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Halothane Depression of Myocardial Slow Action Potentials

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Effects of halothane on myocardial electrophysiologic and contractile properties were studied by simultaneous measurement of action potentials (APs) and contractions in guinea pig papillary muscle. Muscles were stimulated by field electrodes and normal responses measured before, during, and after recovery from halothane application. Halothane was administered in 0.5 per cent to 4 per cent concentrations in 5 per cent CO_2–95 per cent O_2 bubbled through standard Tyrode perfusing solutions. Slow action potentials were then induced with 10^{-7} M isoproterenol in partially depolarized muscles (typically −40 mV in 26 mM K^+ media). AP characteristics and accompanying contractions were again measured before, during, and after halothane application.

The maximum rate of rise ($+\dot{V}_{max}$) of the normal (fast) AP was not depressed in any concentration of halothane, although amplitude and duration were decreased in 3 per cent halothane. In contrast, halothane depressed $+\dot{V}_{max}$ of the slow AP to 61 per cent, 28 per cent, and 14 per cent of control, in concentrations of 1, 2 and 3%, respectively. Decreased duration and decreased amplitude (85% of control of the slow AP), or loss of excitability (4 of 7 muscles) occurred in 3 per cent halothane. Initially, halothane application caused a 5 per cent enhancement of tension with both fast and slow APs. In 0.5 per cent halothane, contractions subsequently declined to steady-state levels of 66 per cent (fast AP) and 76 per cent (slow AP) of control. Contractions were depressed linearly with log dose to 18 per cent (fast AP) and 5 per cent (slow AP) of control in 3 per cent halothane.

Halothane concentrations of 1 per cent and greater inhibit slow ($Na^+ - Ca^{++}$) channels which mediate the slow action potentials. The negative inotropic effect of halothane may be due in part to decreased Ca^{++} influx through the slow channel. The negative inotropic effect of 0.5 per cent halothane, in which the slow AP is unaffected, suggests that additional mechanisms, not involving the slow channel, also participate in the negative inotropic action of halothane. (Key words: Anesthetics, volatile: halothane. Heart: action potentials; calcium; cyclic AMP; contractility; myocardial depression.)

THE MECHANISM by which clinical concentrations of halothane depress myocardial contractility remains uncertain. Halothane was reported to decrease the activity of actomyosin ATPase[1,2] and myofibrillar tension production in disrupted fibers.[3] Other work suggested that the availability or delivery of calcium to the contractile proteins is decreased.[4–6]

It is now clear from voltage clamp studies that complex ionic flows are responsible for modulating the cardiac action potential. During the plateau of the action potential, there is an inward flux of Ca^{++} ions through a kinetically slow ion transport system (channel).[7] Blockade of this channel by specific blockers such as verapamil has a dramatic negative inotropic effect, but permits continued electrical activity with nearly normal action potentials.[8] Ca^{++} influx via the slow (Ca^{++}–Na^+) channels is in part responsible for the Ca^{++} which activates contractile activity during the cardiac action potential.[9] Previous workers have demonstrated a marked electromechanical dissociation (reduced contractions with normal action potentials) upon exposure to halothane.[10,11] Small changes in action potential plateau duration occurred, but were not implicated in the negative inotropism.

The purpose of the present study was to determine whether halothane exerts any effect on the slow channels, and consequently upon contraction. We have examined the effect of halothane upon the slow action potentials and accompanying contractions, and compared that to its effects upon the normal action potential and its contraction.

Methods

Guinea pigs (200–500 g) were stunned by a blow on the head and the heart was removed. Papillary muscles were dissected from the freshly excised hearts in oxygenated Tyrode solution. The cut end of the muscle was affixed with pins to the Sylgard® resin (Dow-Corning) base of a chamber (4-ml volume). The tendinous end of the muscle was connected to a Grass® FT03 force displacement transducer. Muscles were stimulated through 1 × 0.5 cm field electrodes positioned approximately 8 mm on either side of the preparation (see Appendix). Pulses of 1-ms duration (Grass® Model S5 stimulator) were applied at a frequency of 0.2 to 0.4 Hz (usually 0.3 Hz). Tyrode solution was circulated at a rate of 10 ml/min through the chamber. Most experiments were performed at 35–37°C, although a few experiments were performed at 32°C to enhance the contractile response; the halothane effects were not different at these two temperatures. In each experiment, temperature was controlled to ± 0.5°C.

Glass microelectrodes filled with 3 M KCl (resistance 8–20 MΩ), connected to a WPI Model 750 preamplifier, were employed to impale muscle fibers and monitor the transmembrane potential. Electrical responses were differentiated to obtain the rate of rise ($+\dot{V}$). Action potentials and their time differential were photographed from an oscilloscope. In addition, the electrical response

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was converted to digital form and analyzed online by a Challenger® II microprocessor computer (Ohio Scientific). The action potentials were analyzed for maximum rate of rise ($+\dot{V}_{max}$), amplitude, and time required for 10 per cent, 50 per cent, and 90 per cent repolarization from the peak amplitude. The $+\dot{V}_{max}$, amplitude, and 90 per cent duration of successive action potentials were sampled in groups of 7 to 10, and averaged at regular intervals during the course of experiments. Tension (P) and rate of force development (dP/dt) were continuously recorded on a strip chart recorder. In previous work[12] the dose-dependent depression of P and dP/dt by halothane was found to be parallel and nearly equal, therefore only tension was employed as a measure of contractile activation.

The normal Tyrode solution consisted (in mM) of 143 Na^+, 128 Cl^-, 4.7 K^+, 2.5 Ca^{++}, 1.2 Mg^{++}, 25 HCO_3^-, 11 glucose. Elevated potassium solution (26 mM) was prepared by isosmotic substitution of NaCl with KCl. A mixture of 95 per cent O_2–5 per cent CO_2 was bubbled through sintered glass into two reservoirs, one of which served as control (no halothane). The gas supply to the other reservoir passed in series through a Fluotec® 3 halothane vaporizer (Cyprane Ltd., England) so that known percentages of halothane were added to the gas bubbled through solution. The vaporizer calibration and halothane concentration of solutions perfused over the muscle were analyzed by gas chromatography. Halothane 1 per cent in 95 per cent O_2–5 per cent CO_2 yielded a solution concentration of 0.34 mM (66 mg/l), with a linear response verified to 3 per cent halothane. The halothane-containing gas was bubbled through the solution for a minimum of 30 min prior to experimental application. Gas chromatographic analysis showed equilibration to be complete within this period. The halothane-containing solution or control solution applied to the muscle could be rapidly interchanged (90 per cent chamber exhange time of 80 s).

Experiments were first performed upon muscles in normal Tyrode solution. Subsequently, the slow action potential responses were elicited from each muscle by placing it in increased potassium media (26 mM) which depolarized these muscles to approximately −40 mV, inactivating the fast sodium current responsible for the rapid depolarization of the normal action potential. Isoproterenol ($5–10 \times 10^{-8}$ M) was then applied to enhance the slow action potential response to electrical stimulation (0.8 mM EDTA was included to retard oxidation of isoproterenol). In two experiments, the response was activated by 2 mM theophylline in the presence of propranolol (5×10^{-6} M), instead of by isoproterenol. This was done in order to eliminate any possible halothane effect mediated by interaction with β-adrenergic receptors or by catecholamine release from nerve terminals.

In the presence of halothane, stimulation intensity and duration frequently had to be increased by 50 per cent or more to maintain the same latency period, and thereby eliminate any possible variation in $+\dot{V}_{max}$ caused by changing latency. In certain experiments, the Ca^{++} concentration was elevated by adding $CaCl_2$ directly to the perfusing media in order to determine the interaction with halothane. The resulting change in osmolarity was less than 3 per cent.

Each of 25 muscles was tested in three (occasionally two or four) different halothane concentrations (0.5, 1.0, 2.0, 3.0 per cent, and rarely 4 per cent) for both fast and slow responses. In initial experiments, halothane was applied in increasing concentrations, with recovery periods between. Subsequently, concentrations were applied in random sequence. No difference in dose-response was noted between these groups. Exposure to each concentration was maintained for 3–10 min beyond reaching steady-state depression of contractions (10–22 min). The recovery period following exposure was 35–60 min, with a stable restoration of the twitch response for 10–30 min. Only impalements of single cells which were maintained through the control, experimental, and recovery periods were included in the study of AP variables, with four to nine different preparations tested. Values during halothane were compared to the average control/recovery value by paired *t* test. Recovery was complete with regard to AP variables, whereas the contractile responses often showed a gradual decrease over the course of a 2 to 8 h experiment (with or without halothane exposure) of approximately 10 per cent per hour.

Results

Halothane Effects upon Normal Action Potentials and Contractions

The average resting potential in the myocardial cells studied was −91 ± 1 mV (mean ± SEM). No systematic change was noted with halothane application and an average resting potential identical to control was observed (−91 ± 1 mV).

As demonstrated previously by others,[12,13] halothane caused a dose-dependent depression of papillary muscle twitch tension. Although there was substantial variation among preparations in the degree of steady-state depression for a given halothane concentration, there was a linear relation between log-dose and effect over the range studied (fig. 1, open circles). Measurements of the action potentials (APs) in contracting muscles revealed little change up to halothane concentrations of 2 per cent. Beyond 2 per cent halothane, the normal AP was slightly decreased in amplitude, and narrowed, so that the plateau was shortened. The effects upon twitch tension and simultaneous AP variables are summarized in table 1.

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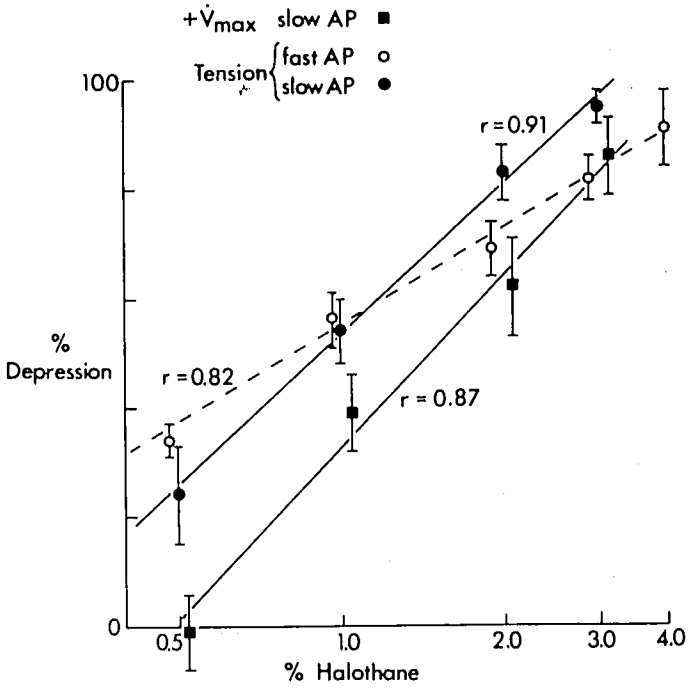

FIG. 1. Dose-response of tension and slow action potential $+\dot{V}_{max}$ to halothane. Values plotted are per cent depression of contractions accompanying normal APs (open circles), contractions accompanying slow APs (closed circles), and $+\dot{V}_{max}$ of the slow AP (filled squares). $+\dot{V}_{max}$ for the normal AP was not depressed and is not plotted. Points represent the means ± SEM, and respective r values for log-linear regressions are indicated.

Figure 2 shows the changes in normal AP configuration recorded in two cells from one muscle. At 2 per cent halothane (fig. 2*A*), the action potential showed two distinct peaks and a decreased plateau duration. Occasionally, the plateau phase almost disappeared with continued administration of 3 per cent halothane (fig. 2*B*). These prominent AP changes appeared more gradually than the contractile depression. While contractions were reduced to very low levels in 2 and 3 per cent halothane, action potentials were always elicited. In this experiment, $+\dot{V}_{max}$ of the AP was reversibly increased. In one of three experiments in 4 per cent halothane, however, increased stimulus intensity and duration failed to cause a regenerative AP.

The time course of contractile depression and accompanying APs are shown in figure 3. The twitch response declined with a roughly exponential time course ($t_{1/2}$ = 85 s). Such an exponential decline was noted in all preparations, and the rate increased with increasing halothane concentration. The transient twitch enhancement seen in figure 3 was observed at all halothane concentrations studied in 68 per cent of the experiments. The average peak enhancement in all fibers studied was 5.5 ± 1.9 per cent (mean ± SEM, $P < 0.02$).

In three experiments, the external Ca^{++} concentration was increased from 2.5 mM to 5 mM in the presence of halothane contractile depression. The depressed contractions (14 per cent of control) were increased by 45 ± 7 per cent ($P < 0.05$). Removal of the halothane in the presence of elevated Ca^{++}, however, resulted in a restoration of tension to 170 ± 12 per cent ($P < 0.05$) of control. The action potential $+\dot{V}_{max}$ was slightly depressed in elevated Ca^{++}, both in the absence and presence of halothane. All variables returned to control values when standard Tyrode solution (2.5 mM Ca^{++}) was reintroduced.

Halothane Effects upon Slow Action Potentials and Accompanying Contractions

The average resting potential of the fibers in the elevated potassium solution was −41 ± 1 mV, and halothane caused no detectable change. Twitch tension accompanying the slow APs was usually over twice as great as that with normal APs. Halothane depressed contractions in response to slow action potentials with a linear response to log-dose (fig. 1, filled circles), as observed for fast action potential contractions.

Slow APs elicited from these partially depolarized papillary muscles had a mean $+\dot{V}_{max}$ of 14.4 ± 1.0

TABLE 1. Effects of Halothane on Contractions and Normal Action Potential Parameters

	n	Tension	$\dot{V}_{max}$	Amplitude	90 Per Cent Duration
Halothane Concentration (Per Cent)					
0.5	4	66 ± 3†	102 ± 5	99 ± 1	100 ± 1
1.0	6	44 ± 5†	97 ± 3	97 ± 2	99 ± 4
2.0	9	31 ± 5†	156 ± 45	97 ± 2	95 ± 4
3.0	8	18 ± 4†	124 ± 12	84 ± 3†	76 ± 8*
Control Values ± SEM		97 ± 13 mg	238 ± 14 V/s	125 ± 3 mV	208 ± 5 ms

Values in halothane expressed as per cent of control, mean ± SEM.
* $P < 0.05$ for difference from control by paired t statistic.
† $P < 0.01$.

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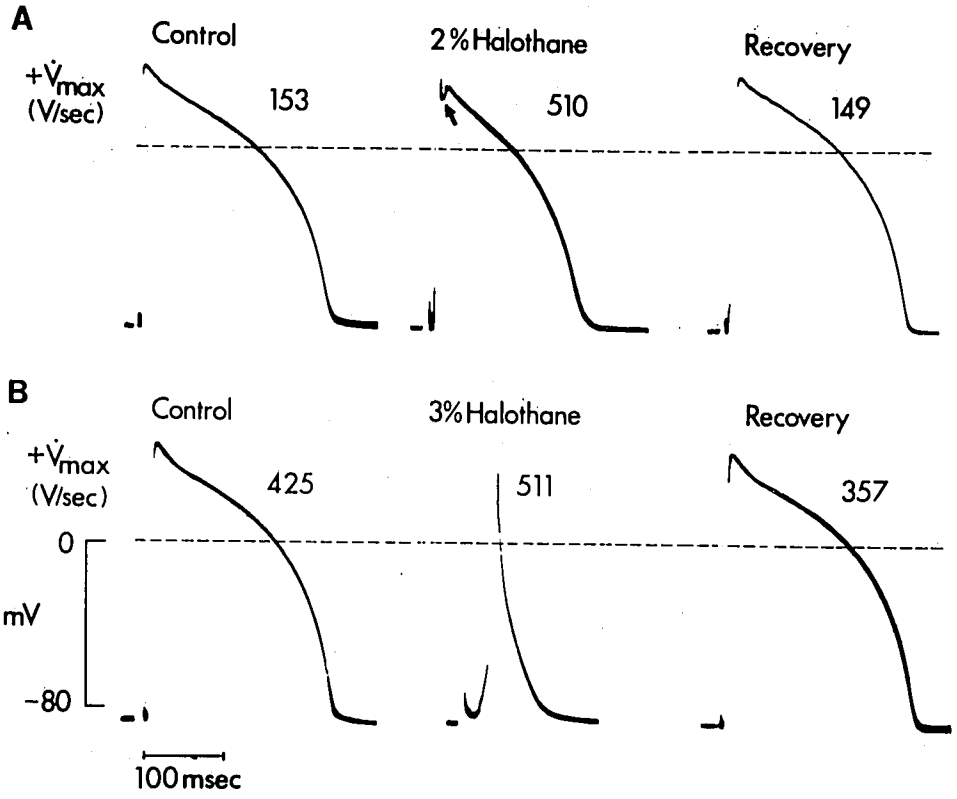

FIG. 2. Effects of 2 per cent and 3 per cent halothane on normal action potentials. The value of $+\dot{V}_{max}$ is given in the upper right corner of the respective action potentials. *A* and *B* represent experiments in two different cells in the same papillary muscle. *A*. 2 per cent halothane exposure. Note the development of a notch (indicated by arrow) and reduction of plateau after 10 min in the presence of halothane. Recovery AP was recorded after 5 min of halothane washout. *B*. 3 per cent halothane exposure. Note total abolition of plateau after 12 min of halothane exposure. Recovery AP recorded after 26 min of halothane washout.

V/s. In 0.5 per cent halothane there was no effect upon slow APs. Halothane concentrations ≥ 1 per cent, however, resulted in a dose-dependent decrease in $+\dot{V}_{max}$ which also had a linear relation to log-dose (fig. 1, squares). Typical effects of 1 per cent and 2 per cent halothane upon slow APs are shown in figure 4 (same muscle as in fig. 2). The AP shown in 2 per cent halothane was markedly depressed (fig. 4*B*). In four of seven

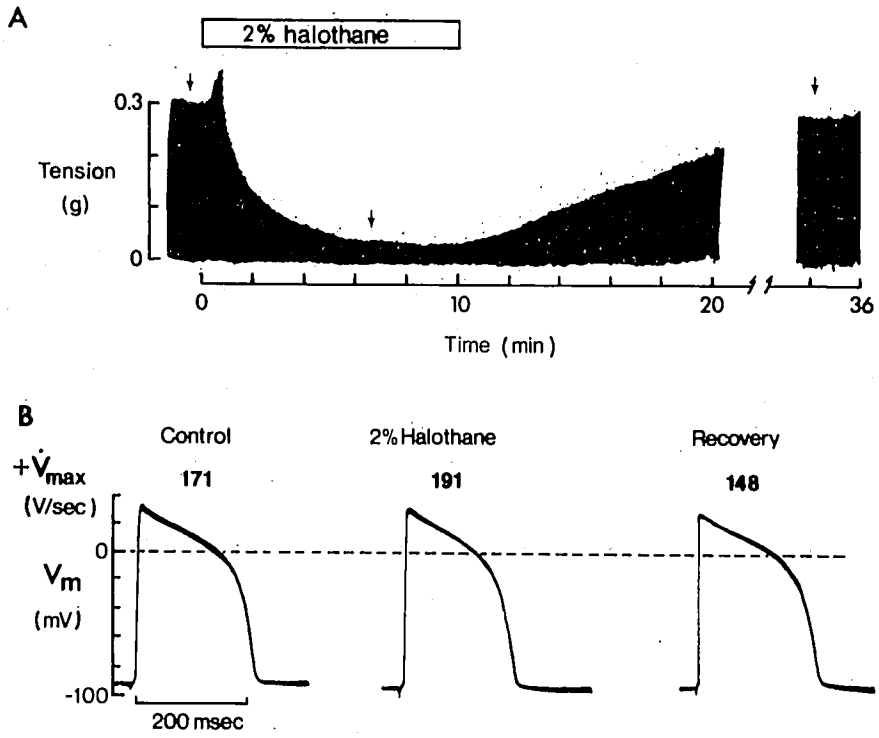

FIG. 3. Halothane effects upon twitch tension and normal action potentials. *A*. Tension trace is a continuous recording of contractions at 0.3 Hz stimulus frequency. The period of halothane exposure is denoted by the bar. *B*. Action potentials were recorded as indicated by the arrows above the tension trace. $+\dot{V}_{max}$ is noted above each action potential. AP amplitude is unchanged, while duration is reduced by 6 per cent in the presence of halothane.

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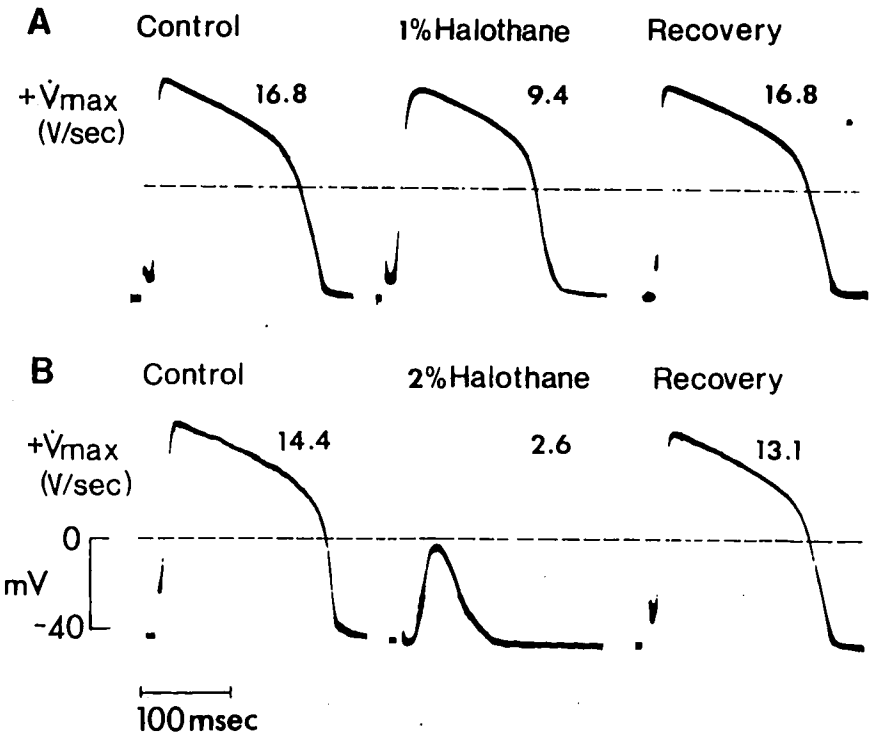

FIG. 4. Effects of 1 per cent and 2 per cent halothane on slow action potentials. The value of $+\dot{V}_{max}$ is given in the upper right corner of the respective action potential. *A* and *B* represent experiments in two different cells in the same papillary muscle, which was also studied in the experiments illustrated in figure 2. *A*. 1 per cent halothane exposure. Recovery AP was recorded after 9 min of halothane washout. *B*. 2 per cent halothane exposure. Recovery AP was recorded after 26 min of halothane washout.

cells studied, 3 per cent halothane prevented a regenerative AP from being elicited. Slow AP duration was significantly reduced at 2 and 3 per cent halothane, while a significant decrease in amplitude (to 85 ± 1 per cent of control, $P < 0.01$) was only noted at 3 per cent halothane (table 2).

As with the normal APs, a transient enhancement of contractions occurred during the first 5 to 10 twitches upon exposure to halothane. Although not present uniformly, the average peak twitch enhancement in all muscles studied was 4.5 ± 1.2 per cent ($P < 0.01$).

Figure 5 demonstrates the time courses of the effects of 2 per cent halothane on slow AP characteristics and twitch tension and their reversal by increased external Ca^{++}. It is apparent that the time course of the depression of twitch tension is more rapid than the effect upon slow AP characteristics. Increasing external Ca^{++} from 2.5 mM to 5.0 mM results in a similar time course of increase in $+\dot{V}_{max}$ and twitch tension. Subsequent removal of halothane results in $+\dot{V}_{max}$ and tension both surpassing their control values. Finally, return to normal Ca^{++} results in return to control values. The gradual increase in $+\dot{V}_{max}$ and contraction to control during recovery probably represents continued halothane washout. Thus, Ca^{++} not only reversed the twitch depression of halothane, it also dramatically corrected the depressed $+\dot{V}_{max}$ of the slow AP (in contrast to the lack of significant effect on $+\dot{V}_{max}$of the normal fast AP). The exponential time

TABLE 2. Effects of Halothane on Contractions and Slow Action Potential Parameters

	n	Tension	$\dot{V}_{max}$	Amplitude	90 Per Cent Duration
Halothane Concentration (Per Cent)					
0.5	4	76 ± 9	101 ± 7	99 ± 1	101 ± 0.4
1.0	7	46 ± 6†	61 ± 7†	97 ± 2	93 ± 4
2.0	8	17 ± 5†	38 ± 9†	79 ± 9	58 ± 11*
3.0	7	5 ± 3†	14 ± 7†	85 ± 1†	63 ± 5†
Control Values ± SEM		223 ± 10 mg	14.4 ± 1.0 V/s	82 ± 2 mV	147 ± 8 ms

Values in halothane expressed as per cent of control, mean ± SEM.
* $P < 0.05$ for difference from control by paired *t* statistic.
† $P < 0.01$.
‡ Four of seven experiments with loss of excitability (*i.e.*, action potential) not included in average.

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course of contractile depression with 2 per cent halothane application has a $t_{1/2}$ of 75 s, while the $t_{1/2}$ of the depression in $+\dot{V}_{max}$ was 275 s.

In six experiments in the presence of halothane (2 or 3 per cent), the depressed contractions (10 ± 3 per cent of control) accompanying slow APs were increased in 5 mM Ca^{++} by 3.8 ± 0.4 times ($P < 0.01$). After washout of halothane and recovery to steady state in 5 mM Ca^{++}, tension was 2.3 ± 0.2 times the control value in normal (2.5 mM) Ca^{++} ($P < 0.01$). There was significantly more enhancement ($P < 0.05$) in the presence of halothane. The slow AP $+\dot{V}_{max}$ showed an enhancement with increased Ca^{++} in the absence (20 ± 10 per cent, $P < 0.05$) and presence of halothane (170 ± 105 per cent, $P < 0.02$); however, the difference in the enhancement was not significant.

The effects of halothane upon tension and slow APs induced with 2 mM theophylline, in the presence of 5 × 10^{-6} M propranolol to provide β-adrenergic receptor blockade, were identical to those muscles stimulated with isoproterenol.

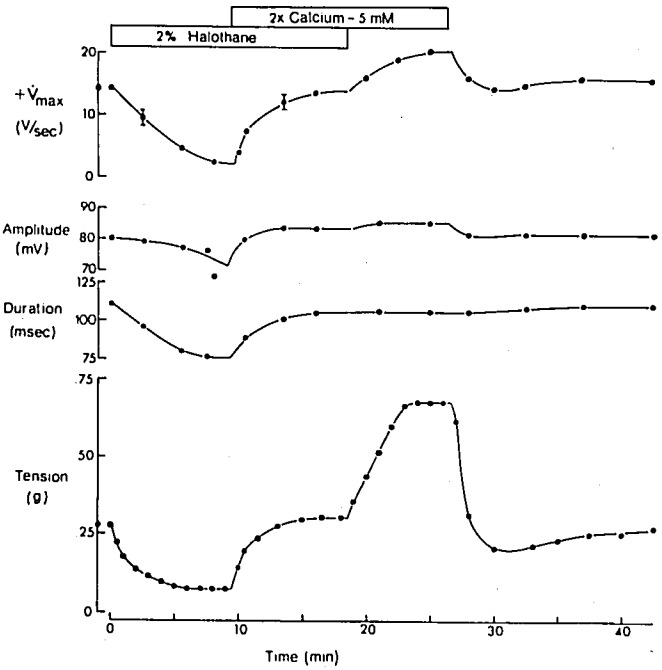

FIG. 5. Time courses of changes in slow action potential variables and tension of a papillary muscle preparation in response to halothane exposure and increased external calcium. Bars denote period of exposure to 2 per cent halothane and 2× external Ca^{++} (increased to 5.0 mM from control value of 2.5 mM). Points for $+\dot{V}_{max}$, amplitude and duration are averages of seven consecutive measurements at the time plotted. The SEM was within the size of the plotted solid circle except when $+\dot{V}_{max}$ was about 10 V/s. Error bars upon the $+\dot{V}_{max}$ values represent a greater variation in $+\dot{V}_{max}$ observed only near this value. See text for further details.

Discussion

The present results show that halothane produces a pronounced depression of the inward calcium current that enters the myocardial cell through the voltage-dependent slow channels, and that this may be a major mechanism by which halothane exerts its well-known myocardial depressant effect. Halothane concentrations of 1 per cent and greater caused a dose-dependent depression of a slow Na^+-Ca^{++} current, as demonstrated by reduction of the slow action potential rate of rise.

These effects of halothane were discerned by functionally removing the fast Na^+ current by partially depolarizing the myocardial cell to about −40 mV, employing 26 mM K^+ solution.[14-16] Although slow channels are unaffected by depolarizations to about −40 mV,[17] the cells become inexcitable since there is insufficient inward current to allow a regenerative AP. The number of active slow channels (which transmit both Ca^{++} and Na^+ ions)[16-18] in ventricular muscle can be increased by elevating the intracellular cyclic adenosine monophosphate (cAMP) level.[19-21] Catecholamines, histamine, methylxanthines, or exogenous cAMP sufficiently increase the number of active slow channels to permit a regenerative AP.[14,16,21] The induced action potentials resemble the plateau component of the normal fast action potential in shape and duration. However, the maximal upstroke velocity ($+\dot{V}_{max}$) of the rising phase (*i.e.*, phase 0) of the slow action potential is about 10 times slower than that of the normal fast AP, reflecting the comparatively lower density of the slow inward current. The $+\dot{V}_{max}$ of the slow AP is approximately proportional to the depolarizing current flow into the fiber.[22] At 3 per cent halothane, the slow AP usually could not be elicited, suggesting that a sufficient fraction of the slow channels had been inactivated so that a regenerative action potential was no longer possible. Consistent with present results on ventricular muscle, halothane caused a dose-dependent decrease in the rate of depolarization (phase 0)[23] and in action potential amplitude[24] in cells of the sinoatrial node. These cells have a slowly rising AP ($+\dot{V}_{max}$ less than 10 V/s) mediated by slow channels.[7]

The mechanism of halothane depression of slow channels is unknown. Halothane could decrease slow channel conductance by altering the fluidity of the sarcolemma,[25] and thereby affect the membrane lipoproteins presumably responsible for slow channel gating. However, if one assumes that alteration in fluidity would affect the gating of fast and slow channels equally, this mechanism cannot apply since fast sodium channel gating is unaffected. If halothane somehow decreases intracellular cAMP, then this could account for the decrease in slow channel conductance. For example, Gangat *et al.*[26] have reported that 2 per cent halothane decreased the isoproterenol activation of myocardial adenylate cyclase by

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about 30 per cent (however, when the substantial baseline activity is taken into account, the decline in activity was about 10 per cent). In two experiments in this study, halothane was effective in blocking slow APs which were induced with 2 mM theophlline in the presence of propranolol, suggesting β-adrenergic effects are not important. Further experiments are required to explore slow channel depression by halothane independently of β-adrenergic effects.

The dose-dependent decrease in twitch tension produced by normal APs as observed in our study is in agreement with what has been found previously. For example, Goldberg and Ullrick[12] found parallel dose-dependent reductions in peak tension (from 10 per cent to 53 per cent, for 0.1 per cent and 2.35 per cent halothane) and maximum rate of tension development of rat myocardial tissue, while the time to peak amplitude was relatively unaffected. Sugai *et al.*[13] found dose-dependent depression by halothane of force-velocity curves, shortening, power, and work performed. In our experiments, the halothane depression of twitch tension accompanying slow APs had a dose dependence not significantly different from that with normal APs. Although the muscle is partially depolarized in the slow AP situation, resting tension is unchanged and excitation-contraction (EC) coupling is not markedly altered.[14] The enhanced contractile response seen with slow APs is probably secondary to increases in contractility of myofibrils caused by catecholamines,[27] the altered accumulation of Ca^{++} by sarcoplasmic reticulum (SR),[28] and enhanced Ca^{++} influx through the increased number of active slow channels.[14,16,20] The similarity in halothane dose-response for the fast and slow AP situations indicates that the basic EC coupling mechanisms are similarly affected.

The depression of contractility by halothane has been attributed to a number of mechanisms. Merin *et al.*[1] and Price *et al.*[2] found dose-dependent depression of the calcium activation of myocardial actomyosin ATPase with halothane concentrations of 1 per cent and greater. Su and Kerrick[3] found that the calcium-activated tension response of mechanically disrupted myocardial fibers was depressed to 92 per cent and 75 per cent of control by 1 per cent and 4 per cent halothane, respectively. Although the depression of myocardial twitch tension at the same concentrations is much greater, the depression of myocardial tetanic contractions[4,5] is similar to that of the skinned fiber preparation.[3] For example, Rusy[5] found that 1.5 per cent halothane depressed twitch contraction of isolated rabbit heart to 22 per cent of control, while sustained myocardial contractions (3 Hz stimulus, in presence of 10 mM caffeine and 10 mM Ca^{++}) were only depressed to 82 per cent of control. It was suggested that the primary effect of halothane was to decrease Ca^{++} delivery during the normal twitch.

In addition to increasing intracellular cAMP by inhibiting phosphodiesterase, methylxanthines directly alter myocardial metabolism of Ca^{++}, probably by increasing Ca^{++} release from the SR.[29] Such an effect may account in part for the positive inotropism and delayed relaxation observed with these drugs, and permits a sustained contraction (tetanus) as observed by Rusy.[5] However, the 2 mM theophylline employed in the present study has been previously shown to cause only small enhancement of papillary muscle twitch.[30] Since in the present study, halothane depressed slow AP twitches stimulated by theophylline as well as by isoproterenol, a depressant mechanism distinct from that observed in isoproterenol stimulated muscle did not seem to be occurring.

The delivery of Ca^{++} to contractile proteins involve three major mechanisms (although there is some controversy over the relative contribution of each): 1) Ca^{++} influx through the slow channel, 2) Ca^{++} release from the SR, and 3) Ca^{++}-Na^{+} exchange across the sarcolemma.[9,31]

The Ca^{++} influx through the slow channel (in normal external Ca^{++} of 2.5 mM) has been estimated to provide up to 20 per cent of the Ca^{++} for myofibrillar activation.[32] With increased external Ca^{++}, and with cAMP activation of more slow channels, the influx of Ca^{++} will increase. In agreement with the results of Price,[4] we found it possible to partly reverse the depression of the contractile force in the presence of halothane by elevation of external Ca^{++}. The positive inotropism of Ca^{++} is paralleled by similar increases in slow action potential $+\dot{V}_{max}$ representing Ca^{++} influx. Thus, the reversal of halothane depression by increased external Ca^{++} is at least in part mediated by increased Ca^{++} influx through the slow channel. While figure 1 shows a clear correlation between depression of tension and $+\dot{V}_{max}$ of the slow AP (and hence Ca^{++} current), about 30 per cent of the twitch was depressed at a concentration of halothane (*i.e.*, 0.5 per cent) in which $+\dot{V}_{max}$ was unaffected. Furthermore, the time course of the contractile depression was more rapid than that for $+\dot{V}_{max}$ depression, thus partially dissociating the two effects. Therefore, while blockade of slow channels undoubtedly contributes to twitch depression at 1 per cent and greater halothane, this does not appear to be the primary mechanism for the effects at lower concentrations.

With regard to Ca^{++} release from the SR, Fabiato and Fabiato[33] have shown that increases of Ca^{++} in the medium of skinned myocardial cells causes release of Ca^{++} from the SR, suggesting influx of Ca^{++} during the AP may trigger Ca^{++} release. Consequently, depression by halothane of the slow channel Ca^{++} influx may affect Ca^{++} release from the SR. In addition, the transient tension enhancement observed in this study with initial

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application of halothane (while action potentials were unchanged) suggests a direct effect upon the SR. Ohnishi[34] found that 0.6 mM halothane (1.76 per cent) increased Ca^{++}-triggered release of Ca^{++} from isolated SR. Su and Kerrick[35] found that ≥ 1 per cent halothane caused an increased tension response in cardiac fibers lacking sarcolemma when stimulated by 2 mM caffeine for release of Ca^{++} from the SR (fibers were previously loaded with Ca^{++} in the absence of halothane). Such an effect certainly might contribute to enhanced twitch. They also found that when fibers were Ca^{++} loaded in the presence of halothane, the subsequent caffeine contractions were reduced, implying less Ca^{++} uptake by the SR. However, work on isolated SR by Lain *et al.*[36] found extremely high concentrations of halothane (7 mM) were required to depress Ca^{++} uptake of cardiac SR. More recently, Blanck and Thompson[37] found that halothane could either stimulate or depress Ca^{++} uptake by isolated myocardial SR vesicles, depending upon ATP concentration. Although reuptake is not consistently affected, if halothane enhances release of Ca^{++} from the SR, this would lead to Ca^{++} depletion of SR, with less eventually available from this source for contraction.[38]

Finally, extracellular Ca^{++} probably enters during the action potential plateau through Na^{+}-Ca^{++} exchange.[31,39,40] (During diastole, the exchange is reversed causing net calcium efflux and contributing to relaxation.) Porsius and Van Zwieten[6] described a decreased rate of Ca^{++} exchange in isolated rat atria in the presence of halothane. Such a decreased Ca^{++} exchange could reflect an effect upon Ca^{++} transport through the slow channel, but an effect upon Na^{+}-Ca^{++} exchange could also contribute.

The normal fast ventricular AP persisted in the presence of halothane in spite of marked reduction in contractile force. Although little change in the characteristics of atrial APs has been noted,[10,11] the ventricular AP of sheep showed a 32 per cent decline in duration and 48 per cent decline in overshoot potential with 2 per cent halothane; Purkinje fiber APs showed a similar reduction.[11] In the present study, significant reductions in the normal AP duration and amplitude became apparent at 3 per cent halothane, while $+\dot{V}_{max}$ was unaffected, indicating that halothane did not depress the fast Na^{+} current. Although we have no explanation for the effect, in some experiments $+\dot{V}_{max}$ was actually increased in the presence of halothane (*e.g.*, fig. 2). The reduction in the amplitude and duration of the AP plateau by halothane probably reflects reduced slow inward current, since this current contributes to the electrogenesis of the plateau.[7]

In summary, the present study demonstrated a depression of the myocardial slow inward current by halothane in concentrations used clinically, thus implicating yet another mechanism in the depressant effect of this anesthetic. Therefore, halothane could effect multiple steps in the excitation-contraction coupling process: 1) myofibrillar proteins, 2) Ca^{++} release by the sarcoplasmic reticulum, and 3) the function of the myocardial slow channel.

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Appendix

Differentiation of Action Potentials

The maximal upstroke velocity ($+\dot{V}_{max}$) of the rising phase of the action potential can be obtained by recording the first derivative of the action potential. This can be done using a resistance-capacitance (RC) circuit (time constant of 10^{-5} s for the normal fast action potential and 10^{-3} s for the slow action potential). The circuits for differentiation are calibrated by means of a ramp generator, and checked against photographs of the rising phase of the action potential taken at fast sweep speeds. $+\dot{V}_{max}$ is found from the peak excursion of the first derivative of the rising phase, and is expressed as volts per second. $+\dot{V}_{max}$ gives a relative measure of the intensity of the inward ionic current that flows during the action potential.[22] Alternately, the $+\dot{V}_{max}$ can be calculated by computer directly from the rising phase of the action potential after conversion from analog to digital format.

Field Stimulation

For electrical stimulation of the papillary muscles, rectangular current pulses are delivered by means of parallel platinum plate electrodes placed in the bath. The stimulating electrodes are positioned parallel to the long axis of the muscle and spans the entire length of the preparation, thereby exposing it to a uniform electric field.

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