Anesthesiology
52:385–389, 1980

Effects of Enflurane on Functionally Skinned Myocardial Fibers from Rabbits

Judy Y. Su, Ph.D., and W. Glenn L. Kerrick, Ph.D.†*

Enflurane, at clinical concentrations, decreases the contractility of isolated intact cardiac muscle. The authors investigated the intracellular mechanism(s) of this depression by examining the Ca^{2+} activation of the contractile proteins and Ca^{2+} uptake and release from the sarcoplasmic reticulum (SR) using functionally skinned fibers from right ventricular papillary muscle of rabbits. This preparation permits control of intracellular ionic composition (*p*H 7.0, 20 C). The [Ca^{2+}]–tension relationship and caffeine-induced tension transient (as a measure of the amount of Ca^{2+} release) were analyzed. Enflurane significantly but only slightly depressed the maximum Ca^{2+}-activated tension (10 per cent decrease at 5 per cent enflurane) and did not change the [Ca^{2+}] required for half-maximal activation of the fibers. In contrast, enflurane markedly inhibited the Ca^{2+} uptake by the SR (30–85 per cent decrease at 2.5–7.5 per cent enflurane). The inhibition was dose-dependent. Ca^{2+} release from the SR with 25 mM caffeine was not changed at low concentrations of enflurane (1–5 per cent), but was decreased at high concentration (25 per cent decrease at 7.5 per cent enflurane). Enflurane (1–7.5 per cent), however, increased (13–44 per cent) the submaximum caffeine (2 mM)-induced Ca^{2+} release from the SR, and the effect was not dose-dependent. The aforementioned effects were reversible. These results are similar to those previously reported for halothane. It is concluded that enflurane may induce myocardial depression mainly by inhibiting Ca^{2+} uptake by the SR. (Key words: Anesthetics, volatile: enflurane. Heart: contraction; papillary muscle. Ions: calcium.)

AT CLINICAL CONCENTRATIONS enflurane directly decreases myocardial contractility.[1] Like halothane, it decreases peak isometric tension and maximal rate of rise of tension development and prolongs the time to peak tension in isolated intact papillary muscle preparations. Therefore, Brown and Crout[1] speculated that enflurane and halothane may have a common mechanism of action. Ca^{2+} plays an important role in the contraction of muscles. The current view of contraction is as follows[2,3]: The depolarization of muscle plasma membrane causes an influx of Ca^{2+} and triggers a release of Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm. The increased cytoplasmic free [Ca^{2+}] binds to troponin, resulting in actin–myosin interaction, thus tension generation or muscle shortening. Enflurane could affect one or more of the above-mentioned steps in the contractile process, resulting in myocardial depression. We have recently reported that halothane significantly but slightly depressed the Ca^{2+} activation of the contractile proteins[4] but markedly depressed Ca^{2+} uptake by the sarcoplasmic reticulum (SR)[5] in functionally skinned myocardial cells. The purpose of this study was to investigate whether the effects of enflurane on the Ca^{2+} activation of the contractile proteins and Ca^{2+} uptake and release from the SR in functionally skinned myocardial cells are similar to those of halothane, as suggested by Brown and Crout.[1]

Methods

The functionally skinned fiber preparation used in this study was originally described for use in skeletal muscle by Kerrick and Krashner[6] and applied to cardiac muscles.[4,5] The functionally skinned muscle fiber preparations (sarcolemma-disrupted) were prepared by homogenization of right ventricular papillary muscle from rabbits (2–3 kg) in relaxing solution (7 mM EGTA). The two ends of a fiber bundle were fastened and one end placed on a tension transducer and immersed in bathing solutions. The cells of the fiber bundle are permeable to ions and large molecules; this permits control of intracellular ionic composition, while at the same time, tension can be monitored. High [EGTA] (7 mM) was used to control free [Ca^{2+}] in the bathing medium; thus, small amounts of Ca^{2+} uptake or release from the SR would not affect the Ca^{2+}-activated tension development of the myofibers. Caffeine has been shown to release Ca^{2+} from the SR.[7] By using low [EGTA] (0.05 mM) and caffeine, a tension transient indicating the amount of Ca^{2+} release from the SR could be manifested.

The Ca^{2+} activation of the contractile proteins was accomplished by immersion of each fiber bundle sequentially in control (no enflurane), test (enflurane), and control solutions (fig. 1). Each control or test phase consisted of contracting the fiber bundle in a submaximum Ca^{2+}-activating solution (*p*Ca 5.6 to 5.0) and in a maximum Ca^{2+}-activating solution (*p*Ca 3.8) and relaxing the fiber bundle in a relaxing solution (*p*Ca

* Research Assistant Professor, Department of Anesthesiology.
† Associate Professor, Department of Physiology and Biophysics.
Received from the Departments of Anesthesiology and Physiology and Biophysics, School of Medicine, University of Washington, Seattle, Washington 98195. Accepted for publication November 23, 1979. This work was supported by grants HL 20754 from National Institutes of Health, in part by a Research Starter grant from Pharmaceutical Manufacturer Associations and by the Ohio Medical Products, Inc., Madison, Wisconsin. Presented in part at the meeting of the American Society for Pharmacology and Experimental Therapeutics, Columbus, Ohio, 1977, and at the annual meeting of the American Society for Anesthesiologists, Chicago, October 1978.
Address reprint requests to Dr. Su.

0003-3022/80/0500/0385 $00.75

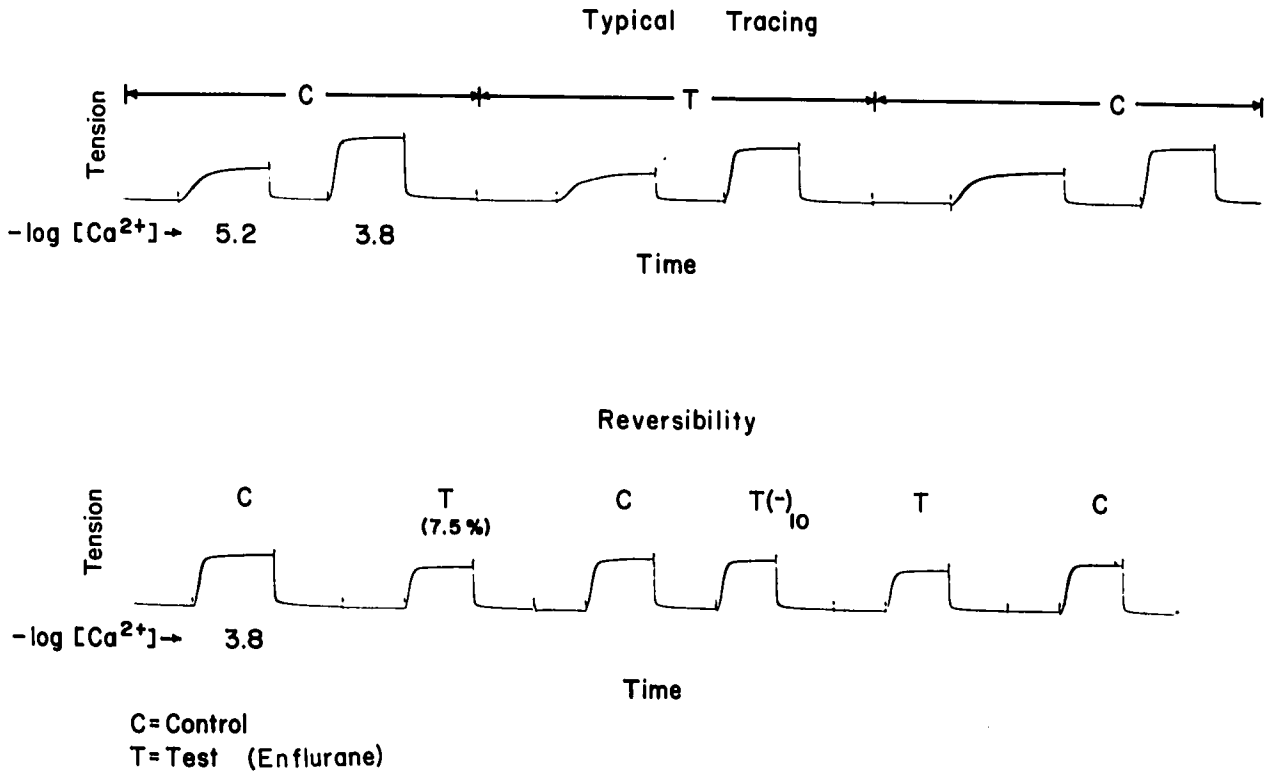

FIG. 1. Typical tracings for Ca^{2+}-activated tension development and reversibility of enflurane-induced depression. *Upper tracings*: each preparation was immersed in a submaximum [Ca^{2+}] (pCa = 5.2), a maximum [Ca^{2+}] (pCa = 3.8), and control solutions (C), followed by test solutions (T) and finally control solutions (C). *Lower tracings*: T(7.5 per cent) = test solution saturated with 7.5 per cent enflurane, $T(-)_{10}$ = T (7.5 per cent) solution was bubbled with pure N_2 for 10 min for removal of enflurane.

> 9) between contractions. The bathing solutions contained [Mg^{2+}], 1; [K^+], 70; [Mg ATP^{2-}], 2; [creatine phosphate^{2-}], 15; [EGTA total], 7 (in mM); imidazole, propionate (major anion) and [Ca^{2+}] (expressed as $-\log [Ca^{2+}] = pCa$ = 3.8, 5.0, 5.2, 5.4, 5.6, or >9). Ionic strength was adjusted to 0.15 and pH 7.00 (±0.02) by varying the amount of imidazole propionate at 20 C (±1). The method of preparing the solutions has been described.[4] Isometric tension developments from baseline (no tension) to steady state were compared between test conditions and the mean of the two bracketing control steady-state tensions for each preparation by t test for paired data.[8] The submaximum tensions generated with submaximum pCa were expressed as percentages of the maximum tension at pCa 3.8.[4]

The exact protocol for generation of the caffeine-induced tension transient was the same as that reported earlier.[5] Briefly, the fiber bundle was immersed sequentially in five different solutions: 1) to empty Ca^{2+} (relaxing solution and 25 mM caffeine), 2) to wash away caffeine (relaxing solution), 3) to load Ca^{2+} (pCa 6.5; EGTA, 7 mM), 4) to wash away the high EGTA concentration (pCa 6.5; EGTA, 0.05 mM), and finally 5) to release Ca^{2+} (caffeine, pCa 6.5; EGTA, 0.05 mM). A tension transient was produced that was used as a measure of Ca^{2+} release from the SR. The composition and preparation of the bathing solutions were essentially the same as those used for Ca^{2+} activation of the contractile proteins[4] with the exception of [Mg^{2+}], 0.1; [EGTA] total, 7 or 0.05 in mM; and 15 units/ml creatine phosphokinase and methansulfonate (major anion).

Each test measurement was bracketed with two control measurements. Three test measurements were carried out at each enflurane concentration by immersion of the fiber bundle in solutions equilibrated with specified enflurane concentrations: 1) from solutions 2 through 5 (uptake and release), 2) from solutions 2 through 4 (uptake only), and 3) solution 5 (release only). The saturation of enflurane in the bathing solutions for the above-mentioned studies has been de-

TABLE 1. Effects of Enflurane on Maximum Ca^{2+}-activated Tension Development (Mean ± SE)

	Enflurane, Per Cent		
	1 (n = 21)	2.5 (n = 17)	5.0 (n = 32)
Per cent of control	97 ± 1*	93 ± 1*	90 ± 1*

* $P < 0.05$ compared with control, t test for paired data.

scribed.[4] One set of solutions saturated with enflurane, regulated through a Verni-Trol® vaporizer, was diluted with N_2 and was used as the test solutions, and another set of solutions was saturated with 100 per cent N_2 for use as control solutions. The concentrations (partial pressure expressed as percentage of 1 atm) of enflurane in the solutions were assayed by gas chromatography.[9] The data were analyzed by comparison of the areas of the caffeine-induced tension transients by t test for paired data.

Results

Enflurane (1, 2.5, and 5.0 per cent) slightly but significantly decreased (3–10 per cent) the Ca^{2+}-activated tension at any Ca^{2+} concentration (submaximum and maximum [Ca^{2+}]), and the effect increased with concentration of enflurane (fig. 1; table 1). The enflurane-induced depression was reversible (fig. 1) when the fiber bundle was immersed in control solutions (C) after test solutions (T), irrespective of enflurane concentration tested. Complete recovery of the tension ($T(-)_{10}$) from enflurane depression occurred in test solutions, T (7.5 per cent), after nitrogen had been bubbled through the test solutions for removal of enflurane.

We calculated the data to percentages of the maximum tension (100 per cent for the maximum tension) to check the sensitivity of the Ca^{2+} activation of the regulatory proteins. There was no difference between control and enflurane-treated pCa–tension curves (fig. 2).

Enflurane, 2.5–7.5 per cent, markedly depressed the caffeine-induced tension transient in both uptake and release phases (30–85 per cent) (figs. 3 and 4, *open circles*) and in the uptake phase only (38–85 per cent) (fig. 4, *crosses*). The depression was dose-dependent and reversible. Enflurane, 2.5 or 5 per cent, did not change the 25-mM caffeine-induced tension transient and depressed (25 per cent) the tension transient

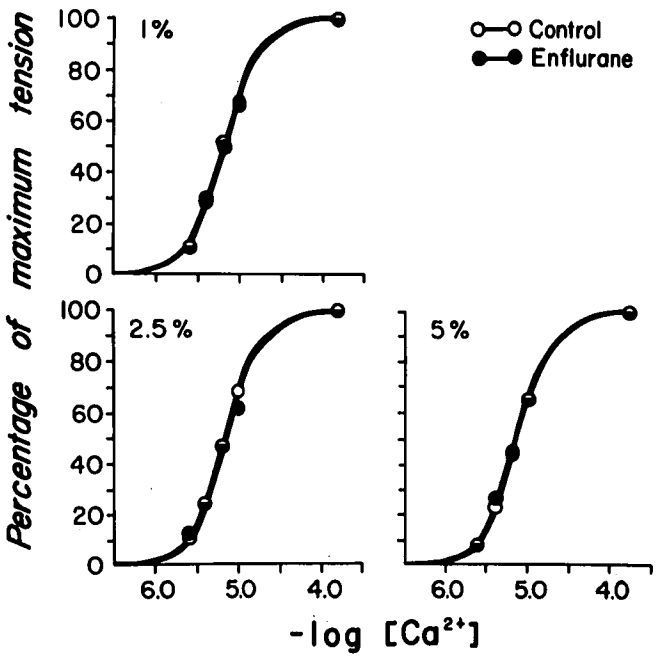

FIG. 2. Effects of various enflurane concentrations on the [Ca^{2+}]–tension (percentage of the maximum tension as 100 per cent) relationship.

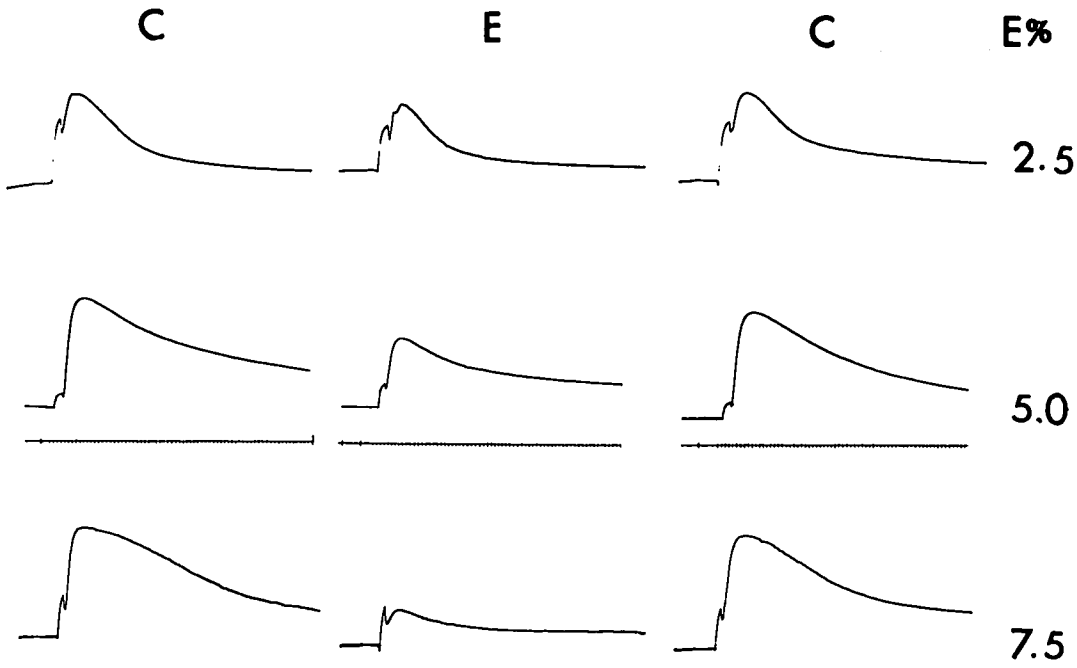

FIG. 3. Typical tracings showing the effects of various enflurane concentrations on caffeine-induced tension transient. C = control. E = enflurane in both uptake and release phases, E (per cent) = enflurane concentrations in per cent of partial pressure. Each row represents one preparation.

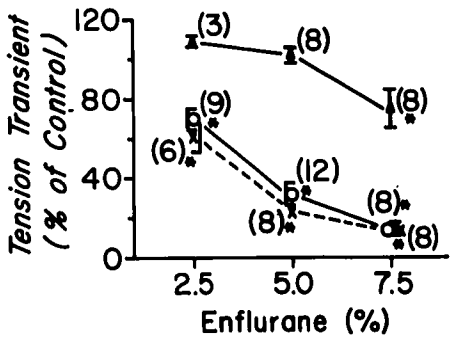

FIG. 4. Effects of various enflurane concentrations on caffeine-induced tension transient mean ± SE (n). O——O = uptake and release; ×---× = uptake only; ▲——▲ = release only. * $P < 0.01$ compared with control by t test for paired data.

at 7.5 per cent concentration in the release phase (fig. 4, *triangles*).

Discussion

We investigated the effects of enflurane on the intracellular structures involved in contraction, the contractile proteins and the SR, using functionally skinned myocardial cells from rabbits. Enflurane, 1–5 per cent, significantly but only slightly decreased the maximum Ca^{2+}-activated tension and did not change the submaximum Ca^{2+}-activated tension (table 1; fig. 1). The decrease in the maximum Ca^{2+}-activated tension could be interpreted as due to a decrease in either the number or the strength of the cross-bridges formed. One might expect changes in the actomyosin or myofibrillar ATPase activities to reflect an effect of enflurane. However, there is no information on the effects of enflurane on these enzymatic activities. The calculated submaximum Ca^{2+}-activated tension as percentage of the maximum tension (100 per cent) was not changed by enflurane, so that Ca^{2+} binding to the regulatory proteins is not affected (fig. 2).

The caffeine-induced tension transient is believed to be due to Ca^{2+} release from the SR rather than from the mitochondria or disrupted sarcolemma[5]; therefore, the decrease in areas under the tension transients by enflurane suggests an inhibition of Ca^{2+} uptake by the SR. The most interesting observation in this study was that enflurane markedly inhibited Ca^{2+} uptake by the SR (fig. 4), and the extent of depression was comparable to that observed in the isolated intact papillary muscle.[1]

This inhibition of Ca^{2+} uptake by the SR could be due to an inhibition of Ca^{2+} active transport (ATP-dependent) or an increase in passive Ca^{2+} permeability (leaky membrane) through the SR membrane, resulting in low Ca^{2+} in the SR. To test whether enflurane would enhance Ca^{2+} release from the SR (which could not be demonstrated in maximally released condition in the presence of 25 mM caffeine), we also used the submaximum caffeine concentration (2 mM). We found that increases in tension transients occurred (13–44 per cent) (fig. 5) in the Ca^{2+} release phase containing enflurane (1–7.5 per cent) so that it enhanced Ca^{2+} release or impaired Ca^{2+} uptake during Ca^{2+} release from the SR. However, no dose–response relationship (fig. 5) was found. Thus, the mechanism of enflurane-induced depression of Ca^{2+} uptake by the SR may not be as simple as suggested. An actual direct measurement of the amount of Ca^{2+} release from the SR would help to resolve the above speculations. It is not known how enflurane depresses the Ca^{2+} uptake in the isolated SR.

The reversibility (figs. 1 and 3) of the preparations indicates that time-dependent deterioration could not be responsible for the enflurane-induced depression in either Ca^{2+}-activated tension or caffeine-induced tension transients observed in this study.

The effects of enflurane on the intracellular sites are qualitatively similar to that of halothane,[5] but lesser in degree. At equal concentrations, the effects of enflurane on the maximum Ca^{2+}-activated tension and the Ca^{2+} uptake and release from the SR are only half those of halothane.[5] This finding is in agreement with observations in the isolated intact cat papillary muscle.[1] This is strong evidence that the SR is responsible for the myocardial depression. Brown and Crout[1] showed that at equal MAC values, enflurane

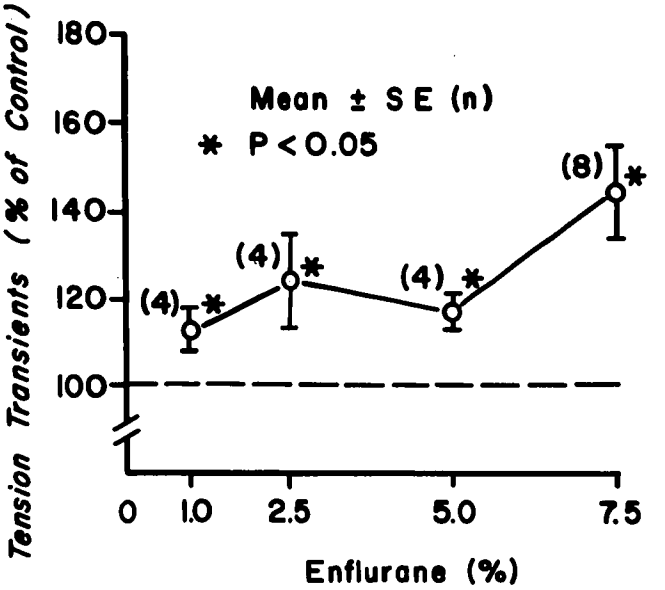

FIG. 5. Effects of various concentrations of enflurane at release phase only on 2 mM caffeine-induced tension transient.

depressed the isometric tension of the isolated intact papillary muscle to a slightly greater extent than halothane did. It is difficult to compare the inhibition of Ca^{2+} uptake by the SR between these two anesthetics with the same MAC values, since the MAC value for enflurane has not been tested in rabbits and there is a wider variation in MAC values for enflurane than for halothane among different animal species.[10]

In the isolated intact cardiac muscle preparation, the inhibition of Ca^{2+} uptake by the SR could cause the subsequent steady-state decrease in Ca^{2+} release from the SR, resulting in the decreased myocardial contractility.

In summary, enflurane, like halothane, slightly depressed the maximum Ca^{2+}-activated tension and markedly inhibited Ca^{2+} uptake by the SR in functionally skinned myocardial cells from rabbits. The depression was dose-dependent and reversible. We conclude that enflurane induces myocardial depression mainly by inhibiting Ca^{2+} uptake by the SR.

The authors thank Ms. Marina Cheung for excellent technical assistance.

References

1. Brown BR, Crout JR: A comparative study of the effects of five general anesthetics on myocardial contractility: I. Isometric conditions. ANESTHESIOLOGY 34:236–245, 1971
2. Weber A, Murray JM: Molecular control mechanisms in muscle contraction. Physiol Rev 53:612–673, 1973
3. Ebashi S: Excitation–contraction coupling. Annu Rev Physiol 38:293–313, 1976
4. Su JY, Kerrick WGL: Effects of halothane on Ca^{2+}-activated tension development in mechanically disrupted rabbit myocardial fibers. Pflügers Arch 375:111–117, 1978
5. Su JY, Kerrick WGL: Effects of halothane on caffeine-induced tension transients in functionally skinned myocardial fibers. Pflügers Arch 380:29–34, 1979
6. Kerrick WGL, Krasner B: Disruption of the sarcolemma of mammalian skeletal muscle fibers by homogenization. J Appl Physiol 39:1052–1055, 1975
7. Endo M: Calcium release from the sarcoplasmic reticulum. Physiol Rev 57:71–108, 1977
8. Snedecor GW, Cochran WG: Statistical Methods. Ames, Iowa, Iowa State University Press, 1967
9. Fink BR, Morikawa K: A simplified method for the measurement of volatile anesthetics in blood by gas chromatography. ANESTHESIOLOGY 32:451–455, 1970
10. Eger EI II: Anesthetic Uptake and Action. Baltimore, Maryland, Williams and Wilkins, 1974, pp 1–25