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Differential Depression of Myocardial Contractility by Halothane and Isoflurane In Vitro

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Depressant effects of halothane and isoflurane on isolated right ventricular guinea pig papillary muscle bathed in Tyrode's solution at 37° C were examined. Contractions were elicited by stimulation through external field electrodes while tension was recorded continuously and the intracellular cardiac action potential (AP) was monitored simultaneously by microelectrodes. The time differential of tension (dT/dt) and of membrane potential ($\dot{V}$) was determined electronically and recorded also. Contractions after rest and at stimulation rates of 0.1, 0.25, 0.5, 1, 2, and 3 Hz were studied. With normal APs, isoflurane (1.3 and 2.5%) depressed peak tension significantly less at high frequencies than did equivalent doses of halothane (0.75 or 1.5%). Isoflurane depressed dT/dt max less than halothane at all frequencies. At 0.3 Hz stimulation, isoflurane (1–4%) significantly increased the normal AP duration by 7–11%. Slow calcium-dependent APs and accompanying contractions were studied in partially depolarized muscles (−40 to −45 mV resting potential in 26mM K^+ Tyrode's solution) stimulated with 0.1 μM isoproterenol. Following rest and at 0.1, 0.25, 0.5, 1, 2, and 3 Hz, both isoflurane (1.3% or 2.5%) and enflurane (1.7% or 3.5%) markedly depressed the late-peaking slow AP contraction observed with low-frequency stimulation. Halothane (0.75% or 1.5%) caused a similar contractile depression (40–60%) at all frequencies. In contrast, isoflurane depressed early peaking tension and the dT/dt max at frequencies greater than 1 Hz significantly less than did halothane or enflurane. At 0.3 Hz, 2% and 4% isoflurane caused 9% and 17% depression of slow AP maximum rate of depolarization ($\dot{V}_{max}$), but significantly prolonged the AP duration. Isoflurane altered the pattern of tension development in a different manner than halothane, suggesting differing mechanisms of myocardial depression by these anesthetics. (Key words: Anesthetics, volatile: enflurane; halothane; isoflurane. Heart: action potential; contractility; papillary muscle; rate response.)

ALTHOUGH THE volatile anesthetics depress myocardial contractility in a dose-dependent manner,[1] the mechanisms responsible for this effect are not completely understood. High concentrations of halothane inhibit myofibrillar adenosine triphosphatase (ATPase),[2] but functional,[3–5] radioisotopic,[6] and electrophysiologic[7] studies suggest that the contractile depression arises in part from an inhibition of Ca^{++} entry into the myocardial cell. Studies at the subcellular level[8,9] as well as contractile studies[5,10,11] suggest that halothane and isoflurane alter uptake and release of Ca^{++} by myocardial sarcoplasmic reticulum. Studies in humans and animals suggest that isoflurane may depress myocardial contractility[12–14] less than halothane, although assessment of contractility *in vivo* is difficult, and the observed changes in myocardial performance could represent alterations in the peripheral circulation. A previous *in vitro* study[15] suggests that, at equivalent anesthetic concentrations, isoflurane depresses contractility to the same degree as halothane.

Myocardial contractions ultimately represent Ca^{++} binding to, and subsequent activation of, myofibrils. However, the contributions of the various cellular mechanisms for calcium delivery (slow channel Ca^{++} entry, Ca^{++}–Na^+ exchange, and sarcoplasmic reticular uptake and release) to the myocardial contraction are not completely defined at present.[17–20] Furthermore, the magnitude and pattern of tension development may be altered dramatically by different frequencies of stimulation and with different inotropic stimulations, reflecting these different routes of Ca^{++} delivery to myofibrils. Therefore, the effects of halothane and isoflurane were compared in a variety of experimental settings that accentuate certain patterns of tension development associated with specific cellular mechanisms.

Methods

The heart was removed from anesthetized (diethyl ether) guinea pigs and quickly transferred to oxygenated Tyrode's solution at room temperature. Right ventricular papillary muscles (0.7 to 4 mg weight) were excised, the cut end was pinned to the base of a chamber, and the tendinous end was attached by a strut to a Grass FTO3 force transducer. Individual muscles were superfused with Tyrode's solution (concentrations in mM: Na, 143; K, 4.7; Cl, 128; Ca, 2.5; Mg, 2.0; SO_4, 2.0; HCO_3, 25; glucose, 11; EDTA, 0.1) at 37° C. This solution was circulated through the chamber (8 ml/min) from heated reservoirs through which 95% O_2/5% CO_2 was bubbled, maintaining *p*H at 7.45 ± 0.5. Muscle length was adjusted to the least resting tension that produced maximum active tension. Based on muscle length, weight, and density, the cross-sectional muscle area ranged from 0.33 to 1.02 mm^2. Preparations were equilibrated for 1 h during constant stimulation at rates of 0.5 to 1 Hz, with intermittent short periods of stimulation at 0.1 to 2 Hz to verify muscle integrity and to define performance.

The muscles were field-stimulated by stimuli of 0.5 ms duration through stainless steel electrodes placed along the wall of the chamber. For most experiments, intracellular potential was monitored by conventional 3 M® KCl filled microelectrodes attached to a WPI VF-1® pream-

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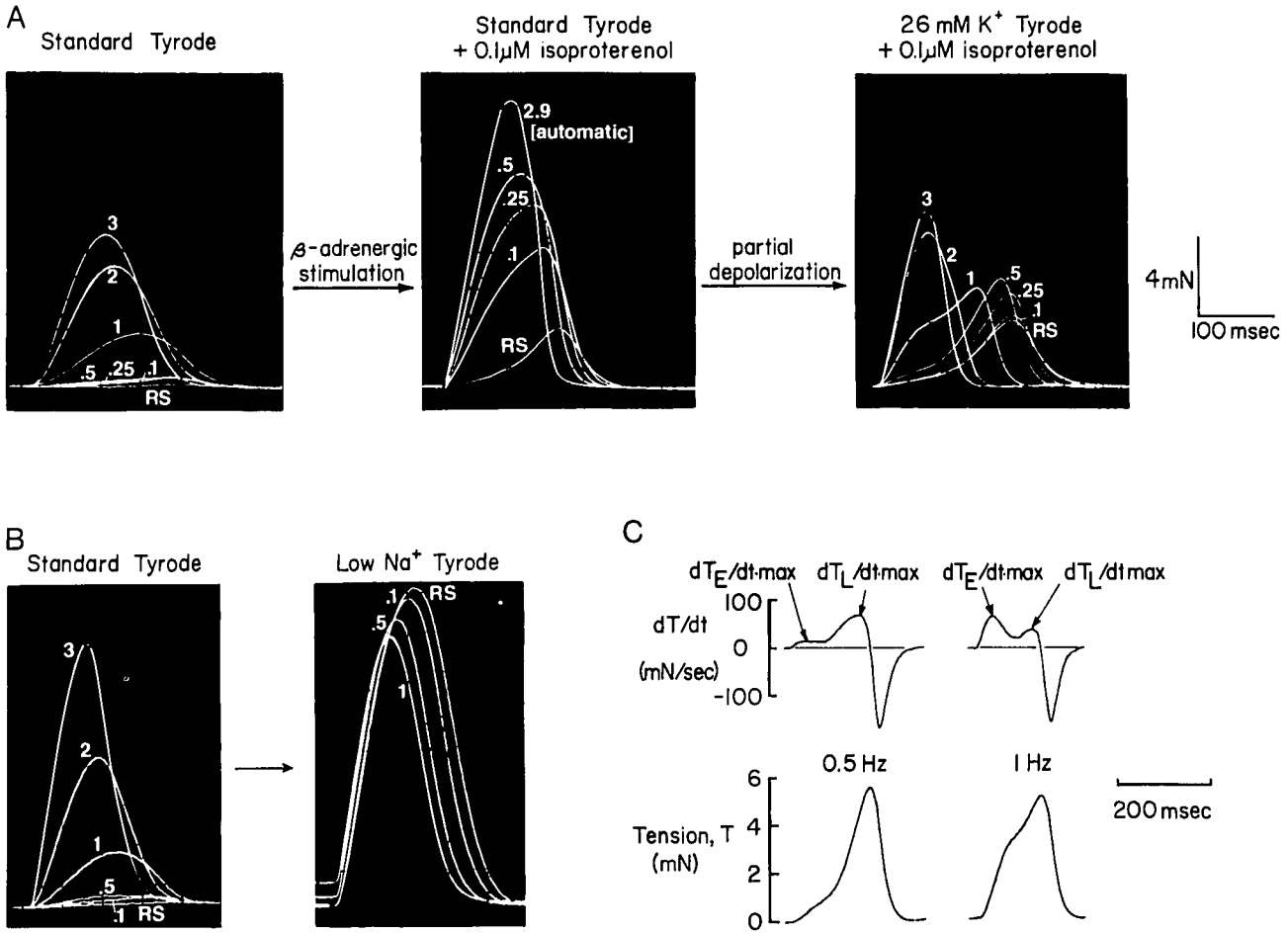

FIG. 1. *A.* The steady-state twitch responses observed at the frequencies indicated and also following rested state (RS). The left panel shows the typical pattern of frequency-dependent inotropy observed in the standard Tyrode's solution. The middle panel shows the contractile responses in standard Tyrode's with 0.1 μM isoproterenol. Note the marked enhancement of late tension development in the RS contractions and enhanced rate of initial tension development even at 0.1 Hz. Automaticity prevented recording of responses at frequencies greater than 0.5 Hz; the tension response during automatic rate of about 3 Hz is shown. The right panel demonstrates the twitch responses in the same muscle in 26 mM K Tyrode's solution with 0.1 μM isoproterenol, which produces propagated slow (calcium-dependent) action potentials. *B.* The steady-state twitch responses in a muscle at the various frequencies indicated, either in standard Tyrode's solution, or in 40 mM Na Tyrode's (with 200 mM sucrose to maintain isotonicity). Above 0.5 Hz the resting tension increased, and above 1 Hz a partial contracture developed, with deterioration of muscle performance. *C.* The tension recording *(lower tracings)* and the simultaneous time differential of tension, dT/dt *(upper tracings)*, shown for 0.5 and 1 Hz. The responses are the same as those noted at these stimulation frequencies in the right panel, part *A.* (26 mM K–isoproterenol). The maximum rate of early tension development (dT_E/dt max) and the maximum rate of development of the late-peak tension (dT_L/dt max) are indicated on the dT/dt tracing.

plifier. Bath potential, monitored through an agar bridge–Ag/AgCl electrode, was subtracted from the intracellular potential to give the membrane potential (V_m). Stimulation intensity was adjusted to provide a latency between the stimulus and the action potential (AP) of 5–10 ms; intensity was adjusted to maintain the same latency duration throughout. In the absence of intracellular recording, stimulus intensity was approximately twice threshold. Membrane potential, muscle tension, and the first derivative of each signal were displayed on a Tektronix D11 storage oscilloscope and were recorded on a Gould 2400 recorder and/or a Hewlett-Packard 3960 FM tape recorder for later playback and analysis. The maximum rate of depolarization of the AP ($\dot{V}_{max}$) was also recorded from the oscilloscope screen.

Muscles were studied under three different experimental conditions to characterize fully the effects of volatile anesthetics on myocardial contraction.

A. Standard Tyrode's Solution (normal action potentials). After 10–15 min rest, a rested state contraction (RSC) was elicited, followed by stimulation rates of 0.1, 0.25, 0.5, 1.0, 2.0, and 3 Hz, which were imposed sequentially. Each stimulation rate was maintained until steady-state contractile responses were present for five to ten contractions. Figure 1*A* *(left panel)* shows typical tension responses at each frequency for a muscle in standard Ty-

rode's solution. In all muscles, developed tension increased as the stimulation frequency increased (*i.e.*, a "positive staircase" response). There was excellent reproducibility of the frequency dependence after rest. Peak tension declined with stimulation at 2–3 Hz for more than 50 s, probably secondary to muscle fatigue and hypoxia in the core of the muscle, and prolonged stimulation resulted in permanent deterioration in peak tension. Brief (30–40 s) stimulation at 2–3 Hz produced reproducible peak tensions with little deterioration in peak contractile force over the 2–8-h course of an experiment.

The vigorous contractions at higher stimulation frequencies (2–3 Hz) frequently dislodged the intracellular electrode, preventing continuous recording from a single cell throughout the experiment. Because continuous recordings were necessary for analysis of AP characteristics, a series of experiments was performed with isoflurane with continuous 0.3 Hz stimulation, a stimulation frequency that allowed intracellular recording for prolonged periods and comparison with prior studies.[7,21]

Experiments were also attempted in standard Tyrode's solution (normal APs) with 0.1 μM isoproterenol stimulation of the muscles. The β-adrenergic stimulation increases: 1) Ca^{++} influx into the muscle by increasing the number of activatable slow channels so that more Ca^{++} enters with each depolarization,[22] and 2) internal Ca^{++} stores by increasing the ability of the myocardial sarcoplasmic reticulum to accumulate Ca^{++}.[23] Figure 1*A* (*middle panel*) shows the steady-state tension responses for the same muscle as that in the left panel (standard Tyrode's solution only), but with 0.1 μM isoproterenol stimulation. In the absence of isoproterenol, responses in the rested state and up to 0.5 Hz show very slow development of tension with a small late peak. With isoproterenol stimulation, the RSC shows the slow initial tension development observed in its absence, but there is an enhanced late maximum tension peak as previously described.[24] Automaticity occurred with stimulation frequencies above 0.5–1 Hz, preventing a complete study of frequency responses; therefore, detailed studies in this condition were not performed.

B. Tyrode's Solution (26 mM K^+) with Isoproterenol Stimulation (slow APs). If muscles treated with 0.1 μM isoproterenol are partially depolarized to −40 to −45 mV in 26 mM K^+ Tyrode's (K^+ substitution for the osmotic equivalent of Na^+), fast sodium channels become inactivated and propagated slow APs due to inward ionic flux through the slow (calcium) channel result. Slow APs were observed with typical $\dot{V}_{max}$ of 15 to 25 V/s, which is proportional to ionic flow through the slow (calcium) channel.[25] Enhanced, late tension peaks, identical to RSCs in standard Tyrode's with isoproterenol, occur (see fig. 1*A*, *right panel*) after rest and at low stimulation frequencies. This late peaking response is thought to be due to Ca^{++} that enters the myocyte with the initial depolarization, is taken up into the sarcoplasmic reticulum of the cell, and is then subsequently released near the end of the same AP depolarization.[26] The partial depolarization in 26 mM K^+ serves to accent this late peak by decreasing the enhanced early rate of tension development observed in standard Tyrode's with isoproterenol (see fig. 1*A*, *middle panel*) at low frequencies (0.1–0.5 Hz). As the stimulation frequency is increased to 2–3 Hz, the slow AP contractions show much more rapid early tension development with no initial delay, a pattern similar to that observed in standard Tyrode's solution at 2–3 Hz.

These slow AP contractions were studied from rest to stimulation frequencies of 3 Hz, in the same manner as described for the normal AP contractions. Due to the difficulty of maintaining intracellular impalements in muscles at higher stimulation frequencies, a series of slow AP experiments was also performed at 0.3 Hz, similar to those previously described for halothane[7] and enflurane.[21]

C. Tyrode's solution (40 mM Na^+). In contrast to the late-peaking response in partially depolarized, isoproterenol-treated muscles, an early-peaking contraction at low stimulation frequencies is elicited by superfusing the muscle with low sodium Tyrode's solution,[26] which in the present study contained (in mM): Na, 40; K, 4.7; Cl, 23; Ca, 2.5; Mg, 2.0; SO_4, 2.0; EDTA, 0.1; sucrose, 200 (to maintain osmolality). Figure 1*B* shows the tension responses in a muscle in normal Tyrode's and during exposure to low sodium Tyrode's (RSC to 1 Hz). Stimulation of muscles in low Na Tyrode's at frequencies of greater than 0.5 Hz resulted in large aftercontractions and increased resting tension. Contractures developed and muscle deterioration ensued at stimulation frequencies of 2–3 Hz. Therefore, stimulation frequency was typically maintained at 0.01 Hz, (similar to RSC responses) and 0.1 Hz. The vigorous contractions prevented stable intracellular recording.

Halothane, isoflurane, or enflurane was equilibrated with solution in one reservoir by passing the O_2/CO_2 through the appropriate calibrated, temperature-compensated vaporizer for at least 20 min before application to the preparation. Following control measurements, muscles were exposed for at least 15 min to the anesthetic-equilibrated solutions, which was sufficient time to produce stable effects in pilot experiments. An anesthetic potency ratio for halothane:isoflurane:enflurane was estimated as 1:1.7:2.3 based on relative MAC values in humans and dogs.[27] The concentration of anesthetic in solution was verified in periodic experiments by gas chromatography. Recovery responses were recorded at least 20 min after washout of anesthetic from the chamber. Identical stimulation programs were employed for control, anesthetic exposure, and recovery periods. After muscle responses recovered to control levels, a muscle was usually treated to the same or another anesthetic

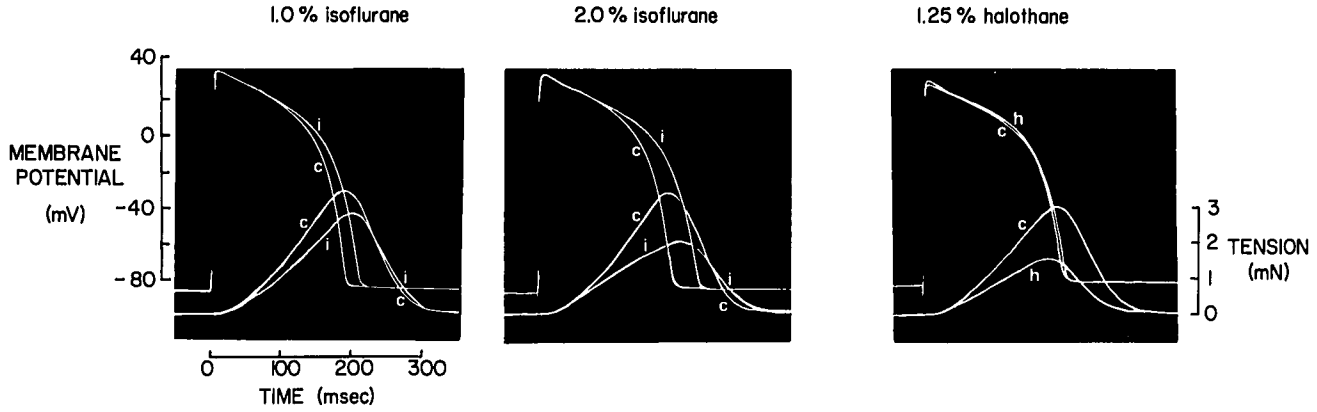

FIG. 2. Simultaneously recorded normal action potentials (AP) and twitch responses at 0.3 Hz in the same papillary muscle. The control responses (c) and during isoflurane (i) or halothane (h) exposure are shown. The AP responses throughout the control and isoflurane exposure periods were recorded from the same cellular impalement. The AP responses during the subsequent control period and halothane exposure were recorded from a closely adjacent region and demonstrated an identical $\dot{V}_{max}$.

agent. In the latter case, equivalent anesthetic concentrations of anesthetics could be compared in the same muscle. Results in four to seven muscle exposures were studied for each anesthetic in each condition and for each stimulation series (rest up to 3 Hz, or 0.3 Hz, see following). The sequence of anesthetic exposure was random, with no apparent carry-over of depressant effects.

The rate of tension development was determined in order to quantitate anesthetic effects on specific aspects of tension development. Figure 1*C* shows the tension and differential signal (dT/dt) at 0.5 and 1 Hz obtained under condition B. The maximum dT/dt observed early (dT_E/dt max) or late (dT_L/dt max) was measured during control, recovery, and anesthetic response for RSC up to 3 Hz. In experiments with only a simple, single period of tension development (standard Tyrode's or low sodium Tyrode's), only a single dT/dt max was quantified. In these muscles studied, the maximum tension response (standardized for cross-sectional area) at 2–3 Hz stimulation ranged from approximately 2–14 $mN \cdot mm^{-2}$.

Values of peak tension, dT/dt, and AP characteristics ($\dot{V}_{max}$, amplitude, and duration) during anesthetic exposure were expressed as a per cent of the control and recovery values. When the experimental responses were not measured at a time halfway between the control and recovery responses (average = 0.50 control + 0.50 recovery), the average was weighted according to the temporal proximity of the experimental responses to the preceding control and subsequent recovery responses. This was done to reduce possible errors caused by aging of the preparation, although aging effects amounted to less than a 10% change (increase or decrease) from control to recovery. Testing for significant differences ($P < 0.05$) among frequencies and among anesthetics was accomplished by an analysis of variance employing a general linear model and application of Duncan's multiple range test (SAS Institute, Inc., Box 8000, Cary, NC 27511). Anesthetic effects were compared with average control–recovery by Student's *t* test. In a paired study of halothane and isoflurane effects in the same muscle, differences were tested by paired *t* test.

Results

NORMAL AP EXPERIMENTS (CONDITION A)

Figure 2 shows the effects of 1% and 2% isoflurane on the AP and tension development in a papillary muscle stimulated at 0.3 Hz; the effect of an equivalent anesthetic concentration of halothane (approximately 1.7 MAC) is also shown. The late-peaking contractions are typical of those observed in normal Tyrode's at frequencies less than 1 Hz. Effects of isoflurane (1–4%) on normal APs and the accompanying peak tension at 0.3 Hz are listed in table 1. Isoflurane caused no significant change in the normal AP amplitude, $\dot{V}_{max}$, or membrane resting potential (87 ± 2 mV, mean ± SEM), but it significantly prolonged the AP duration. The peak tension was depressed in a dose-dependent manner by isoflurane.

The depressant effects of isoflurane (1.3% and 2.5%) or halothane (0.75% and 1.5%) on the peak tension at various stimulation frequencies are shown in figure 3. Halothane caused similar peak tension depression at all frequencies, and the dT/dt max was uniformly depressed to a similar degree (fig. 3, *right panel*). In contrast, isoflurane (open and filled triangles) depressed peak tension significantly less at greater stimulation frequencies than it did at low stimulation rates. At frequencies greater than 0.5 Hz, 1.3% isoflurane was significantly less depressant than 0.75% halothane; 2.5% isoflurane was less depressant

TABLE 1. Effects of Isoflurane on Contractions and Normal Action Potential (AP) Characteristics (at 0.3 Hz)

	n	Peak Tension	$\dot{V}_{max}$	AP Amplitude	AP Duration to 90% Repolarization
Isoflurane concentration (%)					
1	4	73 ± 2*	97 ± 2	99 ± 1	111 ± 0.5†
2	5	53 ± 4*	102 ± 2	100 ± 1	108 ± 2†
4	5	37 ± 4*	100 ± 1	99 ± 0.4	107 ± 2†
Control values ± SEM		0.16 ± .06 mN	145 ± 21 V/s	127 ± 2 mV	206 ± 11 ms

$\dot{V}_{max}$ = maximum rate of depolarization.
Results (mean ± SEM) expressed as per cent of control–recovery.

* $P < 0.01$ for difference from control by Student's t test.
† $P < 0.05$ for difference from control by Student's t test.

than 1.5% halothane at 3 Hz. Although the depression of peak tension at lesser frequencies (below 0.5 Hz) was not different for isoflurane and halothane, dT/dt max at all frequencies was depressed less by isoflurane than by halothane. In order for the rate of tension development to be depressed less than the peak tension, as with isoflurane, either the duration of tension development must be decreased or the pattern and rate of tension development must be altered by the anesthetic during the contractile cycle. The tension responses in figure 2 suggest that isoflurane caused depression of the tension development in a nonuniform manner. Although the early tension development was little altered, the late peak tension was reduced by 40% due to depression of the rate of tension development. The duration of the contraction was prolonged, with an increase in the time to peak tension. Effects at higher stimulation frequencies are shown in figure 4, which compares the depressant effect of 1 MAC halothane and isoflurane on the pattern of tension development at 0.5, 1, 2, and 3 Hz in the same muscle. At 2–3 Hz stimulation frequencies, tension developed rapidly with an early peak tension. Isoflurane, which has less effect on the rate of tension development than halothane, caused less depression of the early peaking tension at 2–3 Hz than did halothane. Isoflurane did cause a decreased duration of the tension response at 2–3 Hz, resulting in an apparent narrowing of the contraction profile. In experiments in which intracellular impalements could be maintained, no anesthetics depressed normal AP $\dot{V}_{max}$ at any stimulation frequency.

SLOW AP EXPERIMENTS (CONDITION B)

Table 2 gives the effects of isoflurane on the slow AP at 0.3 Hz, elicited in 26 mM K Tyrode's solution with 0.1 μM isoproterenol. As with the normal AP, the slow AP duration was increased while the amplitude was unaffected by isoflurane. While isoflurane depressed slow AP $\dot{V}_{max}$ and tension in a dose-dependent manner, tension was depressed to a much greater extent than was $\dot{V}_{max}$. When muscles (n = 5) in 26 mM K^+ Tyrode's were stimulated with 1.0 μM isoproterenol, 2% and 4% isoflurane depressed the slow AP $\dot{V}_{max}$ to 95 ± 1%(± SEM) and 90 ± 4% of control and tension to 56 ± 3% and 30 ± 4% of

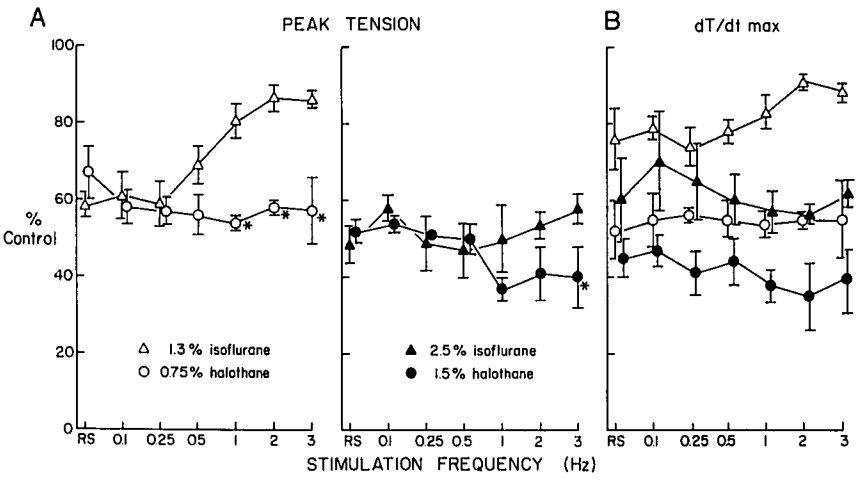

FIG. 3. Effects of approximately equivalent anesthetic concentrations of halothane and isoflurane on tension characteristics for twitches accompanying normal action potentials at the frequencies indicated. Results are expressed as the per cent change from the average control–recovery response and are the mean of four separate muscles studied. Error bars represent ± SEM. RS indicates rested state contraction. *A.* Frequency-dependent depression of peak tension by halothane and isoflurane. A significant ($P < 0.05$) difference between halothane and isoflurane depression was present as indicated by *. Isoflurane (1.3%) was also significantly less depressant at 1–3 Hz than it was for contractions from rested state to 0.25 Hz stimulation. *B.* Frequency-dependent effects on the maximum rate of tension development (dT/dt max). Isoflurane was significantly less depressant than the equivalent halothane concentration at all frequencies.

control, respectively. Thus, increased β-adrenergic stimulation of muscles decreased the sensitivity to isoflurane effects, but peak tension was again depressed far more than $\dot{V}_{max}$.

In five experiments, microelectrodes were maintained in the same cell through exposure to 4.0% isoflurane and 2.5% halothane (approximately 3 MAC) during 0.3 Hz stimulation. Results are listed in table 3. Halothane caused significantly greater depression of slow AP $\dot{V}_{max}$ than isoflurane, while at the same time causing less depression of the late-peak tension. Isoflurane also caused a significant increase in AP duration not seen with halothane.

In addition to different effects on membrane electrical behavior, these anesthetics differed in their effects on the pattern of tension development. Figure 5 shows the contractile responses in a single muscle at rested state up to 3 Hz in the control (C1) and recovery (C2, C3) settings and in the presence of 2.5% isoflurane or 1.5% halothane. Isoflurane markedly depressed and broadened the late tension peak at rested state (RS) to 1 Hz and increased the time to peak tension, while it caused only modest depression of the early peak tension at 2–3 Hz. Halothane depressed the rate of tension development and peak tension to a similar degree at all frequencies. Figure 6 shows the effect of enflurane (3.5%) on contractile responses from RS to 3 Hz on another muscle. In a manner similar to isoflurane, enflurane depressed the late tension peak and delayed the time to peak tension, broadening the tension response. However, at 2 and 3 Hz stimulation, enflurane markedly depressed the rate of tension development in a fashion similar to halothane.

Figure 7*A* plots the average depression of peak tension, the dT_E/dt max, and the dT_L/dt max by 1.5% halothane, 2.5% isoflurane, and 3.5% enflurane at the frequencies studied. Above 1 Hz, the late contractile component was obscured by the early tension development, and no value of dT_L/dt max was measured. Isoflurane and enflurane caused significantly more depression of late peak tension than halothane at frequencies below 2 Hz. Isoflurane depressed peak tension less than either halothane or enflurane at and above 2 Hz. Across the frequency range,

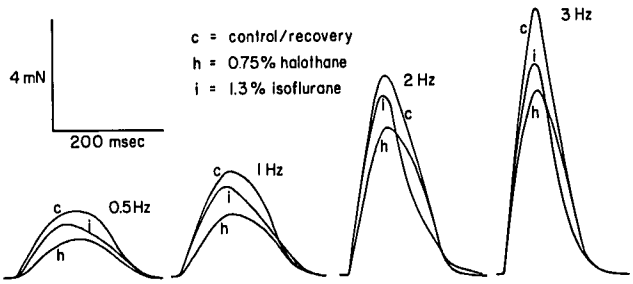

FIG. 4. Effects of 0.75% halothane or 1.3% isoflurane on the contractile response from a papillary muscle at 0.5, 1, 2, and 3 Hz stimulation. The control (c) tension traces demonstrated were recorded following recovery of the muscle from isoflurane (i) exposure and prior to halothane (h) exposure, and are superimposed to permit comparison.

2.5% isoflurane depressed dT_L/dt max more (to about 16% of control) and depressed dT_E/dt max less (to 80–90% of control) than did halothane, which decreased dT_E/dt max and dT_L/dt max by 40 to 60% at all frequencies. Figure 7*B* plots the average depression of contractile characteristics by 0.75% halothane, 1.3% isoflurane, and 1.7% enflurane. Although the contrast is less dramatic among these anesthetics at these lower concentrations, the same pattern exists. Halothane (0.75%) depressed peak tension, dT_E/dt max and dT_L/dt max to 60–70% of control values. In contrast, the peak tension at frequencies below 1 Hz were depressed more by 1.3% isoflurane than at 3 Hz. As observed with the higher concentration (≅ 2 MAC), dT_L/dt max was significantly more depressed by isoflurane. In its depressant effects on contractile characteristics, enflurane was between halothane and isoflurane.

In the presence of isoproterenol and partial depolarization, small amplitude (3–6 mV), late after-depolarizations (LADs) occur approximately 40 to 80 ms following the APs. LADs are frequently observed at 0.5–1 Hz and consistently seen at 2–3 Hz stimulation, accompanied by late aftercontractions (LACs), which are 1–4% of the amplitude of the preceding stimulated contraction. Such LADs and LACs also occur in the presence of high doses

TABLE 2. Effects of Isoflurane on Contractions and Slow Action Potential (AP) Characteristics (at 0.3 Hz)

	n	Peak Tension	$\dot{V}_{max}$	AP Amplitude	AP Duration to 90% repolarization
Isoflurane concentration (%)					
1	5	75 ± 1*	98 ± 0.7†	100 ± 0.7	108 ± 3†
2	5	47 ± 6*	93 ± 1*	99 ± 0.5	126 ± 5*
4	7	23 ± 1*	83 ± 2*	101 ± 2	120 ± 5*
Control values ± SEM		0.85 ± 0.35 mN	17.6 ± 5.3 V/s	80 ± 1 mV	172 ± 10 ms

$\dot{V}_{max}$ = maximum rate of depolarization.
Results (mean ± SEM) are expressed as per cent of control–recovery.

* $P < 0.01$ for difference from control by Student's t test.
† $P < 0.05$ for difference from control by Student's t test.

TABLE 3. Effects of 4% Isoflurane and 2.5% Halothane on Peak Tension and the Slow Action Potential (AP) Characteristics in the Same Muscles

	Peak Tension	$\dot{V}_{max}$	AP Duration	AP Amplitude
Halothane 2.5%	37 ± 4*	72 ± 3*	102 ± 4	99.5 ± 0.8
Isoflurane 4%	24 ± 1*†	84 ± 4*‡	125 ± 6*‡	100.5 ± 1.0
Control–recovery response (mean ± SEM)	2.6 ± 0.6 mN · mm^{-2}	23.5 ± 2.3 V/s	196 ± 9 ms	81 ± 1 mV

$\dot{V}_{max}$ = maximum rate of depolarization.

Results expressed as the per cent of the average control–recovery response.

Each value represents the mean of five muscles studied at 0.3 Hz in 26 mM K^+ Tyrode's with 0.1 μM isoproterenol. Three muscles were studied in the sequence: control, 4% isoflurane, recovery (control for subsequent anesthetic), 2.5% halothane, recovery; in two muscles the sequence was reversed. Recovery of tension was to >90% of control value, and recovery of $\dot{V}_{max}$ was to >97% of control. Intracellular impalements were maintained throughout exposure to both anesthetics.

* $P < 0.05$, significant change from control by Student's t test.

† $P < 0.05$, isoflurane effect significantly different from halothane effect by paired t test.

‡ $P < 0.02$, isoflurane effect significantly different from halothane effect by paired t test.

of digitalis or with increased Ca^{++} concentrations and have been implicated in causing certain cardiac dysrhythmias.[28,29] Such LADs and LACs were consistently depressed by isoflurane and halothane. Table 4 summarizes the effects on LACs of halothane and isoflurane. Both anesthetics reduced the response proportionally more than the preceding tension response.

Anesthetic Effects in Low Sodium (Condition C)

In contrast to the augmented late-peaking tension observed with β-adrenergic stimulation, low Na (40 mM) causes an early peaking, markedly augmented response, the RSC being 40–100 times larger than that observed

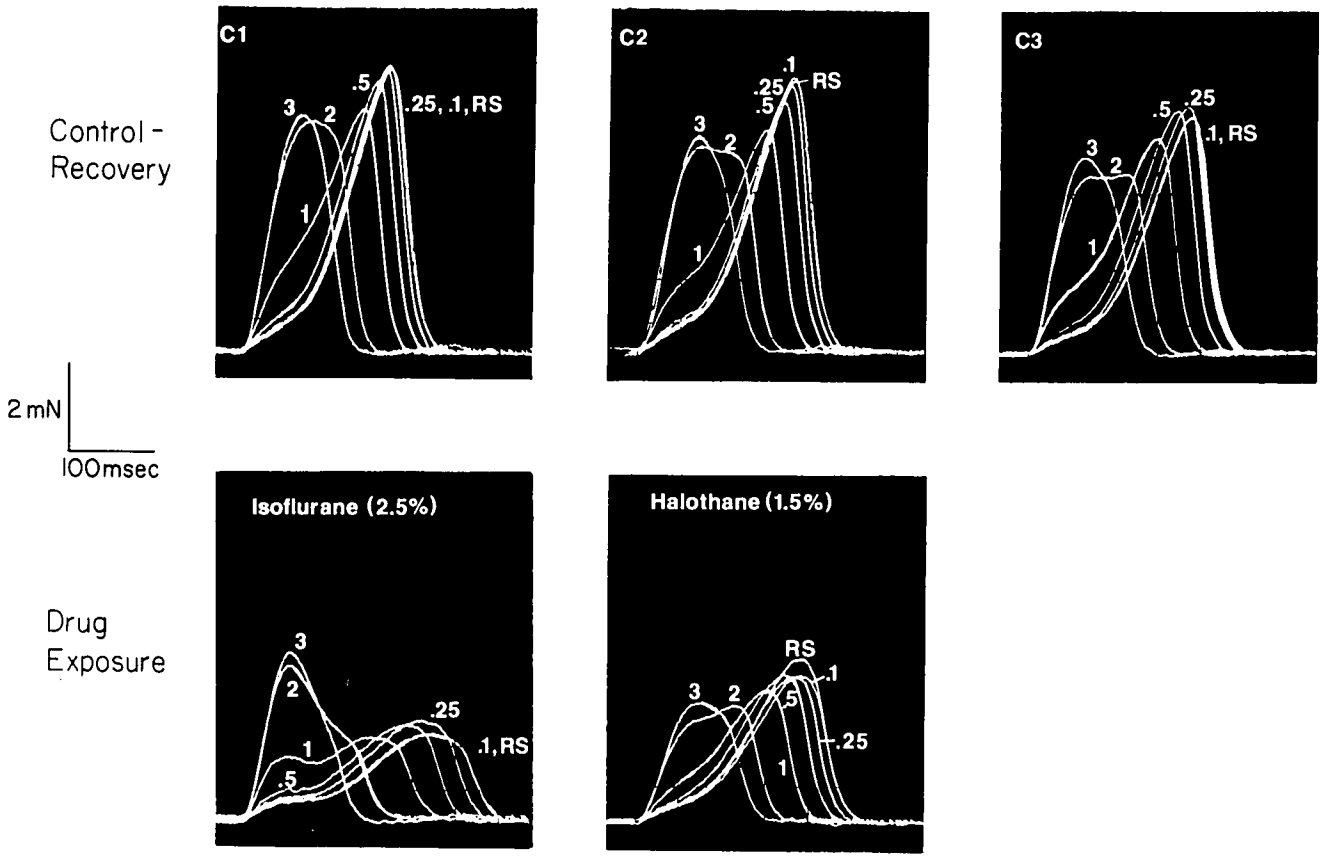

FIG. 5. Effects of approximately 2 MAC isoflurane and halothane on contractions accompanying slow action potentials at the frequencies indicated. All responses are from the same papillary muscle. The initial control response *(C1)* preceded the isoflurane exposure, followed by the subsequent recovery *(C2)* that preceded the halothane exposure and subsequent recovery *(C3)*. RS indicates rested-state contraction.

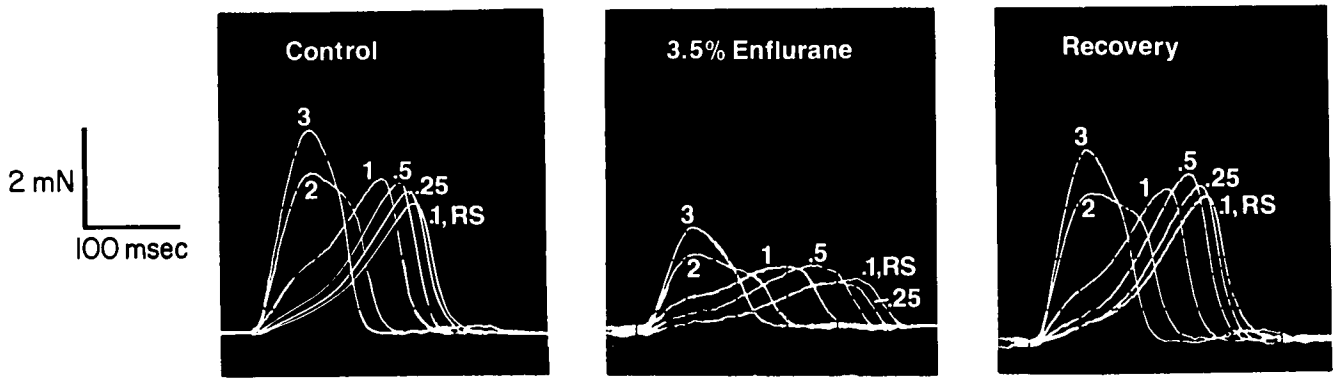

FIG. 6. Effects of approximately 2 MAC enflurane on slow AP contractions at the indicated frequencies.

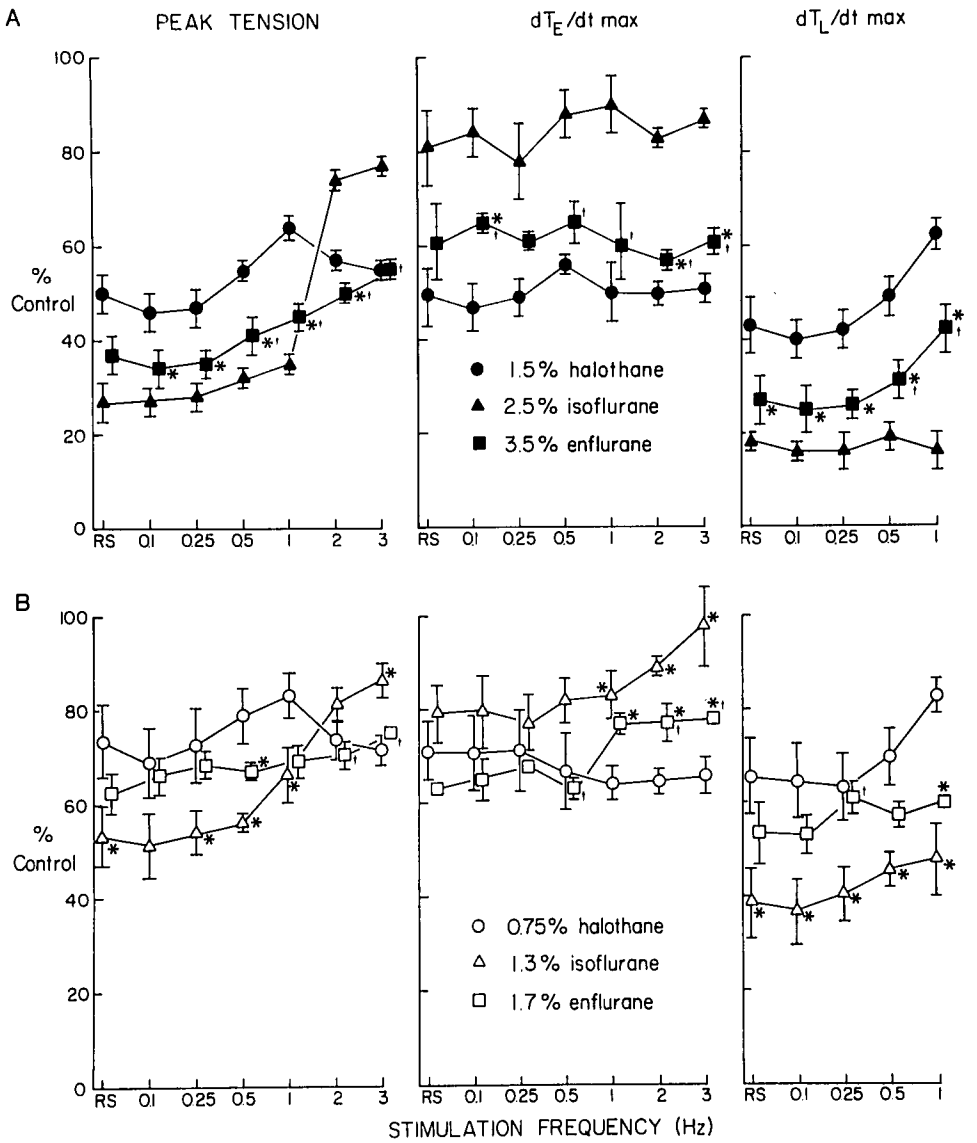

FIG. 7. Effects of equivalent anesthetic concentrations of halothane, isoflurane, and enflurane on the tension development accompanying slow action potentials at various frequencies. Peak tension, the rate of initial tension development (dT_E/dt max), and the rate of late tension development (dT_L/dt max) are expressed as per cent of the average control–recovery response. Each point represents the mean change observed in four (enflurane) or five (halothane) or six (isoflurane) muscles studied. *A.* Effects of approximately 2 MAC anesthetic concentrations. At every frequency, the effect of isoflurane differed significantly from that of halothane ($P < 0.05$, Duncan's multiple range). In the case of peak tension, isoflurane was more depressant from the rested-state contraction up to 1 Hz, but less depressant at 2 and 3 Hz. Isoflurane consistently depressed dT_E/dt max less and dT_L/dt max more than did halothane. Significantly different effects (Duncan's multiple range test, $P < 0.05$) of enflurane from halothane or isoflurane is indicated by * and †, respectively. *B.* Effects of approximately 1 MAC anesthetic concentrations. Significance (Duncan's multiple range, $P < 0.05$) is indicated for: differences between halothane and either isoflurane or enflurane (*); difference between enflurane and isoflurane (†).

TABLE 4. Anesthetic effects on late aftercontractions (at 2 Hz)

Anesthetic Concentration (%)	n	Peak Tension (per cent control)
Halothane		
0.75	4	43 ± 8
1.5	4	22 ± 7
Isoflurane		
1.3	5	41 ± 13
2.5	5	34 ± 11

All values (means ± SEM) were significantly less than control, but not different from each other. Experiments were performed in partially depolarized (26 mM K^+ Tyrode's), isoproterenol (10^{-7} M) stimulated guinea pig papillary muscle. Late aftercontractions typically were about 5% of peak tension observed.

in normal (143 mM Na^+) Tyrode's. Table 5 summarizes the depressant effects of approximately 2 MAC isoflurane, enflurane, and halothane on low sodium contractions at 0.01 and 0.1 Hz. Peak tension and dT/dt were depressed to a similar degree. Both halothane and enflurane caused significantly greater reversible depression than did isoflurane.

Discussion

The depressant actions of halothane and isoflurane on myocardial contractility and electrophysiologic behavior clearly are not identical. Differences can be appreciated even in the study of contractions that accompany normal APs. Whereas halothane depressed peak tension uniformly at all frequencies, isoflurane clearly became less depressant as the stimulation rate was increased to greater, more physiologically relevant frequencies. More subtle differences between these anesthetics are also evident in their effects on the pattern of tension development.

In a previous *in vitro* study, Kemmotsu *et al.*[15] found that the peak force generated by cat papillary muscle stimulated at a rate of 0.2 Hz was depressed to approximately 60% of control by 1 MAC of either isoflurane or halothane; results that are consistent with those observed in the present study at 0.1–0.25 Hz stimulation frequencies. However, in this study stimulation at physiologic frequencies (2–3 Hz) demonstrated a difference in the depressant effects of these anesthetics. Studies that assessed myocardial contractility in animals[13,14] or humans[12] suggested that isoflurane was less depressant than halothane. Horan *et al.*[13] found less depression of canine left ventricular maximum rate of pressure development (dP/dt) by isoflurane than by an equivalent anesthetic concentration of halothane. Merin[14] found similar depression of contractility by equivalent anesthetic levels of halothane and isoflurane, but isoflurane did not significantly increase the left ventricular end diastolic pressure, whereas halothane did. While β-adrenergic stimulation may account in part for the less profound depression of cardiovascular performance associated with isoflurane,[13,14] this present study suggests that the intrinsic depressant effects of these anesthetics differ at physiologic frequencies.

At low stimulation frequencies (≤1 Hz), the late peaking contraction has been attributed to influx of Ca^{++} from the external medium gradually accumulating in the cell.[30–32] More recently, Reiter *et al.*[18,26] have suggested that the Ca^{++} that enters the cell is accumulated in the sarcoplasmic reticulum (SR) during the initial half of the AP, and this Ca^{++} is subsequently released near the end of the AP causing the late peak of tension. This late-peaking contraction is enhanced by β-adrenergic stimulation, which increases Ca^{++} entry[22] as well as increasing Ca^{++} uptake and release by the SR.[23] The late-peaking tension is decreased indirectly by calcium entry blockers such as nifedipine, which decrease the amount of entering Ca^{++} available for accumulation and subsequent release by the SR.[26] The depression by halothane of slow AP $\dot{V}_{max}$, an effect interpreted as a depression of Ca^{++} influx through slow channels,[7,25] is approximately one-half as great as the depression of late peak tension. In a previous study,[7] 0.5% halothane depressed contractions in the absence of a significant reduction in slow AP $\dot{V}_{max}$, also suggesting that halothane may depress other mechanisms of Ca^{++} delivery, such as the SR.[8,10] Nevertheless, depression of Ca^{++} entry probably contributes largely to the contractile depression produced by halothane.

TABLE 5. Effects of Halothane, Isoflurane, and Enflurane on Peak Tension and dT/dt in Low Sodium Medium

Anesthetic	Control-Recovery Tension at 0.01 Hz (mN)	Peak Tension (per cent control)		dT/dt max (per cent control)	
		0.01*	0.1*	0.01*	0.1*
Halothane 1.5%	6.5 ± 2.8	66 ± 2	65 ± 2	67 ± 3	68 ± 2
Isoflurane 2.5%	7.4 ± 3.1	89 ± 3†	92 ± 2†	93 ± 3†	96 ± 2†
Enflurane 3.5%	5.8 ± 2.6	69 ± 3	69 ± 3	70 ± 5	70 ± 5

dT/dt max = maximum rate of tension development.

Values (mean ± SEM) are presented as per cent of average control-recovery response observed for each anesthetic in six different muscles. Depression by isoflurane was significantly (†Duncan's multiple range, $P < 0.05$) less than that for enflurane and halothane, which did not differ significantly.

* Stimulation frequency (Hz).

Like halothane and enflurane,[7,21] isoflurane depressed the rate of depolarization of the slow APs (and presumed slow channel Ca^{++} entry). However, the depression in $\dot{V}_{max}$ of the slow AP by isoflurane is small compared with the much greater depression of late peak tension. When studied in the same muscles, isoflurane depressed slow AP $\dot{V}_{max}$ 42% less than an equivalent anesthetic concentration of halothane; yet, isoflurane caused greater depression of late-peaking tension. The dissociation between $\dot{V}_{max}$ depression and the five-fold greater depression of late peak tension observed with isoflurane suggests that decreased Ca^{++} entry contributes little to the contractile depression produced by isoflurane. An alteration in myocardial SR uptake and/or release would explain the dramatic depression of late peak tension associated with isoflurane. The local anesthetics tetracaine and procaine, which inhibit the release of Ca^{++} from the SR of skeletal or cardiac muscle,[33,34] also cause selective depression of the late-peak tension.† Enflurane depressed and delayed the late peak contraction as did isoflurane, suggesting that enflurane may affect SR function also. The implied effect of isoflurane on SR function reported in the present study contrasts with the interpretation resulting from studies of skinned cardiac muscles. With that preparation, it was concluded that halothane caused greater alteration in SR uptake and release than did equivalent anesthetic concentrations of isoflurane.[10,11]

The greater potency of halothane as compared with isoflurane, in depressing the $\dot{V}_{max}$ of slow APs, correlates with the different actions of these compounds on atrioventricular (AV) conduction.[35] Conduction through the AV node, which is largely responsible for the atrial-His bundle (AH) interval, is mediated by Ca^{++} dependent slow APs. Atlee and Rusy[36] demonstrated that halothane slows AV conduction in dogs, with an increase in the A-H interval. In contrast, Blitt *et al.*[37] and Atlee[35] found that isoflurane caused little or no slowing of AV conduction and no increase in A-H interval in dogs.

A further difference in the effects of these anesthetics on electrophysiologic behavior is the prolongation of the AP observed with isoflurane, but not with halothane. Studies in Purkinje fibers suggest that the intracellular levels of Ca^{++} modulate changes in potassium conductance, which in large part determines AP duration.[38,39] The AP duration is prolonged by decreased intracellular Ca^{++} levels; AP duration is shortened by increased intracellular Ca^{++}. If an increase in intracellular activating Ca^{++} occurs later in the contractile response when isoflurane is present, due to delayed and depressed Ca^{++} release, the increase in potassium conductance may also be delayed. Such an effect might contribute to the delayed repolarization and prolongation of the AP observed with isoflurane in ventricular muscle in this study. It is noteworthy that prolongation of the AP by injection of depolarizing current has a positive inotropic effect,[40] whereas the isoflurane effect increasing AP duration at 0.3 Hz was associated with profound negative inotropy.

With greater frequencies of stimulation (2–3 Hz), these muscles show rapid early tension development, which must represent rapid delivery of activator Ca^{++} to the myofibrils. Such rapid tension development occurs even in the absence of β-adrenergic stimulation. The sources of activator Ca^{++} at these greater stimulation frequencies are not clearly defined, and a number of cellular mechanisms may be involved.[16–20,31,32] Halothane depresses the rapid early tension development and early peak tension more than isoflurane; halothane likewise causes greater depression of the slow AP $\dot{V}_{max}$ and presumed slow channel Ca^{++} entry than does isoflurane. Whether this apparent depression of slow channel behavior is responsible for depression of rapid tension development at these frequencies is not certain, however. For example, halothane and enflurane, which depress tension development at higher stimulation frequencies, also depress the low-Na^+-enhanced contractions. The rapid tension development and myofibrillar activation observed in low Na^+ medium (at low stimulation frequencies) is similar to that at 2–3 Hz stimulation with either normal or slow APs. Yet, these low-Na^+-enhanced contractions show little depression due to nifedipine and are, therefore, not primarily due to Ca^{++} entry through the slow channel.[18,26] Rather, altered function of the Na–Ca exchange, resulting in increased levels of free intracellular Ca^{++} are thought to occur with lowered external Na^+.[41] The Na–Ca exchange is thought to be important in eliminating Ca^{++} from the myocyte after a contraction; however, this is reversible and may be an important path of Ca^{++} entry.[41,42] Halothane and enflurane may alter this mechanism of Ca^{++} membrane transport, resulting in depressed contractions. Isoflurane, although very similar to enflurane structurally, depresses the low-Na^+-enhanced contraction and tension development at 2–3 Hz significantly less than does enflurane or halothane.

While the effects on tension development differ among the anesthetics, both halothane and isoflurane produced similar depressions of LADs and LACs. These phenomena are thought to be due to the cyclic release of internally stored Ca^{++} within the myocardial cell, which activates a transient, inward current and initiates subsequent depolarization.[28,29] Such depolarizations have been suggested as possible sources of ischemic dysrhythmias. Either depression of Ca^{++} entry by halothane or depression of internal release from the SR by isoflurane might be expected to prevent these LADs. Thus, the beneficial antifibrillatory effects observed with isoflurane and halo-

† Lynch C, unpublished observations.

thane in dogs[43] have at least two possible cellular mechanisms.

Certain conclusions may be made from this study that have far-reaching implications. Quite apparently, both *in vivo* and *in vitro* studies of anesthetic depression of myocardial function must be interpreted in light of changes in heart rate. The frequency-dependent depression produced by isoflurane, with decreased depression at physiologic frequencies, needs to be verified in hearts from other species and *in vivo* experiments. Although the three volatile anesthetics in this study represent simple halogenated hydrocarbons (isoflurane and enflurane being molecular methyl–ethyl ether isomers), they have distinctly different actions at the cellular level. Differing actions in the same tissue by such relatively simple molecules suggest that, while anesthetic incorporation into membrane lipids may contribute to their action, distinct and specific anesthetic interactions with certain protein systems that regulate cell functions are also important. Such specific anesthetic effects have implications for the study of anesthetic mechanisms in general.

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