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Differential Effects of Halothane, Enflurane, and Isoflurane on Ca^{2+} Transients and Papillary Muscle Tension in Guinea Pigs

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These studies were designed to examine the effects of inhalational anesthetics on rapid changes in myocardial intracellular Ca^{2+} and Ca^{2+} sensitivity of the contractile apparatus. The effects of halothane, enflurane, and isoflurane on rapid changes in intracellular Ca^{2+} (Ca^{2+} transients as measured with bioluminescent protein aequorin) and contractile characteristics were compared in guinea pig right ventricular papillary muscles. In addition to examination of their potencies at equianesthetic concentrations, the effects of these agents on alterations in Ca^{2+} sensitivity at myofilaments were also investigated. The negative inotropic effects of halothane (0.65 and 1.15%) and enflurane (1.0 and 2.2%) were dose-dependent and closely related to a decrease in Ca^{2+} transients. In the presence of isoflurane (0.77 and 1.6%), the contractile force decreased in a dose-dependent manner, but the decrease was significantly less as compared to that with equianesthetic concentrations of halothane and enflurane. An additional feature observed in the presence of isoflurane was a dissociation between intracellular Ca^{2+} availability and contractile force. Although the magnitude of the Ca^{2+} transients did not change when the percentage of isoflurane was increased from 0.77 to 1.6, the contractile force decreased. Because of these findings, the effects of halothane (1.2%), enflurane (2.2%), and isoflurane (1.6%) on the relationship between intracellular Ca^{2+} and tension developed in the papillary muscle were examined in order to assess myofibrillar responsiveness to Ca^{2+}. The results indicate that only isoflurane slightly but significantly shifted the Ca^{2+}/isometric tension curve toward higher intracellular Ca^{2+} concentrations; no differences were observed in the absence and presence of equianesthetic concentrations of halothane and enflurane. In summary, the weaker negative inotropic effects of isoflurane as compared to halothane and enflurane are associated with less depression of intracellular Ca^{2+} concentrations. Because it was shown previously that these agents produce equivalent depression of transsarcolemmal Ca^{2+} current at equianesthetic concentrations, it appears that halothane and enflurane are more potent in depressing cellular accumulation and release of intracellular Ca^{2+}. Overall, it appears that depression of Ca^{2+} sensitivity probably does not play a major role in the negative inotropic effects of halothane and enflurane. However, a decrease in Ca^{2+} sensitivity by isoflurane appears to be compensated for by smaller depression of Ca^{2+} transient. (Key words: Anesthetics, volatile: halothane; enflurane; isoflurane. Animal: guinea pig. Heart: calcium transients; contractility. Ions: calcium; calcium sensitivity.)

STUDIES IN THE ISOLATED heart and papillary muscle demonstrate that halothane, enflurane, and isoflurane depress myocardial contractility in a dose-dependent manner with equianesthetic doses of halothane and enflurane depressing cardiac function more than isoflurane.[1–6] The mechanism(s) responsible for these differences are controversial. Some investigators have hypothesized that the major effect of isoflurane is *via* inhibition of Ca^{2+} influx, whereas others have attributed the difference to a greater effect on the sarcoplasmic reticulum (SR).[3] Determining which cellular sites are targets for the action of volatile anesthetics is difficult in the intact cardiac preparations because changes in contractile force reflect interaction between Ca^{2+} influx through the sarcolemma; release and sequestration of Ca^{2+} by the SR; activity of membrane Ca^{2+} pumps and ionic exchanges; and the Ca^{2+} sensitivity of the contractile proteins. Likewise, predictions of Ca^{2+} influx drawn from cardiac action potential configuration are complicated by interaction between different ionic fluxes across the sarcolemma.[1,6,7] Despite these drawbacks, increasing evidence suggests important quantitative differences between the depressant action of volatile agents on myocardial function, with equianesthetic doses of halothane and enflurane depressing cardiac function more than isoflurane. Although the direct cardiac depression observed *in vitro* is altered by indirect neural compensatory mechanisms *in vivo*,[8] isoflurane also appears to be a less potent cardiac depressant than either halothane or enflurane in isolated human atrial[9] and ventricular tissue.[10]

It is likely that the negative inotropic[4,8] and chronotropic[11] actions of halothane, enflurane, and isoflurane on the myocardium are related, at least in part, to their inhibition of inward Ca^{2+} current (I_{Ca}) at the sarcolemma.[12] However, because all three agents depressed the I_{Ca} amplitude similarly at equianesthetic concentrations,[12] their quantitatively different effects on cardiac performance[1–3,6,7] could be due to differential actions at other cellular sites. In order to examine these effects more closely, this study was designed to examine the effects of equianesthetic concentrations of halothane, enflurane, and isoflurane on rapid changes in intracellular Ca^{2+} (Ca^{2+} transient) in papillary muscles from the guinea pig and

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the effects of these agents on isometric contractile force. In addition, Ca^{2+} sensitivity curves were obtained in the presence of inhalational anesthetics for comparison of Ca^{2+} sensitivity in the intact cardiac muscle.

Materials and Methods

These experiments were approved by the Medical College of Wisconsin Animal Care Committee.

After intraperitoneal ketamine injection, guinea pigs were decapitated, and the hearts were quickly removed and perfused briefly with cold oxygenated Krebs' solution. Right ventricular papillary muscles having a width of less than 1 mm (mean OD = 0.7 mm) were excised from 31 guinea pigs and mounted horizontally in a single low-volume, high-flow chamber (1-ml volume, 5-ml/min flow). Oxygenated Krebs' solution was composed of the following (millimolar): NaCl 137, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, and dextrose 11.5 (*p*H 7.40 ± 0.05); this solution was equilibrated with a 97% O_2–3% CO_2 mixture and circulated at 30° C as reported earlier.[13]

The papillary muscles were field-stimulated at 0.5 or 1 Hz throughout the experiment with 2-ms pulses at slightly above the threshold strength. The chorda tendineae of the muscle was connected with a fine 10-0 thread to the arm of the miniature isometric force transducer (BG 10, Kulite Semiconductor Products, Inc., Ridgefield, NJ). At the beginning of each experiment, the muscle length was adjusted to a point where the tension developed was maximal. The experiments were conducted at 30° C because it was observed that muscles maintained a stable level of developed tension and light signal for a longer period of time as compared to that obtained at 37° C.

Highly purified aequorin used in this study was obtained from the laboratory of Dr. J. R. Blinks (Rochester, MN). Lyophilized aequorin was reconstituted to a concentration of 2 mg/ml with Ca^{2+}-free distilled water to give an aqueous solution containing 150 mM KCl and 5 mM HEPES buffer at *p*H 7.5. The aequorin solution was filtered and placed into fine microelectrodes. Platinum wire was positioned inside the micropipettes to permit simultaneous recording of cellular potentials while applying nitrogen pressure of up to 100 psi to the pipette. It was necessary to inject at least 50 cells in order to obtain satisfactory light signals. The intracellular aequorin light signals provide a good indication of the overall magnitude and time course of the intracellular myoplasmic Ca^{2+} concentrations.[14,15] The light emitted by aequorin was recorded in a light proof setting using a photomultiplier cathode. Successive contractile forces and light signals were averaged (100 consecutive beats; model 9153 Hewlett-Packard computer) to obtain satisfactory luminescence signal-to-noise ratios. Light signals were expressed in terms of anode current in nanoamperes, while tension was normalized for the cross sectional area of the muscle and expressed as millinewtons per square millimeter. Resting light emission was low and so close to the threshold for detection that it was not feasible to investigate the effects of different anesthetics on the resting photon emission. Anesthetics were tested for their direct effects on the aequorin light emission by injecting aequorin (10^{-8} M) into a Ca^{2+} buffer solution containing 150 mM KCl, 5 mM HEPES, and 3 μM Ca^{2+} (*p*H 7.0). Aequorin light emission was monitored at 30° C.

Individual anesthetics were introduced to the superfusate reservoir for at least 20 minutes *via* calibrated vaporizers. Anesthetic concentrations in the tissue bath were measured during anesthetic exposure using a gas chromatograph with a flame ionization detector. The bath concentrations at 30° C were converted to their equivalent percentages in the gas phase. The mean concentrations (volume percent) were as follows: halothane 0.65 and 1.15, enflurane 1.0 and 2.2, and isoflurane 0.77 and 1.6. These levels of anesthetics are referred to as low and high concentrations. The potency ratios for these agents were reasonably close to the estimated potency ratios for the guinea pig—1:2.15:1.14 for halothane, enflurane, and isoflurane, respectively.[16] Papillary muscles were exposed to the desired concentration of anesthetic in random order for 10 min prior to measurements of contractile force and light signal. After the measurements, there was a 10-min period for anesthetic washout and control measurements. Each papillary muscle was exposed to lower and higher concentrations of all three anesthetics in random order.

To directly examine the effects of halothane, enflurane, and isoflurane on the relationship between intracellular Ca^{2+} and tension development, simultaneous measurements of Ca^{2+} transients and isometric contractile force were performed by increasing the extracellular Ca^{2+} in steps from 1 to 12–17 mM. Myofibrillar responsiveness to Ca^{2+} studies were conducted as a single series of control measurements under different extracellular concentrations of Ca^{2+}, followed by a similar series in the presence of higher concentration of each anesthetic. Contractile force measurements and Ca^{2+} transient changes were taken 10 min after extracellular Ca^{2+} change. Ca^{2+} sensitivity curves were obtained by plotting the peak isometric contractile force and peak light intensity. The light intensity was expressed in terms of the 2.5th root of the anodal current, because the light emission from the aequorin varies approximately in proportion to the 2.5th power of the Ca^{2+} concentration.

Because there was a small reduction in light signal over time, data for each test condition were bracketed (average of values before and after exposure to anesthetic) for data tabulation and statistical analyses. Differences in contrac-

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tile force and Ca^{2+} transient parameters between anesthetics and the respective doses were evaluated using two-way analysis of variance, and the means were compared by the least significant difference test. Comparison of the relationship between peak isometric contractile force and peak aequorin light measured at various extracellular Ca^{2+} concentrations before and after inhalational anesthetics were compared by expressing the above measurements as percentages of maximum response obtained during the control. Their respective regression lines were compared for differences in slopes using a Test for Parallelism.

Results

The direct effects of halothane, enflurane, and isoflurane on aequorin luminescence were tested *in vitro* by injecting aliquots of aequorin (10^{-8} M) into a buffer solution containing several concentrations of the anesthetic agents (halothane 0.8 and 1.6%, isoflurane 1.1 and 2%, and enflurane 1.3 and 2.5%). At these concentrations the anesthetics had no significant direct effect on the intensity of luminescence, indicating that these agents do not interact with aequorin.

The effects of inhalational anesthetic agents at higher concentrations on the Ca^{2+} transients and the contractile force of a typical guinea pig papillary muscle preparation are shown in figure 1. Anesthetic depression of contractile force was accompanied by depression of the intracellular Ca^{2+} signal, and, as illustrated, the depression of the Ca^{2+} transients in the presence of the isoflurane was less than that produced in the presence of halothane or enflurane ($P < 0.05$). The rapid increase in intracellular Ca^{2+} concentration following the initiation of the action potential, as seen in figure 1, is believed to be due primarily to Ca^{2+} release from the SR, and this rapid release is generally believed to involve a Ca^{2+}-induced Ca^{2+} release mechanism.[17,18] Therefore, the Ca^{2+} that enters the cytoplasm *via* Ca^{2+} channels contributes to the Ca^{2+} transient by acting both as a trigger for release of Ca^{2+} and as the primary source of Ca^{2+} for the SR.

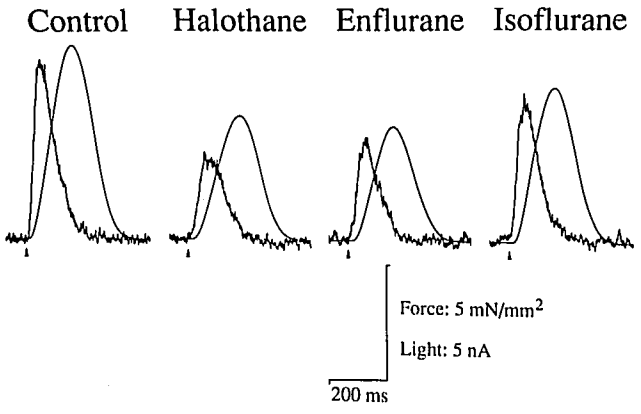

FIG. 1. Effects of higher concentrations of halothane (1.1%), enflurane (2.2%), and isoflurane (1.6%) on aequorin signal and isometric contractions of a single isolated guinea pig papillary muscle. Pacing rate 1 Hz (at the arrowhead), 30° C.

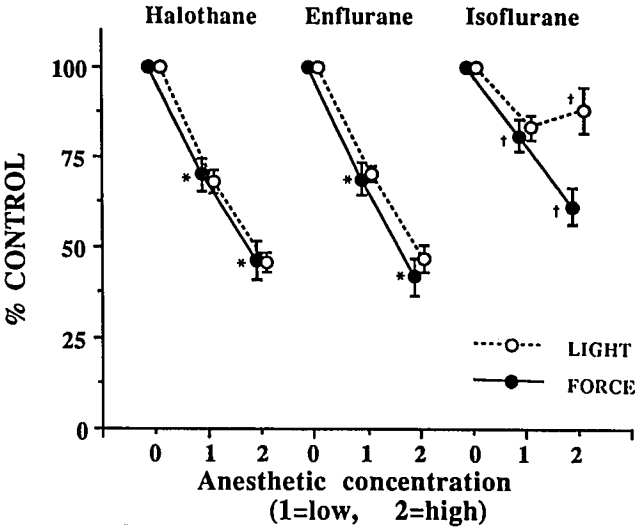

FIG. 2. Effects of halothane, enflurane, and isoflurane at lower and higher concentrations on the peak tension development and peak aequorin signal as percentage of control in the isolated guinea pig papillary muscle. *$P < 0.05$ *versus* 0 (no anesthetic control); †$P < 0.05$ *versus* other anesthetics at the same concentration. n = 13.

The comparative effects of halothane, enflurane, and isoflurane on the amplitude of the Ca^{2+} transients and peak isometric force are summarized in figure 2. The values are means ± SEM as percentages of control. The negative inotropic effects of halothane and enflurane were dose-dependent and closely related to the decrease in intracellular Ca^{2+}. Isoflurane also reduced contractile force in a dose-dependent manner, but the decrease was significantly less as compared to that produced by halothane and enflurane. A difference observed with isoflurane was a dissociation between intracellular Ca^{2+} availability and contractile force. Although the magnitude of the Ca^{2+} transient did not change when the concentration of isoflurane was increased from low to high concentration, the contractile force decreased.

Figure 3 represents the effect of halothane, enflurane, and isoflurane on time to peak amplitude of the aequorin signal and of the isometric contractile force (measured from stimulus artifact to the signal peak). Halothane was the only anesthetic to increase both the time to peak Ca^{2+} transient and time to peak tension. These effects could be due to a smaller Ca^{2+} release from the SR secondary to a lesser Ca^{2+} gradient between the SR and the cytoplasm following the attenuated Ca^{2+} uptake by the SR, although the underlying relationship between these two observations is not known, along with the role of troponin-C Ca^{2+} binding. On the other hand, the falling phase of

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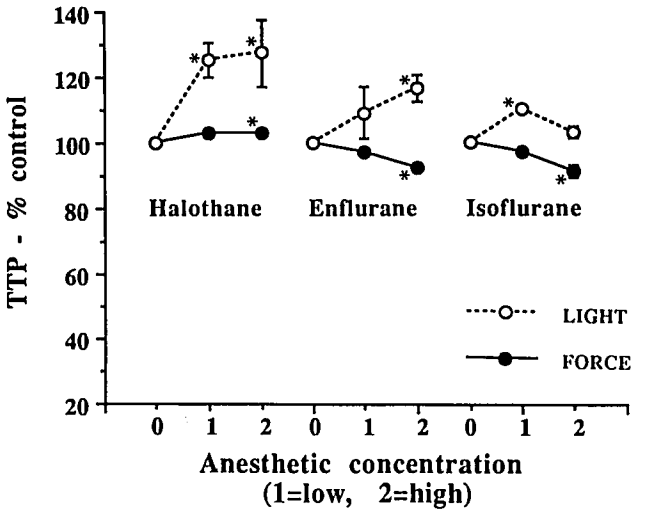

FIG. 3. Effects of two doses of halothane, enflurane, and isoflurane on time duration between the stimulus and the peak amplitude (time to peak [TTP]) of the aequorin signal and isometric contractile force as percentage of control in the isolated guinea pig papillary muscle. Anesthetic concentration: 0, low (1), high (2). *$P < 0.05$ *versus* 0. n = 13.

the Ca^{2+} transient and contractile force contribute to a change in the duration of the force and light that are measured at half of peak amplitude (fig. 4). Generally, a slower falling phase of the aequorin signal may suggest that there is a slower removal of Ca^{2+} from the myoplasm by the SR, a slower Na^+/Ca^{2+} exchange or altered affinity of troponin-C for Ca^{2+}.

The time course of the aequorin signal and the isometric contractile force from a single guinea pig papillary muscle are shown in figure 5. The aequorin signal and contractile force from control are displayed in their true proportions, while the tracings in the presence of all three anesthetics are adjusted electronically to equal control amplitude, to examine the duration and shape of the light signal and the force. As shown, only halothane substantially lengthens the slower falling phase of the aequorin signal. Because electronic adjustments may disproportionately alter certain phases of the signal, similar comparisons were performed after the aequorin signal was increased in the presence of inhalational agents by increasing the extracellular concentration of Ca^{2+}. Under these conditions, halothane was again the most effective in prolonging the duration of aequorin signal (data not shown). These results indicate that halothane was more potent in increasing the duration of the Ca^{2+} transient as measured at half of the peak amplitude, and that isoflurane slightly shortened the Ca^{2+} transients (fig. 4). This abbreviation of the light signal suggests a faster removal of Ca^{2+} from cytoplasm by the SR in the presence of isoflurane as compared to halothane and enflurane.

The changes in sensitivity of troponin-C to Ca^{2+} and/or altered response of the myofilaments to a given level of occupancy of the Ca^{2+} binding sites on troponin-C ("downstream mechanisms") were examined in 18 papillary muscles. This was accomplished by increasing the extracellular concentration of Ca^{2+} to 12 mM during the control and to 17 mM in the presence of higher concentration of inhalational agents. Figure 6 illustrates the changes in intracellular Ca^{2+} and contractile force developed in a single guinea pig papillary muscle at various concentrations of extracellular Ca^{2+} during control and in the presence of isoflurane. Comparisons of the relationship between peak isometric force and peak aequorin light signal measured in the same muscle were made at various extracellular Ca^{2+} concentrations before and after the individual inhalational agents.

Figure 7A shows the findings in a single papillary muscle for the control and in the presence of 0.6 mM halothane. The contractile force and Ca^{2+} transients were normalized to a percentage of the maximum control response for each experiment and plotted as illustrated in figure 7B along with regression lines. Figure 7C and 7D illustrate the same analysis in the presence of enflurane. No differences were found between regression slopes of Ca^{2+} sensitivity obtained in the absence and the presence of equianesthetic concentrations of halothane and enflurane. Only in the presence of isoflurane did we find a slight shift in the Ca^{2+} isometric curve toward the higher intracellular concentrations (fig. 8A), along with a significant ($P < 0.005$) decrease in the regression slope of the Ca^{2+} sensitivity (fig. 8B). Thus, in the presence of isoflurane, a greater Ca^{2+} transient was required to produce similar isometric force seen under control conditions.

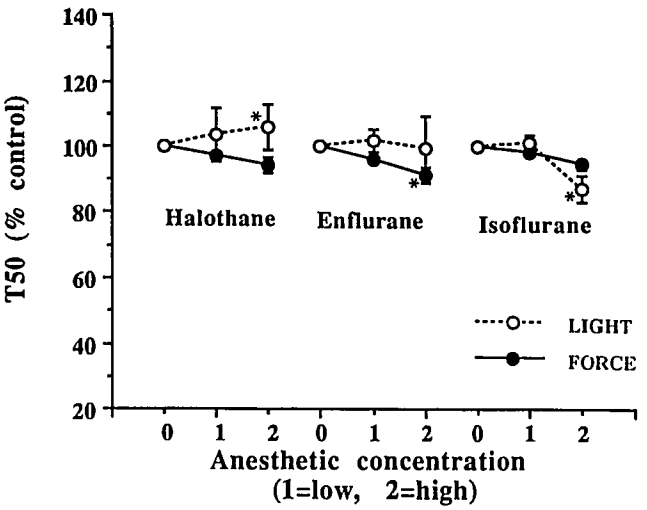

FIG. 4. Effects of two doses of halothane, enflurane, and isoflurane on the duration of aequorin light and contractile force measured at half of peak amplitude (T_{50}) in the guinea pig papillary muscle. Anesthetic concentration: 0, low (1), high (2). *$P < 0.05$ *versus* 0. n = 13.

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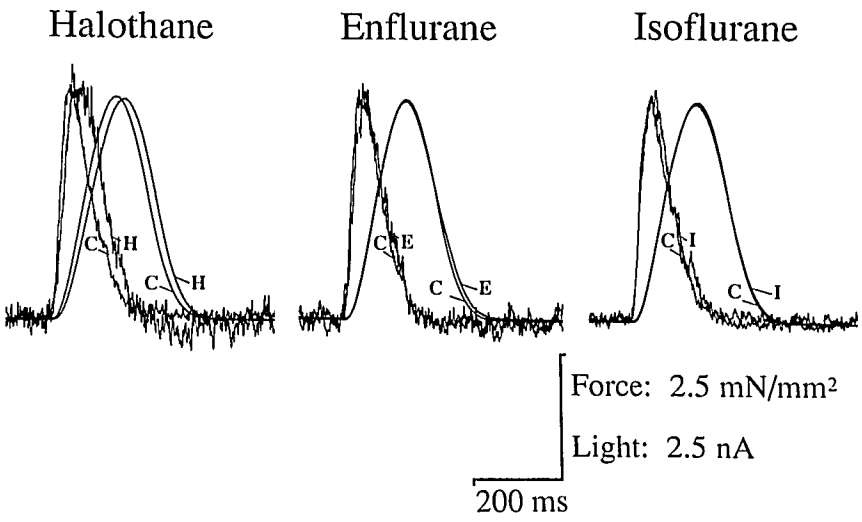

FIG. 5. Effects of higher concentrations of halothane (H), enflurane (E), and isoflurane (I) (1.0, 2.1, and 1.6%, respectively) on time courses of the aequorin signals and isometric contractions from the same guinea pig papillary muscle. Aequorin signals and contractions during the control (C) are displayed in their true proportions. The vertical gain of the signals obtained during anesthetics (H, E, or I) have been adjusted electronically to make the amplitudes of the signals match those recorded during the control.

Discussion

Volatile anesthetics at clinically useful concentrations depress the contractile force of the heart, and these actions in part contribute to significant decrement of cardiovascular homeostasis. Studies in isolated heart and papillary muscle preparations consistently demonstrate that these agents produce dose-dependent decreases of indices of contractility, with equianesthetic doses of halothane and enflurane depressing cardiac function more than isoflurane. The mechanisms underlying the negative inotropic effects of the volatile anesthetics are not fully understood. Contractile force generated in the beating heart is associated with the increase and decrease of intracellular Ca^{2+} ion concentration. There are several mechanisms by which agents may directly alter contractile performance of cardiac muscle. The first group represents "upstream" mechanisms whereby Ca^{2+} transients are influenced mainly by a variety of effectors at the surface membrane and SR. The second group, "downstream" mechanisms, involves changes in sensitivity of troponin-C to Ca^{2+} or an altered response of the myofilaments to a given level of occupancy of the Ca^{2+} binding sites on troponin-C. The sites of action of volatile anesthetics are difficult to separate because a change in the influx of sarcolemmal Ca^{2+} alters the sequestration of Ca^{2+} in the SR and ultimately the level of myoplasmic Ca^{2+} available as the Ca^{2+} transient to activate the contractile proteins. Despite these difficulties, accumulating evidence suggests that the volatile anesthetics act in a number of specific ways, including: 1) effects on the sarcolemmal flux of Ca^{2+}; 2) alteration in SR function; 3) decreasing the level of intracellular ionized Ca^{2+} during systole; and 4) modification of the responsiveness of the contractile proteins to activation by Ca^{2+}.

Although several types of voltage-dependent Ca^{2+} channels exist in various cell types,[19] the Ca^{2+} channels of cardiac muscle include the low-threshold transient (T-type) channels and the high-threshold long-lasting (L-type channels). Although it has been shown that Ca^{2+} influx *via* L-type Ca^{2+} channels is required for SR Ca^{2+} release in heart muscle,[20] the role of Ca^{2+} influx *via* Ca^{2+} channels as a prerequisite for SR Ca^{2+} release has been challenged. One recent study has suggested that the triggered Ca^{2+} may also enter *via* Na^+/Ca^{2+} exchange.[21] In ventricular cells, the L-type channel is predominant, and current through T-type channels is small, decays quickly, and contributes little to the total I_{Ca} during the cardiac action potential.[22] Halothane has been shown to reduce a slow inward current in the isolated rat ventricular cells.[23]

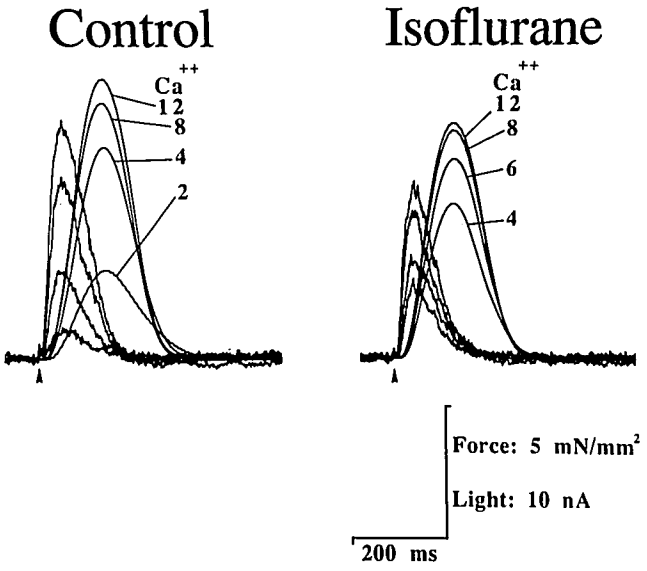

FIG. 6. Effects of various concentrations of extracellular calcium (indicated in millimolar concentrations) on aequorin signal and isometric contraction of a single isolated guinea pig papillary muscle during control and after exposure to 1.6% isoflurane.

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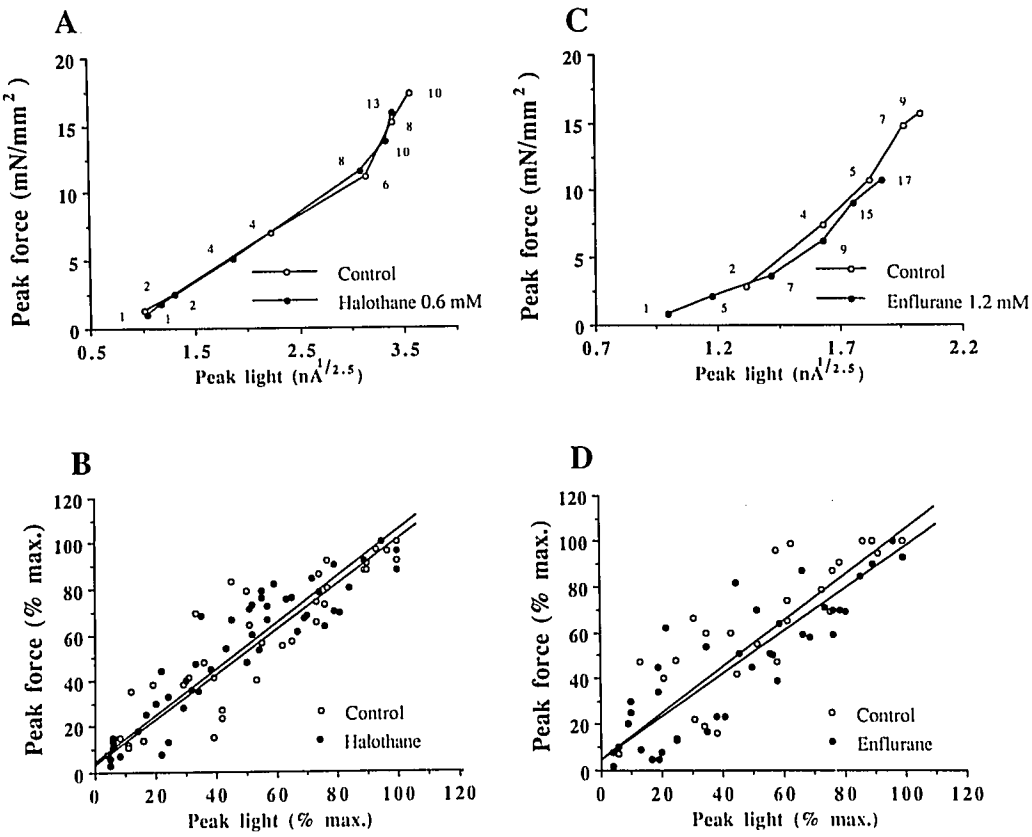

FIG. 7. *A:* Comparison of the relationship between peak isometric contractile force and peak aequorin light measured in the same muscle at various extracellular calcium concentrations before and after halothane (0.6 mM or 1.2%). The 2.5th root of the amplitude of the aequorin signals was used as an indicator of the amplitude of the Ca^{2+} transient. The numbers beside the points indicate extracellular Ca^{2+} in millimolar concentrations. Higher concentrations of extracellular Ca^{2+} produced either no further increase or a decrease in the force developed, and those values are not shown in the graph. *C:* Similar comparison in the presence of 1.2 mM enflurane (2.2%). *B, D:* Summary of the relationships between the peak contractile force and the peak light expressed as a percentage of maximal response along with regression lines obtained during the control and in the presence of anesthetics. Regression lines were not statistically significant for either anesthetic (n = 6 for each agent).

Our previous study[12] showed that halothane, enflurane, and isoflurane, when tested in the same cardiac myocytes under identical conditions, produce equivalent depression of peak I_{Ca} at equianesthetic concentration without shifting the current–voltage relationship for channel activation. Presuming that effects in enzymatically isolated myocytes reflect intact myocardium, it would be unlikely that the differences between anesthetic action on contractile force generation are due to these actions on sarcolemmal Ca^{2+} channels because halothane, enflurane, and isoflurane produce similar depression of I_{Ca}. Other differences between anesthetic effects at the sarcolemmal level, such as the effect on the Na^+/Ca^{2+} exchange and the membrane Ca^{2+} pump, also cannot be completely excluded.

Measurements of changes in free-intracellular Ca^{2+} using the bioluminescent protein aequorin and contractile force, as performed in this study, indicate that the weaker negative inotropic effect of isoflurane as compared to halothane and enflurane is associated with less depression of the peak intracellular Ca^{2+} concentration. The decrease in Ca^{2+} transients by these agents is likely related to the inhibition of I_{Ca} at the sarcolemma,[12] which in turn could affect the quantity of Ca^{2+} released by the SR. Because there was no quantitative difference between the effects of isoflurane and the other two agents on I_{Ca} amplitude in the previous study,[12] the sarcolemmal Ca^{2+} flux is an unlikely site for their differential cellular effects.

While halothane, enflurane, and isoflurane decrease the I_{Ca}, it is expected that over many beats, these fluxes will contribute to the decrease in SR loading and that therefore the SR will release less Ca^{2+}. In addition, it was suggested that these agents also depress the net SR Ca^{2+} uptake and contribute to the negative inotropic effect.[24,25] In addition to the effects of Ca^{2+} uptake, the possibility exists that inhalational anesthetics might also increase the rate of Ca^{2+} leak from the SR during rest and therefore contribute to depletion of SR Ca^{2+} content. In any event,

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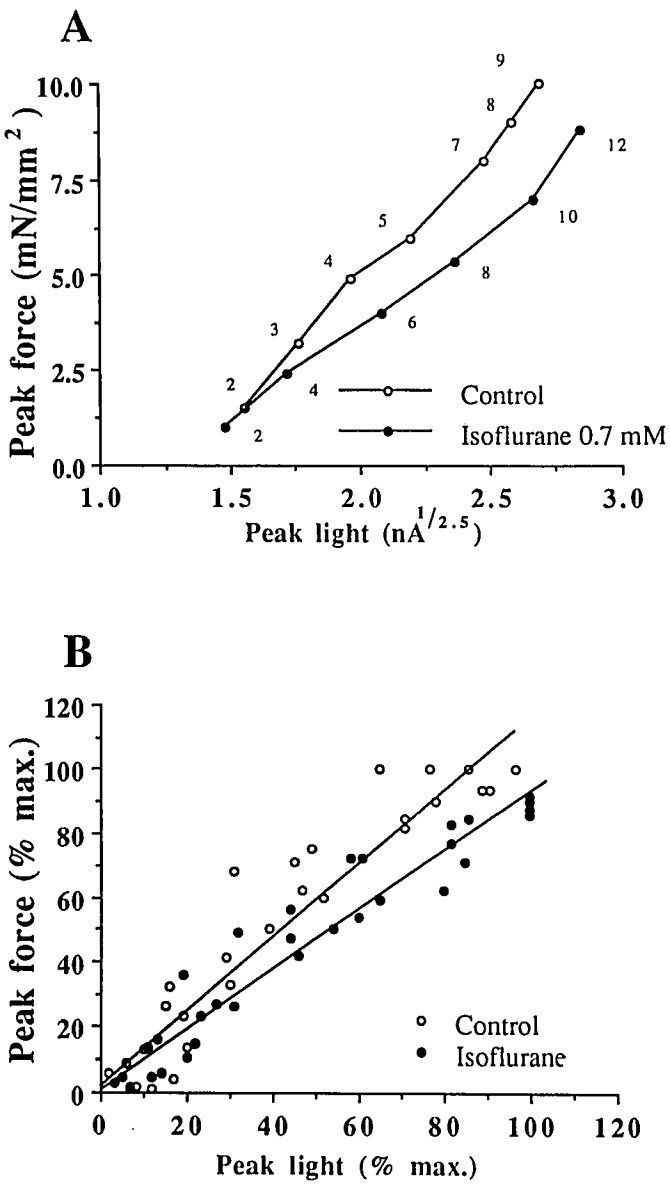

FIG. 8. *A:* Comparison of the relationship between peak isometric contractile force and peak aequorin light measured in the same muscle at various extracellular calcium concentrations before and after isoflurane (0.7 mM or 1.6%). The numbers beside the points indicate extracellular Ca^{2+} in millimolar concentrations. *B:* A significant shift of the regression line was obtained in the presence of isoflurane when tested for parallelism (n = 6).

because Ca^{2+} transients are dominated by Ca^{2+} that is released from SR,[15,26] the most potent negative inotropic anesthetic is expected to have the greatest effect on the SR.

The major determinant of the decline of the Ca^{2+} transient in heart muscle is likely to be reuptake of Ca^{2+} by the SR,[26] although this decline might be modulated by changes in myofibrillar Ca^{2+} sensitivity. The SR Ca^{2+}-pumping ATP-ase appears to be responsible for the majority of the rate of decline of the Ca^{2+} transients once release of Ca^{2+} is over.[27,28] The greater role of the SR for Ca^{2+} reuptake is due to a greater Ca^{2+} ATP-ase affinity for Ca^{2+} and a higher rate of transfer relative to that of sarcolemmal Ca^{2+} ATP-ase. In addition to these pumps, the Na^+/Ca^{2+} exchange also contributes to the decline of the Ca^{2+} transient.[29] Therefore, if anesthetic agents decrease the rate of Ca^{2+} uptake by the SR, it is expected that they would have a corresponding effect on the rate of decline of the aequorin signal. As seen from these results, halothane was most effective in lengthening the time to peak duration of the aequorin signal as well as the time of the Ca^{2+} transient measured at half of peak amplitude. The amplitudes of aequorin signal obtained in the presence of anesthetics also were adjusted electronically for comparisons of their time courses. In addition, because the upper part of the aequorin signal due to this adjustment might be disproportionately prolonged,[26] the time course of aequorin signals that have been increased to the equal magnitude by increases in extracellular Ca^{2+} were also compared with control. During all measurements, in the presence of higher concentrations of halothane, the aequorin signal was longer than that recorded during control measurements, whereas isoflurane at similar concentration decreased the duration at half of peak amplitude of the aequorin signal. This finding might imply that halothane delays the rate of Ca^{2+} removal by both the SR and/or Na^+/Ca^{2+} exchange. In the presence of isoflurane, Ca^{2+} removal is well maintained as indicated by the decrease in T_{50}.

Several studies of anesthetic effects on skinned muscle preparation suggest that volatile anesthetics may depress myofibrillar Ca^{2+} sensitivity.[30,31] Although valuable information was obtained regarding the relationship between intracellular Ca^{2+} and contractile force in the mammalian cardiac tissue using the skinned muscle preparations, extrapolation of the findings to the intact myocytes might be difficult.[32] Specifically, it appears that the myofilament sensitivity to Ca^{2+} may be greater in intact muscle than in skinned preparations. It was reported that the Ca^{2+} required for maximal activation of intact muscle fiber is less than or equal to 1 μM.[29] On the other hand, the concentrations of Ca^{2+} producing maximal contractions in skinned muscles are approximately 10 μM.[33] In intact ferret papillary muscle, Housmans *et al.*[34] have measured force generation and intracellular Ca^{2+} transients in the presence and absence of volatile anesthetics. They concluded that although each agent has an inhibitory effect on myofibrillar Ca^{2+} responsiveness (with isoflurane having the largest effect), the relative magnitude of the effect is small relative to the ability of the agents to decrease Ca^{2+} availability.[35] In the present study, also using intact muscles, inference is made about the Ca^{2+} tension relationship, but one should be cautious about

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comparing these findings with those obtained using skinned muscle, because unlike in the skinned muscle experiments, maximal force was not achieved, and the relationship between the Ca^{2+} and tension was not obtained at steady-state. The results of the present study suggest that while halothane and enflurane do not alter myofibrillar Ca^{2+} sensitivities, isoflurane depresses Ca^{2+} sensitivity despite relatively less influence on the Ca^{2+} transient and overall contractile force development.

In summary, these results support previous findings[36,37] on the differential effects of halothane and isoflurane by direct measurements of intracellular Ca^{2+} transients. Using intact papillary muscle preparations, these studies could not confirm the depressant effects of halothane and enflurane on Ca^{2+} sensitivity obtained from either mechanically[30] or chemically[31] treated cardiac fibers. Our results suggest that depression of Ca^{2+} sensitivity probably does not play a role in the negative inotropic effects of halothane and enflurane. However, a decrease of Ca^{2+} sensitivity by isoflurane may play a role in the more modest depression of contractile force, although this appears to be compensated for by less depression of the Ca^{2+} transient. Furthermore, results obtained from the Ca^{2+} transients are in agreement with results obtained using different methodology[38,39] showing that halothane and enflurane are more potent in depressing cellular accumulation and release of intracellular Ca^{2+} than is isoflurane. These effects of inhalational agents could lead to a decrease in Ca^{2+} content of cardiac cells[40,41] and most likely contribute to the cardiac protection following ischemia and Ca^{2+} paradox.[42]

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