin complex) and the actin-myosin interaction. Therefore, in addition to the force measurements, the evaluation of the direct influence of the anesthetics on Ca^{2+} sensitivity is also important. In skeletal muscle, reports about the effects on Ca^{2+} sensitivity by inhalational anesthetics have been controversial: Tavernier *et al.*[14] observed no effect of halothane on the $[Ca^{2+}]$-force relation of fast skeletal muscle, whereas Ca^{2+} sensitivity of the contractile apparatus of slow-twitch skeletal muscle was decreased. In contrast, Su and Bell[2] observed an increase in Ca^{2+} sensitivity when isoflurane

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Table 1. Bathing Solutions for SR Experiments with Saponin-skinned Trabeculae

	LRS	HRS	HAS	SKS	LS
ATP (mM)	8	8	8	8	8
CP (mM)	10	10	10	10	10
CK (U/ml)	150	150	150	150	150
Ca^{2+} (μM)	—	—	24.9	—	0.4
Mg^{2+} (mM)	0.4	0.4	0.4	0.4	0.4
Na^{+} (mM)	36	36	36	36	36
K^{+} (mM)	66	66	66	66	66
HEPES (mM)	30	30	30	30	30
EGTA (mM)	0.3	30	30	0.3	30
HDTA (mM)	29.7	—	—	29.7	—
Saponin (μg/ml)	—	—	—	50	—

LRS = low relaxing solution; HRS = high relaxing solution; HAS = high activating solution; SKS = skinning solution; LS = Ca^{2+}-loading solution.

or halothane was added to skinned fibers of fast-twitch muscle. However, both volatile anesthetics caused no change in slow-twitch skeletal muscle.[2]

For a more quantitative assessment of Ca^{2+} release from the SR, we developed a novel method to derive Ca^{2+} transients directly from Ca^{2+}-induced force transients.[15,16] This method is based on measuring the force induced by calcium release of skinned muscle together with the Ca^{2+} sensitivity of the contractile proteins of the same muscle fiber to transform subsequently force transients into Ca^{2+} transients. The aim of our study was to compare the effect of sevoflurane with those of isoflurane and halothane on Ca^{2+} release from the SR and to consider the effects of the volatile anesthetics on the Ca^{2+} sensitivity of the contractile apparatus.

Materials and Methods

Muscle Fiber Preparation

After we received approval from the local animal care committee, BALB/c mice were anesthetized for 2–3 min with carbon dioxide and subsequently killed by cervical dislocation. The extensor digitorum longus muscle was isolated in paraffin oil at 4°C, and a small single-fiber bundle containing one to three fibers (between 80 and 150 μm in diameter) was dissected. The fiber preparation was glued to a force transducer pin (AE801; Senso-Noras, Horten, Norway) and a micrometer-adjustable screw using a collagen glue.

Solutions

Table 1 shows the concentrations of the experimental solutions. The high-activating solution and the high-relaxing solution contained 30 mM EGTA to "clamp" free Ca^{2+}, whereas the low-relaxing solution contained 0.3 mM EGTA and 29.7 mM 1,6-diamino exane-N,N,N,N-tetraacetic acid (HDTA), which, in contrast to EGTA, has a very low affinity to Ca^{2+}. The addition of 50 μg/ml saponin to the low-relaxing solution created the skinning solution. Free ion concentrations were calculated using the REACT program from Smith and Miller.[17] Solutions were adjusted to pH 7.0, and ionic strength was calculated to 157 mM. All measurements were performed at room temperature (23°C). The release solution consisted of the low-relaxing solution plus caffeine (30 mM), liquid sevoflurane, isoflurane, or halothane. The solutions to measure the $[Ca^{2+}]$-force relation were obtained by mixing appropriate amounts of high-activating solution with high-relaxing solution and adding 30 mM caffeine, liquid sevoflurane, isoflurane, or halothane.

Liquid sevoflurane, isoflurane, and halothane (0.5–5 μl) was diluted in 5 ml weakly calcium-buffered release solution, high- activating solution, or high-relaxing solution; solubilized with an ultrasound bath for 120 s; and thereafter stirred with a magnet for at least 60 min in an air-tight glass syringe. The anesthetic solution was added into a vial shortly before the muscle fiber was immersed in the solution. For gas chromatographic analysis of the concentration of the anesthetic agents, 400-μl aliquots of the working solutions were transferred into 5-ml vials for head space injections. After the addition of 10 μl chloroform-saturated water (internal standard), the vials were capped with septa with aluminum foil placed beneath them. The vials were stored frozen at −20°C for further analysis.

Chromatographic Conditions

Before the analysis, the samples were incubated for 30–45 min in a thermal block at 75°C to achieve a stable equilibrated gas phase. For gas chromatography mass

selective analysis, 100-μl aliquots were collected using a gastight 500-μl glass syringe. Analysis by gas–liquid chromatography was performed on a Hewlett Packard gas chromatograph (6890 series) combined with a mass selective detector (HP 5973 series; Hewlett Packard, Bad Homburg, Germany) equipped with a Hewlett Packard 5-MS capillary column, 30 m × 0.25 mm with a film thickness of 0.25 μm. Separation conditions included an injector temperature of 120°C and a detector temperature of 280°C with helium as the carrier gas at a starting flow of 0.5 ml/min and increasing after 3 min to a rate of 2 ml/min^2 to 1 ml/min. The temperature gradients were 33°C at 0–2 min and 33–55°C at 2–5.7 min. For quantification, substance-specific ions were used.

The corresponding anesthetic concentrations in volume percentages at 37°C were calculated using the reported Ostwald water–gas partition coefficients in aqueous solutions at 37°C (0.26 for sevoflurane, 0.57 for isoflurane, and 0.67 for halothane),[18] as described by Franks and Lieb.[19]

Protocol

Muscle fibers were kept in the skinning solution for 5 min. The sarcomere length was adjusted from the diffraction pattern of a helium–neon laser to 2.6 μm.[20] Before loading the SR of the fibers with Ca^{2+} for 1 min in the loading solution, which contained 0.41 μM Ca^{2+}, they were placed for 3 s in the release solution and 3 s in high-relaxing solution, and they were equilibrated for 2 min in the low-relaxing solution. Subsequently, they were transferred for 2–4 s into the high-relaxing solution and for 2 min in the low-relaxing solution. The fibers were exposed to the release solution containing 30 mM caffeine or liquid isoflurane–sevoflurane in the low-relaxing solution. The maximum force was measured in the high-relaxing solution at 24.9 μM Ca^{2+}.

As a quality control for permeabilization of the fibers, and because saponin skinning causes some reduction of the Ca^{2+} loading ability of the SR in rat skeletal muscle,[21] the initial caffeine (30 mM) release had to be at least 30% of maximal force; otherwise the fiber was discarded.

To reveal the time constant of decay, a fit by a single exponential function was applied, with $1/\tau$ in the exponent reflecting the time constant of decay.

The $[Ca^{2+}]$-force relation was measured with at least six different Ca^{2+} concentrations ranging from 0.003 μM to 24.9 μM. By nonlinear regression, a Hill curve was fitted to the measured data points by applying the following equation: $y = 10^{-x \cdot H}/(10^{-H \cdot EC50} + 10^{-H \cdot x})$, where $x = -\log[Ca^{2+}]$, which is a modified version of the Hill equation described by Fink *et al.*[22] The Hill coefficient (H) gives an indication of the maximum steepness of the sigmoidal curve. The EC_{50} value indicates the Ca^{2+} concentration for half-maximal isometric force activation as a measure of the sensitivity of the contractile proteins to Ca^{2+}. The correlation coefficients (r values) were calculated to determine the accuracy of the fit. The individual EC_{50} values and Hill coefficients (H) were subsequently applied to transform each force transient directly into a Ca^{2+} transient, by using the individual $[Ca^{2+}]$-force relation as a Ca^{2+} indicator and reversing each point of the force transients into the corresponding free Ca^{2+} level.[15,16] The sensitivity of the Ca^{2+}-regulatory proteins and the corresponding force development directly measures the free myofibrillar $[Ca^{2+}]$ and relates free Ca^{2+} and force. Thus, the $[Ca^{2+}]$-force relation can be used as a bioassay to convert the rather slow force transients from the Ca^{2+} release of the SR into apparent Ca^{2+} transients.

Statistical Analyses

To compare the effects seen with different concentrations and different volatile anesthetics, the effects of caffeine were measured as a control and for standardization in all experiments. The normal distributions of each group were confirmed by applying the Kolmogorov–Smirnov test. One-way analysis of variance was applied for comparisons between groups. When differences between the groups were greater than would be expected by chance, the Bonferroni *t* test was applied. A significant difference was defined as $P < 0.05$. Data are presented as the mean ± SD.

Results

All measurements were evaluated at three levels of concentrations: The lowest dose of 0.6 mM can be calculated to correspond to 5.87 vol% sevoflurane, 2.68 vol% isoflurane, and 2.28 vol% halothane at 37°C.[19] Higher levels (3.5 mM and 7.6 mM) were chosen to evaluate the extent and direction of effects.

Representative Ca^{2+}-induced force transients, $[Ca^{2+}]$-force relations, and the derived SR Ca^{2+} transients from an extensor digitorum longus muscle fiber preparation are shown for sevoflurane in figure 1, for isoflurane in figure 2, and for halothane in figure 3 at 3.5 mM. These recordings show that peak force transients induced by either anesthetic were lower compared with the standard transient of 30 mM caffeine and that force transients

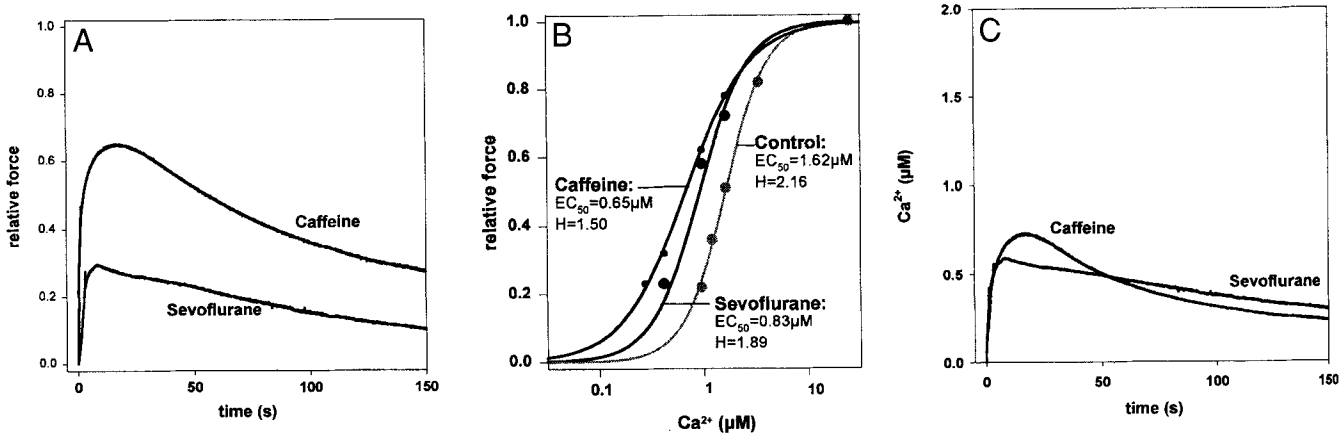

Fig. 1. A representative recording of the effect of 3.5 mM sevoflurane on a muscle fiber bundle shows a calcium-induced force transient (*A*), which is normalized to maximal force obtained in the presence of 24.9 μM Ca^{2+} and indicated by the value 1.0. The $[Ca^{2+}]$–force relation is shown in *B*. The Ca^{2+} transient is derived from the force transient based on the relation defined between $[Ca^{2+}]$ and force of the same fiber (*C*). As a control for the force transient, the caffeine (30 mM) transient of the same fiber is shown. The control for the $[Ca^{2+}]$–force relation without caffeine or a volatile anesthetic is shown in *B*.

of isoflurane and sevoflurane also revealed a slower decay when compared with caffeine (figs. 1A and 1C, 2A and 2C, and 3A and 3C). The individual $[Ca^{2+}]$-force relation revealed greater EC_{50} values for the volatile anesthetics compared with 30 mM caffeine and lesser EC_{50} values when compared with the control, suggesting a Ca^{2+}-sensitizing effect of the contractile proteins by the volatile anesthetics. Hill coefficients of sevoflurane, isoflurane, and halothane were greater compared with caffeine, resulting in a steeper Ca^{2+} dependence of isometric force (figs. 1B, 2B, and 3B). The peak Ca^{2+} transients derived from the force curves were less with sevoflurane, isoflurane, and halothane than were those induced by caffeine comparable to the reduced peak force transients (figs. 1C, 2C, and 3C).

Peak values of isometric force transients induced by sevoflurane, isoflurane (7.6 mM, 3.5 mM, and 0.6 mM), and halothane (3.5 mM and 0.6 mM) are given in figure 4A. Apart from the highest concentrations (7.6 mM), all peak values obtained by application of the volatile anesthetics were less than those of the caffeine transients. At 0.6 mM, the effect induced by halothane was more pronounced than that of isoflurane and sevoflurane, and the effect of isoflurane was greater than that of sevoflurane. The corresponding calcium transients (derived from the force transients based on the $[Ca^{2+}]$-force relation) re-

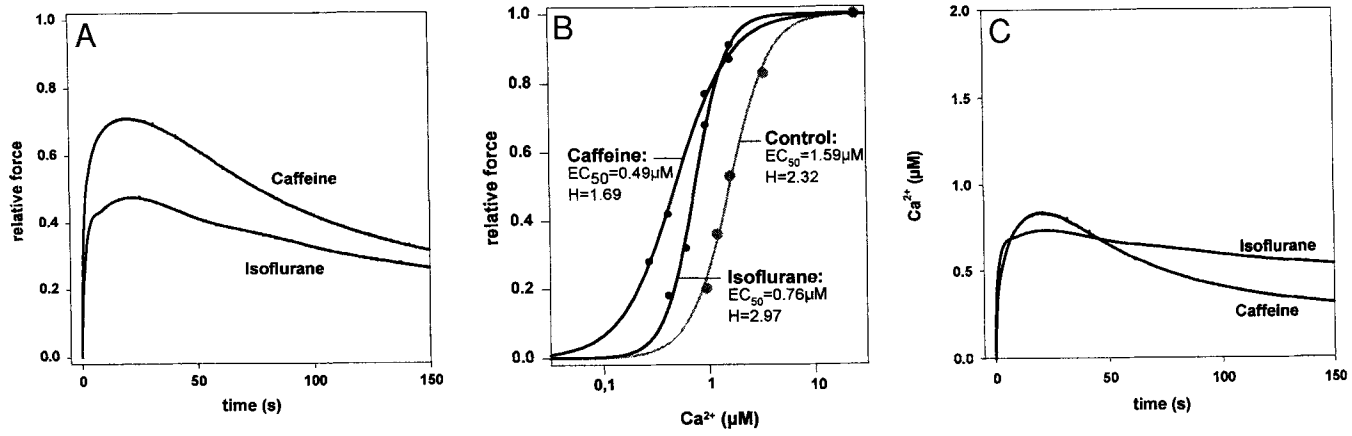

Fig. 2. A representative recording of the effect of 3.5 mM isoflurane on a calcium-induced force transient of a muscle fiber bundle is shown compared with that of 30 mM caffeine (*A*). The transients are normalized to maximal force, as in *A*. $[Ca^{2+}]$–force relations of the same muscle fiber preparation with or without 30 mM caffeine or isoflurane are given in *B*. The Ca^{2+} transients with isoflurane and caffeine are shown in *C*.

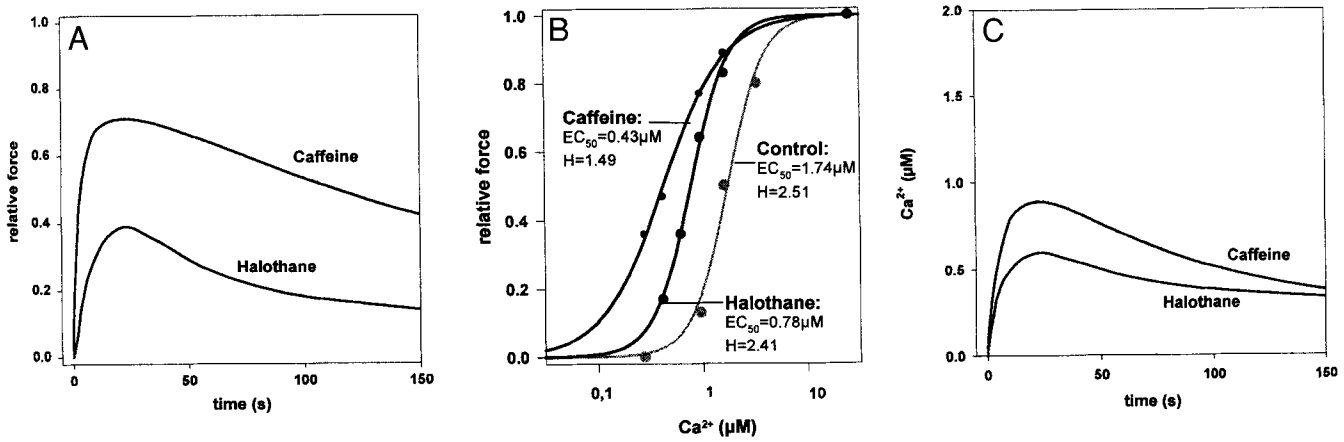

Fig. 3. A representative recording of the effect of 3.5 mM halothane shows a calcium-induced force transient (*A*) normalized to maximal force (see fig. 1A). The $[Ca^{2+}]$–force relation is shown in *B*. The Ca^{2+} transient in the presence of halothane is given in *C*. As a control for the force transient, the caffeine (30 mM) transient of the same fiber is shown. The control for the $[Ca^{2+}]$–force relation without caffeine or a volatile anesthetic is shown in *B*.

vealed similar findings compared with the isometric force transients for all given experimental conditions (fig. 4B). The rate of decay ($1/\tau$) was more reduced after the addition of 3.5 mM sevoflurane and isoflurane compared with equimolar halothane (fig. 5).

The $[Ca^{2+}]$-force relation was fitted to the Hill equation with mean correlation coefficients (r values) greater than 0.993 (SD < 0.05). Mean EC_{50} values under different experimental conditions are summarized in figure 6. In contrast to the changes of EC_{50}, no effect of the volatile anesthetics was observed on the Hill coefficient when compared with the control. However, for 30 mM caffeine ($H = 1.46 \pm 0.31$; $n = 10$; $P < 0.05$), the Hill coefficient was reduced compared with the control value ($H = 2.66 \pm 0.51$; $n = 7$) or compared with that observed with the high or low concentrations of sevoflurane (7.6 mM: $H = 2.60 \pm 0.40$; 3.5 mM: $H = 2.42 \pm 0.78$, $n = 5$; 0.6 mM: $H = 2.84 \pm 0.35$, $n = 3$), isoflurane (7.6 mM: 2.75 ± 0.48; 3.5 mM: $H = 2.44 \pm 0.71$, $n = 5$; 0.6 mM: $H = 3.00 \pm 0.34$ mM, $n = 3$) and halothane (3.5 mM: $H = 2.39 \pm 0.15$, $n = 5$; 0.6 mM: $H = 3.16 \pm 0.19$ mM, $n = 3$); mean ± SD.

The maximal Ca^{2+}-activated force in the presence of the volatile anesthetics revealed increased maximal values for all three volatile agents at the lowest concentration (0.6 mM) and increased values for 3.5 mM and 7.6 mM sevoflurane compared with the control (fig. 7).

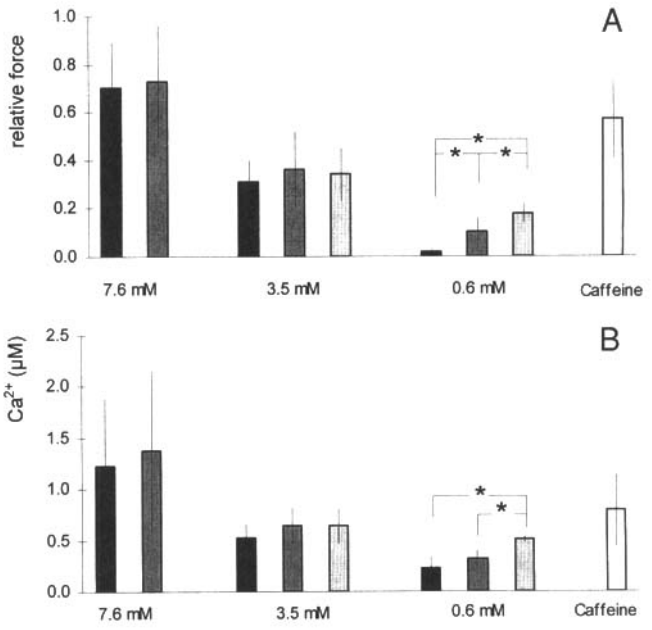

Fig. 4. (*A*) Peak force induced by Ca^{2+} release. Comparison between sevoflurane (*black square*, n = 4–6), isoflurane (*dark gray square*, n = 4–6) and halothane (*light gray square*, n = 4–7) at 7.6 mM and 3.5 mM (to evaluate the extent and direction of the effects) and in a lower concentration (0.6 mM equivalent to 5.87 vol% for sevoflurane, 2.68 vol% for isoflurane, and 2.28 vol% for halothane). Caffeine (30 mM) is shown as a standard and as a control (*open square*, n = 11). The relative force induced by caffeine was significantly greater than 3.5 mM and 0.6 mM of volatile anesthetics ($P < 0.05$, significance not indicated). (*B*) Peak Ca^{2+} transients derived from force transients based on the $[Ca^{2+}]$–force relation. The peak calcium transients induced by caffeine were significantly greater than those induced by 0.6 mM sevoflurane and isoflurane (significance not indicated). Results are mean ± SD. *$P < 0.05$.

Discussion

Little is known about the effect of newer volatile anesthetics on skeletal muscle. However, there have been

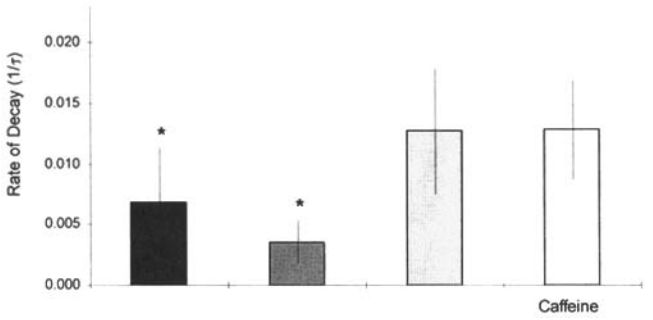

Fig. 5. The effects of 3.5 mM sevoflurane (*black square,* n = 5), isoflurane (*dark gray square*, n = 5), and halothane (*light gray square*, n = 5) on the rate of decay of the force transients. The time constants of the decay were fitted by a single exponential function (correlation coefficients > 0.990 in all fittings). Results are mean ± SD. *P < 0.05 when compared with the time constants of decay induced by caffeine (*open square*, n = 5).

case reports about malignant hyperthermia after administration of sevoflurane,[11-13] and the Ca^{2+}-release channel of the SR has been associated with malignant hyperthermia.[9,10]

Our goal was to evaluate the direct effect of sevoflurane on the SR compared with isoflurane and halothane. By measuring the Ca^{2+} sensitivity of the contractile apparatus of the same muscle fiber, we also calculated the individual Ca^{2+} release of that fiber compared with caffeine. This was possible by using a novel approach developed in our laboratory to transform force transients directly into Ca^{2+} transients.[15,16] It is based on the fact that the sensitivity of the Ca^{2+} regulatory proteins directly measures the free myofibrillar $[Ca^{2+}]$ and relates free Ca^{2+} and force (see also Materials and Methods section). All experiments were performed at room temperature and can be extrapolated only tentatively to the experimental condition expected at 37°C.

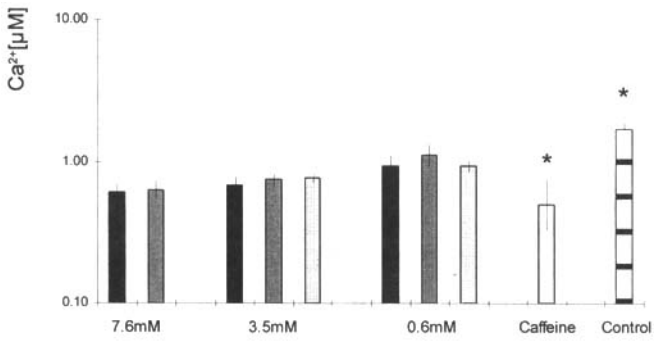

Fig. 6. The EC_{50} values of the $[Ca^{2+}]$–force relation, corresponding to the Ca^{2+}-concentration at half-maximal force. Comparison between sevoflurane (*black square,* n = 3–5), isoflurane (*dark gray square*, n = 3–5), and halothane (*light gray square*, n = 3–5) at 7.6 mM and 3.5 mM (to evaluate the extent and direction of the effects) and in a lower concentration (0.6 mM equivalent to 5.87 vol% for sevoflurane, 2.68 vol% for isoflurane, and 2.28 vol% for halothane). Caffeine (30 mM; *open square*, n = 14) is shown as a standard. Control solution without any substance added (*shaded square,* n = 9). With the exception of 7.6 mM *versus* 3.5 mM, there was a concentration-dependent effect for sevoflurane, isoflurane, and halothane (P < 0.5, significance not indicated). The EC_{50} of caffeine was significantly less compared with 0.6 mM of the volatile anesthetics. The EC_{50} of the control was significantly greater when compared with all other groups. Results are mean ± SD. *P < 0.05.

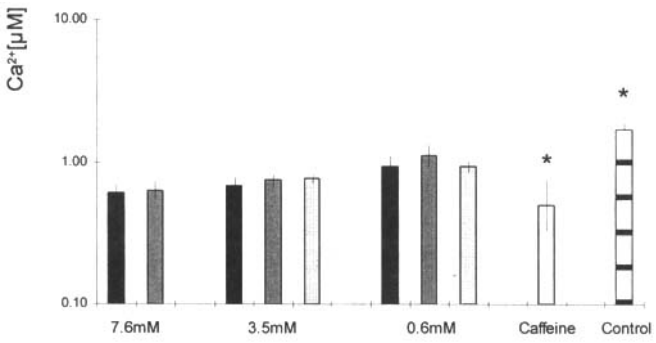

Fig. 7. Maximal Ca^{2+}-activated force in the presence of 24.9 µM Ca^{2+}. Comparison between sevoflurane (*black square,* n = 3–4), isoflurane (*dark gray square*, n = 3–4), and halothane (*light gray square*, n = 3–4) at 7.6 mM and 3.5 mM (to evaluate the extent and direction of the effects) and in a lower concentration (0.6 mM equivalent to 5.87 vol% for sevoflurane, 2.68 vol% for isoflurane, and 2.28 vol% for halothane). Forces were normalized to individual control forces, which were obtained in the absence of agents and are indicated by the value 1.0 on the ordinate axis. Results are mean ± SD; *P < 0.05 compared with the control (*shaded square*).

Interestingly, equimolar concentrations of the more recent agent sevoflurane behaved differently compared with isoflurane and halothane. All agents induced force transients by releasing Ca^{2+} from the SR coupled with a simultaneous increase in Ca^{2+} sensitivity of the contractile proteins. However, at the lowest concentration of 0.6 mM, Ca^{2+}-induced isometric force measurements revealed a significantly reduced peak force transient with sevoflurane when compared with isoflurane and halothane. This finding supports previous data, which were, however, derived from myocardial preparations.[23-25]

To our knowledge, the effect of sevoflurane on Ca^{2+} release from the SR has not been described. It is known, however, that isoflurane and halothane induce Ca^{2+} release. This had been shown indirectly by demonstrating that isoflurane or halothane increase a submaximal caffeine transient.[1-3] In our study, we recorded Ca^{2+}-dependent force transients that were induced by the volatile agents alone.

We also analyzed the Ca^{2+} transients of the SR derived from the force transients and the [Ca^{2+}]-force relation of the same fiber. These results revealed an increased peak Ca^{2+} transient of halothane compared with sevoflurane and isoflurane. Our results suggest a possible direct interaction of sevoflurane with the calcium release channel of the SR. Although the amino acid sequence and three-dimensional structure of the ryanodine receptor have been resolved in part,[26,27] little is known about possible interaction sites between volatile anesthetics and this receptor. Our results suggest that the volatile anesthetics interact differently with their binding region of the release channel; however, future studies defining these sites of action will be necessary.

The shape of the force transient and the corresponding Ca^{2+} transients show that the initial decay during relaxation induced by isoflurane and sevoflurane is slower compared with that of caffeine. One possible factor that influences the decrease in [Ca^{2+}] is the altered activity of the Ca^{2+} adenosine triphosphatase (ATPase) of the SR (SERCA), which pumps the released Ca^{2+} from the cytosol back into the lumen of the SR.[16] Volatile anesthetics have been shown to stimulate the SR Ca^{2+} ATPase activity[28,29] but also to inhibit Ca^{2+} uptake into the SR.[30] Under our experimental conditions, the slower initial relaxation of sevoflurane and isoflurane suggests an inhibition compared with caffeine. A slow initial relaxation has been described when the Ca^{2+} ATPase was blocked with the SR Ca^{2+} ATPase inhibitor cyclopiazonic acid,[16] which revealed a reduced decay similar to that seen in the presence of sevoflurane and isoflurane.

Whereas the influence of sevoflurane on Ca^{2+} sensitivity of the contractile proteins has not been evaluated before, our finding of a significant increase of Ca^{2+} sensitivity after the addition of isoflurane or halothane supports similar observations by Su and Bell.[2] In contrast, when a slow-twitch skeletal muscle (soleus) was evaluated, no effect of halothane on the [Ca^{2+}]-force relation was detected.[2,31] Little is known about the detailed mechanisms of the Ca^{2+}-sensitizing effect. Possible targets include a direct interaction with the tropomyosin-troponin complex, resulting in altered calcium binding to troponin C or in a different shift of tropomyosin. In addition, a direct interaction of isoflurane or sevoflurane with the contractile proteins actin or myosin may be involved. This suggestion is speculative at this stage and more studies are needed for a precise definition of the target protein or proteins. In rat myocardium, however, the method of skinning has been described to be important for the Ca^{2+} sensitivity.[7] An increase in Ca^{2+} sensitivity by isoflurane has been observed, although to a different extent both with saponin skinning and mechanical skinning. Under otherwise identical experimental conditions, a decrease in Ca^{2+} sensitivity was found after Triton X-100 skinning, which also disrupts the SR membrane. Whether the influence of different skinning methods can be transferred easily from cardiac to skeletal muscle is yet to be resolved.

The minimum alveolar anesthetic concentration is used to describe anesthetic potency in an intact organism (*in vivo*), whereas our *in vitro* experiments describe the effect of an anesthetic dose on a single organelle, the SR. Comparison of sevoflurane, isoflurane, and halothane using minimum alveolar anesthetic concentrations may be misleading because the anesthetic concentration in the SR during anesthesia and its proportion to the minimum alveolar anesthetic concentration have not been described in detail yet and remain open because lipid solubility is likely to be important. Thus, we compared isoflurane, sevoflurane, and halothane on an equimolar basis.

When interpreting the action of volatile anesthetics on calcium activation of muscle, the study of sensitivity changes of calcium-activated force and the effects on calcium release and uptake properties of the SR are important and potentially clinically relevant.

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