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Actions of Halothane on the Electrical Activity of Purkinje Fibers Derived from Normal and Infarcted Canine Hearts

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The effects of 0.39 mM halothane (approx. 1.1 vol%) on the action potentials of proximal (false tendon) and distal (apical) left ventricular Purkinje fibers were compared in analogous *in vitro* preparations derived from normal dogs and animals surviving 1 day following acute myocardial infarction. In ten noninfarcted hearts, halothane reduced regional differences in repolarization by decreasing action potential duration (APD_{90}, mean ± SE) in proximal fibers from 300 ± 7 to 277 ± 6 msec ($P \leq 0.01$) without decreasing APD_{90} in distal fibers (control 240 ± 4 msec, halothane 249 ± 5 msec). In ten infarcted hearts, halothane accentuated pathologic differences in repolarization by decreasing APD_{90} in the non-ischemic proximal fibers from 311 ± 8 to 287 ± 7 msec ($P \leq 0.01$), while increasing APD_{90} in the ischemic distal fibers from 375 ± 15 to 406 ± 18 msec ($P \leq 0.01$). Halothane also decreased the overshoot from 32.9 ± 1.0 to 28.4 ± 0.8 mV ($P \leq 0.01$) and $\dot{V}_{max}$ from 356 ± 28 to 300 ± 22 V/s ($P \leq 0.05$) in ischemic fibers. In seven infarcts evaluated by extrastimulus techniques, halothane slowed the conduction of premature impulses and prolonged refractoriness, while, in five of the seven hearts, it reversibly increased the range of coupling intervals which induced probable reentrant responses. In a separate study of seven infarcts, halothane decreased the rate of spontaneous activity originating in the ischemic region. It is concluded that halothane facilitates the occurrence of re-entry while inhibiting the initiation of abnormal impulses in the *in vitro* canine infarction model. (Key words: Anesthetics, volatile: halothane. Heart: action potential; arrhythmias; ischemia.)

THE ELECTROPHYSIOLOGIC ACTIONS of volatile anesthetics on the ischemic heart are of interest because they may influence the occurrence of perioperative arrhythmias in patients with ischemic heart disease or recent myocardial infarction. Studies of the actions of halothane on the transmembrane potentials of non-ischemic ventricular tissues have demonstrated that this agent inhibits automaticity,[1] while abbreviating refractoriness and slowing conduction in a manner which may influence the occurrence of re-entrant arrhythmias.[2] However, no studies have examined the influence of halothane on the abnormal electrical activity of ischemic Purkinje fibers. The mechanisms underlying the occurrence of ischemia-related arrhythmias are often investigated in the canine infarction model of Harris.[3] The ventricular tachyarrhythmias which occur 24 h following coronary artery ligation may originate in subendocardial Purkinje fibers which survive in the infarcted region.[4] These variably depolarized ischemic fibers exhibit abnormal automaticity characterized by enhanced spontaneous diastolic depolarization, as well as oscillatory afterdepolarizations leading to triggered activity.[5,6] On the other hand, appropriately timed premature impulses may undergo conduction block in the ischemic region and propagate sufficiently slowly to permit circulating re-excitation.[7,8] The present study was designed to compare the actions of halothane on the electrical activity of Purkinje fibers derived from non-infarcted and 1-day-old infarcted canine hearts.

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Materials and Methods

Unconditioned mongrel dogs weighing 14–23 kg were anesthetized with intravenous thiopental (10–20 mg/kg), and halothane and the LAD coronay artery was ligated 1.5–2.5 cm from its origin.[3] The animals were given 1–2 mg/kg morphine sulfate for postoperative analgesia and allowed to recover for 22–26 h. Animals in whom an infarct was produced and non-operated control animals were anesthetized with halothane in oxygen and killed by cardiac excision. The left ventricle was rapidly dissected to yield a 4 by 5 cm tissue block,[5] which was mounted endocardial surface upwards in a 50-ml chamber. The tissues were superfused at 25 ml/min with 37° C Tyrode's solution equilibrated with 97% O_2/3% CO_2 (*p*H 7.25–7.35) and of the following composition (mM): NaCl 137, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 1.8, $NaHCO_3$ 12, and dextrose 5.5. Each preparation (fig. 1) included the anterior papillary muscle with its attached false tendon coursing from the basal interventricular septum and portions of the paraseptal free walls and left ventricular apex. The pale apical ischemic zone of infarcted hearts comprised about 60% of the preparations. The visible location of the border zone was confirmed in three hearts by injection of methylene blue dye into the prox-

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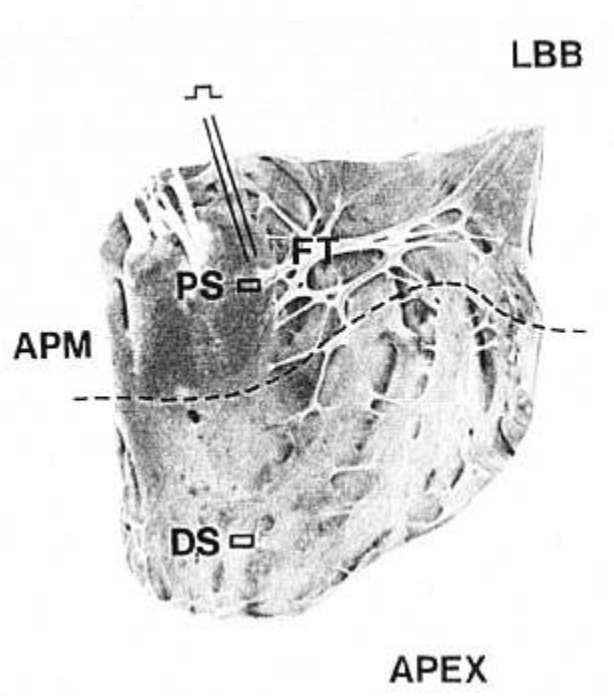

FIG. 1. One-day-old canine infarction preparation including the left bundle branch (LBB), false tendon (FT), and anterior papillary muscle (APM). The border between the non-ischemic and pale apical ischemic zones is indicated by a dashed line. PS, DS = proximal and distal recording sites; ⎍ = extracellular stimulation site.

imal coronary arteries, and histopathologic sections revealed that three to four cell layers of Purkinje fibers were present overlying the necrotic myocardium.

Action Potential Measurements

Following 1–2 h of equilibration action potentials were recorded from the first superficial subendocardial Purkinje fiber encountered utilizing glass microelectrodes (resistance 10–30 MOhms). The preparations were stimulated at 75 bpm using bipolar silver wire electrodes placed at the tip of the papillary muscle in most instances. The drive stimuli were pulses of 2X threshold intensity derived from a programmable digital stimulator.[9] The analogue signals were amplified (WPI M707), sampled utilizing an A/D converter (IS AI-13), and stored in 12-bit digital format in an Apple® II+ computer. The data were processed to yield the following action potential characteristics: in mV, the maximum diastolic potential (MDP), overshoot (OS), and amplitude (Amp); and, in msec, the action potential duration (APD) measured at the 50% and 90% repolarization levels (APD_{50}, APD_{90}). The maximum rate of phase 0 depolarization ($\dot{V}_{max}$) was measured in V/sec by differentiation of the upstroke potential sampled at a rate of 25 kHz.

Action potentials were recorded from ten consecutively impaled fibers located at two analogous 1 by 2 mm sites in each preparation.[10] The false tendon-papillary muscle junction was selected as a proximal site that was always located outside the ischemic zone of infarcted hearts. A distal site was selected 2–3 cm towards the apex from the proximal site, which was always within the ischemic zone of infarcted hearts. Purkinje fibers were identified and differentiated from ventricular muscle fibers by their action potential characteristics.[11] This differentiation was difficult in ischemic fibers due to the low membrane potential and prolonged APD. While most reports indicate that few myocardial fibers survive in the ischemic region,[5] we wished to limit this study to the action potential responses of recognizeable Purkinje fibers. Therefore, we excluded ischemic fibers exhibiting MDP less than 70 mV or loss of the characteristic overshoot of Purkinje fibers. These criteria are similar to those used by other investigators[10] to define a population of moderately depressed "fast response" fibers comprising about 70% of those surviving in the infarcted region.[5] The action potential data from ten fibers were pooled to develop values of the measured characteristics at each site under each condition.

The action potential responses to halothane were studied in ten infarcted and ten non-infarcted hearts. Following control observations, the anesthetic was introduced by switching to perfusate pre-equilibated with halothane by passing the O_2/CO_2 mixture through a calibrated vaporizer. At least 30 min was allowed for equilibration and 45 min for washout before final control measurements were made in experiments lasting about 7 h. There was about a 30% loss of anesthetic through the interconnecting tubing and at the surface of the bath. The equilibrium concentration of halothane in the bath, measured directly by gas chromatography (N = 4), was 0.39 ± 0.05 mM (approximately 1.1 vol%). Conduction of the drive impulses was evaluated during wash-in of halothane by simultaneously recording the phase 0 upstroke of fibers at both sites. The conduction velocity was derived from the measured distance and the conduction time for successful trials, in which both impalements were maintained for at least 15 min.

Arrhythmia Studies

The influence of 0.39 mM halothane on the conduction of premature impulses from the non-ischemic to the ischemic region was evaluated in seven infarcted hearts by an extrastimulus technique.[10] Trains of ten extracellular drive stimuli were applied and followed by an intracellular premature stimulus of variable coupling

TABLE 1. Effects of 0.39 mM Halothane on the Transmembrane Potentials of Subendocardial Purkinje Fibers in Non-infarcted and Infarcted Canine Hearts

	Action Potential Characteristics					
	MDP mV	OS mV	Amp mV	$\dot{V}_{max}$ V/s	APD_{50} msec	APD_{90} msec
Non-infarcted (N = 10)						
Proximal Recording Site						
Control	−82.1 ± 0.5	31.9 ± 1.1	114.1 ± 1.4	432 ± 27	249 ± 7	300 ± 7
Halothane	−81.5 ± 0.7	30.7 ± 0.9	111.8 ± 1.4	410 ± 23	203 ± 5†	277 ± 6†
Control	−79.9 ± 1.1†	31.8 ± 1.1	111.7 ± 1.6	403 ± 23	243 ± 7	301 ± 8
Distal Recording Site						
Control	−81.2 ± 0.7	33.3 ± 0.8	114.5 ± 0.8	401 ± 11	201 ± 5	240 ± 4
Halothane	−81.0 ± 0.9	31.2 ± 1.1	112.6 ± 1.3	415 ± 24	203 ± 6	249 ± 5
Control	−80.5 ± 1.2	33.2 ± 0.7	113.7 ± 1.3	439 ± 10	206 ± 6	251 ± 5
Infarcted (N = 10)						
Proximal Recording Site						
Control	−82.3 ± 0.7	32.7 ± 1.1	115.0 ± 3.4	417 ± 26	260 ± 8	311 ± 8
Halothane	−81.5 ± 1.0	31.4 ± 1.0	113.0 ± 1.4	401 ± 26	221 ± 7†	287 ± 7†
Control	−81.7 ± 0.7	30.9 ± 0.8	112.6 ± 1.2	400 ± 25	262 ± 8	321 ± 8
Distal Recording Site						
Control	−75.8 ± 1.1	32.9 ± 1.0	108.6 ± 1.6	356 ± 28	289 ± 12	375 ± 15
Halothane	−75.8 ± 1.7	28.4 ± 0.8†	104.2 ± 2.1	300 ± 22*	287 ± 9	406 ± 18†
Control	−77.1 ± 1.2	30.5 ± 1.7	107.6 ± 2.1	320 ± 20	298 ± 12	398 ± 16†

MDP = maximum diastolic potential; OS = overshoot; Amp = amplitude; $\dot{V}_{max}$ = maximum rate of phase 0 depolarization; APD_{50}, APD_{90} = action potential duration at 50% and 90% complete repolarization. Values shown represent the mean ± SEM for ten preparations of pooled values from ten fibers at each site under each condition.
* $P \leq 0.05$; †$P \leq 0.01$ compared to initial control (ANOV, range test).

interval and a subsequent pause of 2 s for observation of induced responses. The premature stimuli were depolarizing pulses 2 msec in duration of 2X threshold intensity delivered directly through a recording microelectrode located adjacent to the extracellular stimulating electrodes. Stimuli were applied to non-ischemic fibers located at the false tendon-papillary muscle junction in three of the initial ten infarcts, and to fibers located at the septal origin of the false tendon[8] in an additional four preparations. The onset of the intracellular stimulus artifact was used to demarcate the upstroke of the resulting action potential. In successive trains, the coupling interval was reduced until capture no longer occurred. The effective refractory period (ERP) was measured as the minimum interval between proximal responses at which the premature impulse conducted to the distal site. The functional refractory period (FRP) was measured as the minimum interval between responses obtained at the distal site.

The effect of halothane on spontaneous activity was evaluated in seven infarcted hearts. Following stabilization, two microelectrodes were used to confirm that the earliest activity originated within the ischemic region and at least one impalement was maintained at all times throughout subsequent interventions. The preparations were exposed to each of three progressively higher concentrations of halothane for 30 min, and 35–75 min was allowed for washout at each level. The bath concentrations obtained were (N = 7) 0.41 ± 0.03, 0.86 ± 0.06, and 1.22 ± 0.07 mM. The spontaneous rate was determined by counting the action potentials observed during the last 10 min under each condition. The actions of halothane on single automatic fibers were also investigated in a few small preparations (approx. 6 × 8 mm) dissected from the infarct[6] and mounted in a corner of the tissue bath. A mapping technique was used to locate the ischemic focus initiating spontaneous activity, and the action potentials were recorded continuously during exposure to 0.39 mM halothane.

The action potential characteristics obtained were evaluated by analysis of variance (ANOV), and Duncan's multiple range test was used to compare mean values obtained under different conditions.[12] The *t* test for non-independent samples was used to assess changes at a single recording site, and the values obtained at the different sites or in the different preparations were evaluated by Student's *t* test. A probability level of 0.05 or less was considered significant.

Results

Action Potential Characteristics

The action potential responses of fibers in each type of preparation are summarized in table 1. In non-infarcted hearts, the control action potentials at the two

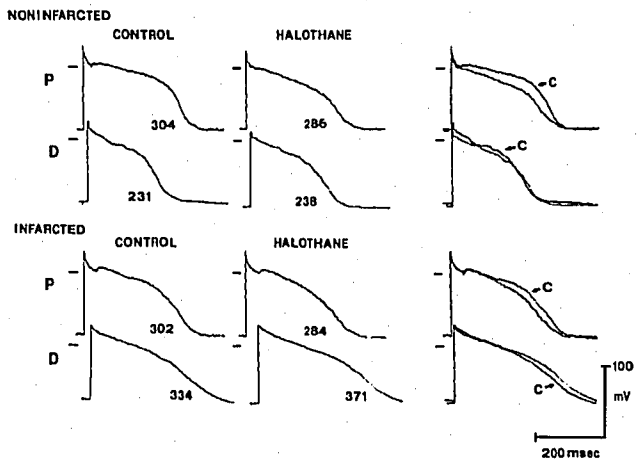

FIG. 2. Simultaneously recorded single proximal (P) and distal (D) Purkinje fiber action potentials from typical non-infarcted and infarcted preparations (75 bpm, Cycle Length 800 msec). APD_{90} is indicated in msec below each action potential, and the control action potential on the superimposed tracings to the right is labeled C. Exposure to 0.39 mM halothane abbreviated APD_{90} in the proximal fibers of both preparations, but prolonged APD_{90} in the ischemic distal fiber of the infarcted heart.

sites differed only in that APD_{50} and APD_{90} were longer ($P \leq 0.01$, Student's t test) in proximal fibers than in distal fibers. Exposure to 0.39 mM halothane reversibly decreased (ANOV, range test) APD_{50} and APD_{90} in the proximal fibers without altering repolarization of the distal fibers or changing any other measured characteristic. In infarcted hearts, the control characteristics of the non-ischemic proximal fibers did not differ from those of the proximal fibers of non-infarcted hearts. However, the ischemic distal fibers exhibited ($P \leq 0.01$) reduced membrane potential, action potential amplitude, and rate of phase 0 depolarization ($\dot{V}_{max}$), as well as markedly prolonged APD_{50} and APD_{90} relative to the analogous distal fibers of non-infarcted hearts. APD_{90} of the ischemic fibers increased by 23 ± 8 msec in the final control period, while the other characteristics did not show any time-dependent changes. Exposure of infarcted hearts to halothane reversibly decreased APD_{50} and APD_{90} in the proximal fibers, just as it did in non-infarcted hearts. On the other hand, the ischemic distal fibers exhibited decreased overshoot and $\dot{V}_{max}$, as well as prolongation of APD_{90} relative to the initial control values. However the final control mean values of the overshoot, V_{max} and APD_{90} did not differ significantly from those in the presence of halothane, indicating that its actions on ischemic fibers were not fully reversible. The decrease of the oveshoot in ischemic distal fibers on exposure to halothane (−4.5 ± 1.3 mV) was greater ($P \leq 0.05$) than the decrease occurring in the analogous distal fibers of non-infarcted hearts (−1.2 ± 0.7 mV), while the reduction of $\dot{V}_{max}$ (−56 ± 22 V/s) and increase of APD_{90} (31 ± 9 msec) only tended to be greater ($P \leq 0.10$) than that found at the distal site of non-infarcted hearts.

Figure 2 illustrates the difference between the actions of 0.39 mM halothane on repolarization of single proximal (P) and distal (D) Purkinje fibers in each type of preparation. Under control conditions in the non-infarcted preparation (left upper panel), APD_{90} of the proximal fiber exceeded that of the distal fiber. Exposure to halothane (center) reduced the difference between the action potential durations of the two fibers by decreasing APD_{90} in the proximal fiber. On the other hand, in the infarcted preparation (left lower panel), the control APD_{90} of the nonischemic proximal fiber was shorter than that of the ischemic distal fiber. Exposure to halothane (center) increased the difference between the action potential durations of the two fibers by decreasing APD_{90} in the proximal fiber and increasing APD_{90} in the distal fiber. The panels to the right show the action potentials obtained in the presence of halothane (unlabeled) superimposed on the control tracings (labeled C). In the non-infarcted heart, halothane reduced the difference between the repolarization times of fibers in the two regions, while, in the infarcted heart, it accentuated the disparity between the repolarization times of fibers located within the non-ischemic and ischemic regions.

EFFECTS OF HALOTHANE ON INDUCED ARRHYTHMIAS

In non-infarcted hearts, the conduction velocity of non-premature impulses was 2.1 ± 0.2 m/s (N = 8), and there was no change on exposure to 0.39 mM halothane. In eight infarcted hearts, the conduction velocity obtained on exposure to halothane (1.6 ± 0.2 m/s) did not differ from the control value of 1.9 ± 0.1 m/s.

The responses of a non-infarcted preparation to premature stimulation are shown in figure 3A. Under control conditions (circles), there was little increase in the interelectrode conduction time (y axis) as the coupling interval (x axis) was decreased to the effective refractory period. Exposure to halothane decreased the ERP and FRP in parallel with the abbreviation of the proximal action potential without change in the threshold current for intracellular stimulation. The conduction times of early premature impulses in the presence of halothane (squares) did not increase, presumably because impulses initiated in the proximal fiber continued to propagate towards more fully repolarized distal fibers, even in the presence of the agent. In contrast, the infarcted preparation illustrated in figure 3B exhibited marked prolongation of the conduction times of early premature stimuli, even under control conditions

(open and filled circles). Decremental conduction presumably occurred because early premature impulses encountered less well repolarized, and, therefore, more refractory, fibers on propagation into the infarcted region. Exposure to halothane (open and filled squares) slightly decreased the ERP, increased the delay of conduction of premature impulses over a wide range of coupling intervals, and prolonged the FRP. The conduction times of premature impulses initiated at similar coupling intervals were increased, presumably because the propagating impulse encountered ischemic fibers exhibiting further reduction of $\dot{V}_{max}$ and increased APD_{90} in the presence of halothane.

Figure 4 shows the action potential responses of the same infarct to progressively earlier premature stimuli.

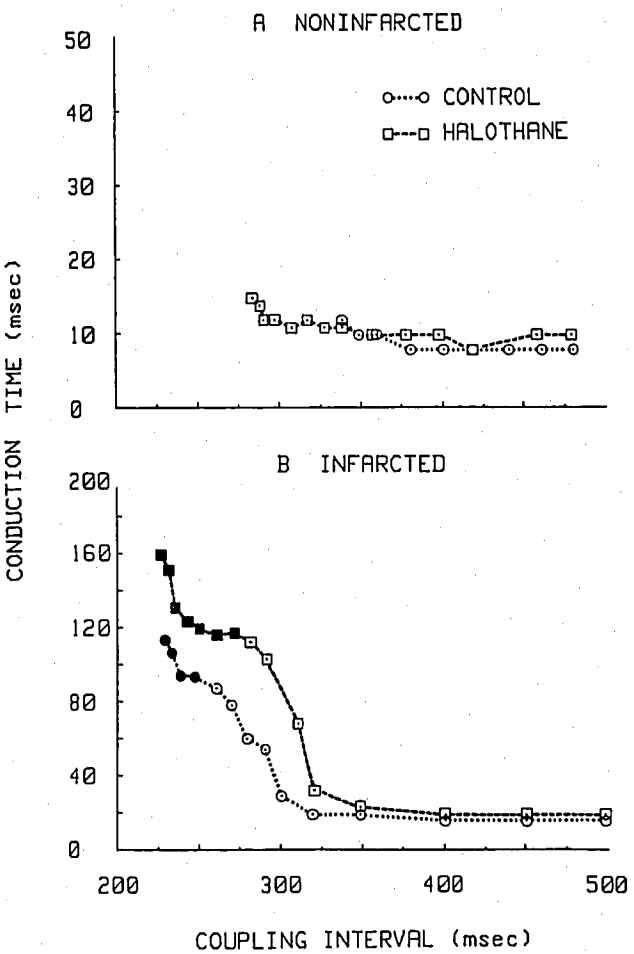

FIG. 3. Effects of 0.39 mM halothane on the conduction of premature impulses in a non-infarcted and infarcted preparation. The X axis is the coupling interval (prematurity) and the Y axis is the interelectrode conduction time. The circles are control conduction times and the squares times in the presence of halothane. Halothane increased the delay of conduction of premature impulses in the infarcted heart. The filled symbols represent impulses followed by the repetitive activity shown in figure 4.

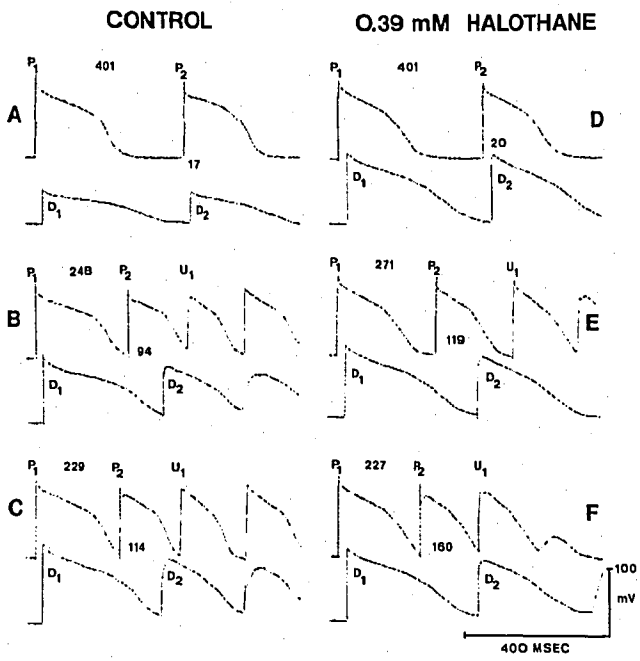

FIG. 4. Effects of halothane on the generation of unstimulated repetitive responses (U_1) in an infarction preparation. *A, D.* Responses of single proximal and distal fibers to a drive stimulus (P_1, D_1) and a subsequent late premature impulse (P_2, D_2) applied at a coupling interval of 401 msec. *B, E.* Latest premature impulse producing U_1. *C, F.* Earliest premature impulse inducing U_1. The repetitive response zone increased from a control "width" of 19 msec (248–229 msec, *B–C*) to 44 msec (271–227 msec, *E–F*) in the presence of halothane. The low amplitude of the D_1 and D_2 responses in *A* was due to deterioration of the impalement.

In each panel, the proximal (P_1) and distal (D_1) responses at the left resulted from a basic drive stimulus, while the second pair (P_2, D_2) are the responses to a premature stimulus. The coupling intervals (P_1-P_2) and conduction times of the premature impulses (P_2-D_2) are indicated in msec between the respective action potentials. Under control conditions, as the coupling interval was reduced, the conduction time of the premature impulse increased to a maximum of 114 msec at the ERP in panel C. Premature impulses applied within the 19 msec range of coupling intervals between 248 msec (panel B) and 229 msec (panel C) were consistently followed by unstimulated repetitive responses (labeled U_1), which re-excited the proximal fiber and then the distal fiber in a reciprocating manner. The range of coupling intervals inducing the arrhythmia, referred to as the repetitive response zone, is indicated by the filled circles in figure 3B. As shown to the right in figure 4, exposure of the infarcted heart to halothane increased the maximum conduction time to 160 msec (panel F) and increased the "width" of the repetitive reponse zone to 44 msec, from a coupling interval of 271 msec (panel E) to 227 msec (panel F). This action appeared to

TABLE 2. Effects of 0.39 mM Halothane on Conduction, Refractoriness, and the Generation of Repetitive Activity in Seven Infarcted Hearts

	ERP	$IECT_{ERP}$	FRP
Control	273 ± 15	125 ± 18	367 ± 16
Halothane	271 ± 14	190 ± 29†	408 ± 24*
Control	287 ± 12	126 ± 16	384 ± 16

Width of Repetitive Response Zone and Range of Coupling Intervals Inducing Repetitive Activity in Each Preparation

Preparation	Control	Halothane	Control
1	0	0	0
2	0	0	0
3	19 (229–248)	44 (227–271)	35 (232–267)
4	18 (242–260)	51 (265–316)	25 (277–302)
5	3 (226–229)	57 (216–273)	0
6	0	41 (316–357)	0
7	0	63 (285–348)	29 (280–309)
Mean ± SE	6 ± 3	37 ± 10	13 ± 6

ERP = effective refractory period; $IECT_{ERP}$ = interelectrode conduction time for impulse at the ERP; FRP = functional refractory period. All values are in msec representing mean ± SE.

* $P \leq 0.05$; † $P \leq 0.01$ compared to preceeding and following control.

be due to the increased delay of conduction of later premature impulses in the presence of halothane (filled squares, fig. 3B) such that they attained the delay of about 100 msec associated with re-excitation.

The responses of seven infarcted preparations to premature stimulation are summarized in table 2. Exposure to 0.39 mM halothane did not decrease the ERP, but reversibly prolonged the interelectrode conduction time for premature impulses initiated at the ERP and increased refractoriness as assessed by the FRP. Repetitive activity occurred in three of the seven infarcts in the initial control period. Two additional hearts exhibited these responses only on exposure to halothane, and the arrhythmia disappeared in two preparations on change to control perfusate. In each instance, halothane increased the "width" of the repetitive response zone, and the range of coupling intervals inducing the arrhythmia decreased on washout of the agent.

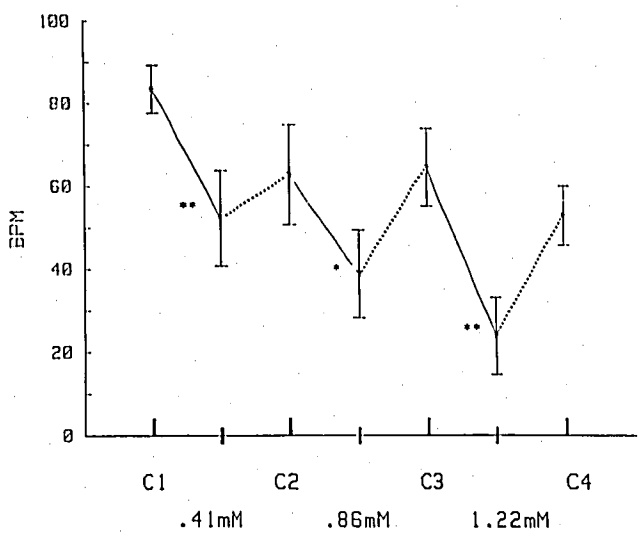

FIG. 5. Influence of halothane on the rate of spontaneous activity in seven infarcted preparations. X axis: periods of control (C1 to C4) and halothane (mM) exposure. Y axis: average spontaneous rate during 10-min observation period (bpm ± SEM). The rate decreased relative to the preceeding control at each level of halothane. *$P \leq 0.05$. **$P \leq 0.01$.

EFFECTS OF HALOTHANE ON SPONTANEOUS ARRHYTHMIAS

The effects of halothane on the spontaneous activity of infarcted preparations are summarized in figure 5. All preparations initially exhibited rapid rhythms with intermittent irregular impulses and occasional pauses. The average spontaneous rate, measured over a 10-min period under each condition, decreased from 84 ± 6 to 53 ± 7 bpm between the initial and final control periods. The rate decreased on exposure to each concentration of halothane and increased significantly on return to control perfusate, except following 0.41 mM halothane. Compared to the rates observed during the immediately preceeding and following control periods, 1.22 mM halothane produced a greater decrease ($P \leq 0.05$) in rate (−60 ± 12%) than that due to 0.41 mM halothane (−32 ± 10%). However, analysis of the 10-min average rate obscured the fact that halothane often changed the character of the spontaneous rhythm.

On seven trials in five infarcts, halothane reversibly converted apparently continuous rhythms to a pattern of intermittent short bursts alternating with slower activity. These changes occurred twice at each of the lower concentrations (0.41 and 0.86 mM) and three times on exposure to 1.22 mM halothane. The findings in one peparation are illustrated to the left in figure 6 by a slow speed continuous record of the activity of a single ischemic fiber during wash-in and wash-out of halothane. The panels to the right show the action potentials obtained from a non-ischemic fiber (top) and the same ischemic fiber (bottom) at the times indicated by *A* to *D* on the continuous record. At seven min of halothane exposure, the control rhythm was interrupted by a pause (panel A) and followed by a series of bursts of progressively shorter duration. Each burst exhibited an abrupt onset and termination and the same non-reciprocating activation pattern as the control rhythms. The rate of activity during the bursts (60 ± 4 bpm, seven trials) in the presence of halothane was slightly lower ($P \leq 0.05$) than the rate of continuous

activity observed under control conditions (76 ± 6 bpm). In contrast, the slow activity between bursts was characterized by an initial pause, suggesting overdrive inhibition of an automatic mechanism, followed by several spontaneous action potentials which appeared to trigger the subsequent bursts of rapid activity. During the slow rhythm, the ischemic fiber sometimes exhibited spontaneous phase 4 diastolic depolarization (panels B and D) or a delayed afterdepolarization (panel C, arrow). In each reversible trial, change to control perfusate was associated with increasing duration and frequency of the bursts until the control continuous rhythm was restored (panel D) as the result of the initiation of a single long "continuous" burst. Thus, the action of halothane decreasing the average spontaneous rate of infarcted preparations (fig. 5) was largely associated with inhibition of the type of rapid activity producing bursts.

The actions of halothane on ischemic fibers initiating spontaneous activity in two small preparations dissected from infarcted hearts are shown in figure 7. The preparation illustrated by the upper tracings exhibited continuous rhythmic activity under control conditions (panel A) associated with spontaneous phase 4 diastolic depolarization. Exposure to halothane (panel B) reduced the slope of diastolic depolarization and decreased the rate of spontaneous activity from 89 to 80 bpm. The preparation illustrated by the action potentials at the bottom of figure 7 was quiescent under control conditions and did not exhibit spontaneous activity in the absence of an initiating stimulus. However, the application of three drive stimuli at 1-s intervals (dots, panel C) induced a subthreshold afterdepolarization (arrow), while four drive stimuli consistently initiated a burst of triggered activity. Exposure to halothane (panel D) decreased the amplitude of the subthreshold afterdepolarization (arrow) and abolished the triggered responses to four or more drive stimuli.

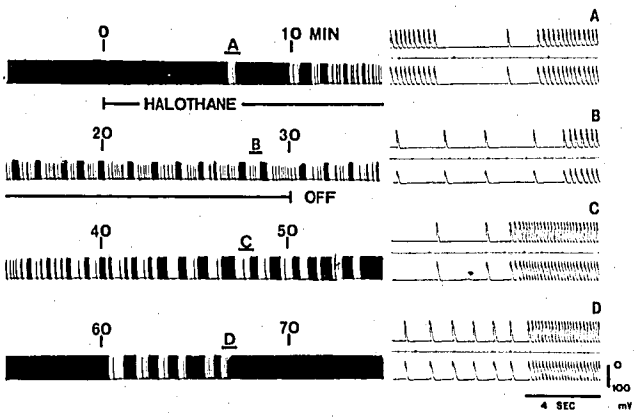

FIG. 6. Effects of 0.57 mM halothane on spontaneous arrhythmias in an infarction preparation. Left panels: continuous recordings of an ischemic fiber action potential. The elapsed time from the beginning of halothane exposure is given in minutes. Right panels: simultaneous recordings from a nonischemic fiber (top each panel) and the same ischemic fiber (below) at the times indicated by *A–D* on the continuous record. *A*. Change from continuous activity to alternating bursts and slow activity on halothane exposure. *B, C*. Occurrence of spontaneous phase 4 diastolic depolarization and a delayed afterdepolarization (arrow, *C*) in the ischemic fiber during the slow rhythm. *D*. Resumption of continuous rapid activity on wash-out of halothane as the result of a single burst.

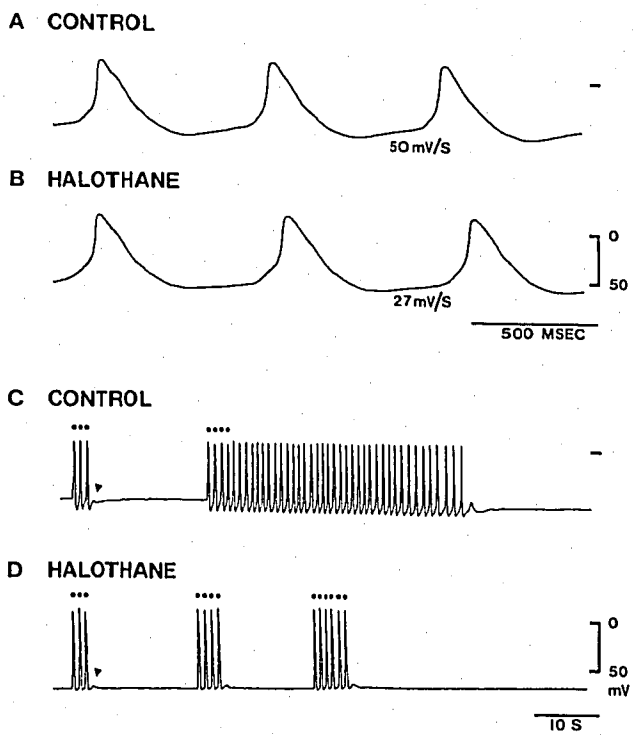

FIG. 7. Responses of single ischemic fibers to 0.39 mM halothane in two small infarction preparations. The upper panels show reduction of the slope of spontaneous phase 4 diastolic depolarization (mV/s) in an ischemic pacemaker fiber. The lower recordings show abolition of triggered activity induced by extracellular stimuli in a fiber exhibiting delayed afterdepolarizations (arrows). The action potentials indicated by dots were initiated by stimuli applied at 1-s intervals (see text for details).

Discussion

The present study differs from previous investigations by evaluating the actions of halothane on Purkinje fibers located in different regions of the normal and infarcted canine left ventricle. The recording sites selected and multifiber technique used to assess the regional responses were by design similar to those employed by Allen *et al.* in an investigation of the actions of lidocaine in this model.[10] The responses to halothane were superimposed on well known control variations in Purkinje fiber action potential duration (APD) in each

type of preparation. These differences may be summarized[7] as follows: while, in normal hearts, APD increases along the course of the false tendon to reach a maximum in fibers close to the papillary muscle junction (proximal site) and then decreases towards the apex (distal site), in infarcted hearts, the APD of ischemic fibers is greatly prolonged relative to that of fibers located in the non-ischemic region. Regional variations of Purkinje fiber APD in the normal ventricle have been attributed to differences in the degree of electrotonic coupling to myocardial fibers[13] and to differences between the contributions of specific ionic currents to repolarization in individual fibers.[14] In infarcted hearts, the abnormal properties of ischemic fibers have been related to depression of the membrane pumping mechanism with accumulation of extracellular K^+ and intracellular Na^+, Ca^{++}, and H^+ ions, as well as other substances released by the necrotic myocardium.[15] The use of large preparations to assess regional effects has several disadvantages, including delay in equilibration and wash-out of the agent and tissue movements which readily dislodge the microelectrodes and effectively prevent long-term measurement of changes in single fibers. The present study was also limited to a selected "moderately depressed" group of recognizeable Purkinje fibers to exclude possible impalement of surviving ischemic muscle fibers. Further studies will be required to assess the responses of severely depressed single fibers (MDP ≤ -70 mV) which were occasionally observed to depolarize and become inexcitable on exposure to halothane.

Action Potential Changes

Several studies have demonstrated that halothane alters the action potential of non-ischemic Purkinje fibers. Hauswirth[2] reported that halothane produced a dose dependent decrease in the overshoot and APD, while $\dot{V}_{max}$ and the conduction velocity decreased at a concentration of 2 vol%. Reynolds *et al.*[1] noted inhibition of spontaneous diastolic depolarization and increased slope and shortening of the plateau phase, as well as delay of terminal repolarization resulting in little change in APD. The present study indicates that 0.39 mM halothane (approx. 1.1 vol%) has important differential regional actions on Purkinje fibers in non-infarcted and infarcted canine hearts. These actions reduced regional differences in repolarization in non-infarcted hearts by decreasing APD_{90} in proximal fibers without altering APD_{90} in distal fibers. In contrast, in infarcted hearts, halothane accentuated regional differences in repolarization by reducing APD_{90} in the non-ischemic proximal fibers, while tending to further increase the abnormally prolonged APD_{90} of fibers located within the infarcted region. In the presence of halothane, the latter ischemic fibers exhibited significantly reduced overshoot and $\dot{V}_{max}$, and increased APD_{90} relative to the initial control values, but not on comparison to final control values (ANOV, range test). This lack of reversibility could be due to true irreversible actions of halothane, incomplete wash-out of the tissue soluble agent, or deterioration of the preparation. While we are unable to account for this result, the importance of these changes in the phase 0 and repolarization characteristics of ischemic fibers is suggested by the reversible increase in refractoriness (FRP) which occurred in infarcted hearts exposed to halothane.

Although the mechanisms by which halothane alters the action potential of Purkinje fibers are not known, a number of studies have emphasized the possible role of inhibition of the slow inward current (I_{si}) and the transmembrane Ca^{++} ion influx[16] in mediating the negative inotropic and action potential effects of halothane on ventricular muscle fibers. Lynch *et al.*[17] reported that halothane depressed slow action potential responses in ventricular muscle fibers partially depolarized by high K^+ ion concentrations, and suggested that halothane may reduce the plateau amplitude and duration in myocardial fibers by inhibiting I_{si}. Recently, Ikemoto *et al.*[18,19] examined the actions of halothane on inward membrane currents in enzymatically dispersed single ventricular myocytes utilizing voltage clamp techniques. They confirmed that halothane reduced the slow inward current, and also demonstrated that this agent inhibits inward sodium current (I_{Na}). The latter action was not associated with use dependence, and could produce a decrease in $\dot{V}_{max}$ of phase 0 by reducing maximum sodium conductance. Assuming that halothane also depresses I_{si} and I_{Na} in Purkinje fibers, its effects on the action potential characteristics in the canine infarction model may be compared to those of more conventional anti-arrhythmic agents known to have actions on Na^+ and Ca^{++} channels.

The regional actions of halothane in non-infarcted hearts, limited to about a 7% shortening of APD_{90} in the proximal fibers, were qualitatively and quantitatively similar to those of lidocaine reported by Allen *et al.*[10] The mechanism by which this local anesthetic produces greater shortening of the action potential in Purkinje fibers exhibiting intrinsically long APD is thought to involve inhibition of the "sodium window current," a component of the depolarizing inward sodium current (I_{Na}) which persists during the long plateau phase in some fibers.[14,20,21] The findings in non-infarcted hearts suggest that halothane may, in part, abbreviate repolarization in Purkinje fibers by a similar action on Na^+ channels inhibiting the sodium window current. Although a "lidocaine-like" action of halothane is consis-

tent with the observed decrease of APD_{90} in the non-ischemic proximal fibers of infarcted hearts, some other mechanism is required to account for its action increasing APD_{90} in ischemic fibers, since lidocaine slightly decreases APD_{90} in these fibers.[10] It is of interest that verapamil has been shown to increase refractoriness in infarcted hearts and prolong APD_{100} to a greater extent in ischemic fibers than in non-ischemic fibers.[22] While calcium channel blockade may be expected to depress the plateau phase of the action potential, the secondary reduction of intracellular Ca^{++} ion concentration may also alter outward K^+ ion currents delaying repolarization[23] and possibly depolarize ischemic fibers.[24] It is speculated that the action of halothane inhibiting I_{si} may be related to the increase of APD_{90} observed in the ischemic fibers and the prolongation of the FRP found in infarcted hearts.

The similarities between the observed regional actions of halothane on repolarization and those reported for lidocaine and verapamil in this model suggest that halothane may alter the action potential of Purkinje fibers by analogous mechanisms involving inhibition of the major inward currents carried by both Na^+ and Ca^{++}. However, the findings do not exclude several other possible actions by which an agent may alter repolarization.[25] The relative sensitivity of ischemic fibers to the actions of halothane reducing the overshoot and $\dot{V}_{max}$ could be due to inhibition of either I_{Na} or I_{si} during the upstroke of the action potential. Agents inhibiting I_{Na} may have a greater action reducing $\dot{V}_{max}$ in partially depolarized ischemic fibers due to voltage dependent inactivation of Na channels.[10] On the other hand, agents inhibiting the remaining I_{si} in depolarized fibers would also be expected to decrease $\dot{V}_{max}$ to a greater extent than in normal fibers.[22] While the changes in the overshoot and $\dot{V}_{max}$ of the ischemic fibers were not associated with a decrease in the conduction velocity of non-premature impulses, conduction may have been depressed in localized areas of the infarct and not have been detected by the method used to assess conduction to a single distal site. However, these changes probably contributed to the increase in the conduction delay of premature impulses and the prolongation of the FRP found in infarcted hearts.

Effects of Halothane on Ischemia-Related Arrhythmias

The results indicate that halothane has both arrhythmogenic and anti-arrhythmic actions in infarcted canine hearts, and suggest that these actions are related to facilitation and inhibition of activity generated by different mechanisms. The pro-arrhythmic action was manifested by an increase in the range of coupling intervals at which premature impulses conducted sufficiently slowly into the infarcted region to produce unstimulated responses which re-excited the non-ischemic region. This action appears to be related to differences in its effects on the phase 0 and refractory characteristics of ischemic and non-ischemic fibers, which are largely analogous to the reported "toxic" or arrhythmogenic effects of lidocaine in this model.[7,8,10] It has been demonstrated in other studies that this type of repetitive activity is due to a re-entrant mechanism associated with unidirectional conduction block and slow conduction of the premature impulse around areas of increased refractoriness in the infarcted region.[15] While 0.39 mM halothane further slowed the conduction of premature impulses, this action did not appear to inhibit or block subsequent re-excitation. The findings support the hypothesis of Hauswirth[2] that the actions of halothane slowing conduction and abbreviating repolarization in ventricular tissues may facilitate the occurrence of re-entrant arrhythmias.

On the other hand, halothane exhibited an anti-arrhythmic action on infarcted hearts, manifested by a decrease in the average rate of spontaneous activity originating within the ischemic region. The enhanced rhythmicity of infarcted preparations has been attributed to abnormal automaticity.[5] This type of abnormal pacemaker activity in depolarized ventricular fibers[26] can generate complex patterns of arrhythmias in association with small changes in the maximum diastolic potential or the degree of entrance and exit block around an ischemic focus.[27] Other investigators have demonstrated the initiation of afterdepolarizations and triggered activity in ischemic fibers by both "slow background automaticity" and single applied stimuli.[6] The pattern of alternating bursts and slow activity often observed in the presence of halothane (fig. 6) was consistent with initiation of triggered activity by a separate slow automatic focus, while the increase in rate on wash-out of halothane appeared to be due to resumption of continuous triggered activity. However, the observed changes in the pattern of the arrhythmia could also be produced by an increase in the degree of conduction block around a focus of abnormal automaticity. The findings in a few small preparations (fig. 7) suggest that the action of halothane slowing the rate of abnormal impulse initiation in infarcted preparations could be due to depression of either triggered activity or abnormal automaticity. Since both of these forms of activity occur in depolarized fibers at membrane potentials associated with slow action potential responses, it is probable that the observed anti-arrhythmic actions of halothane involve reduction of I_{si} and the intracellular Ca^{++} ion concentration. An additional action of halothane altering the binding or release of Ca^{++} ions from

the sarcoplasmic reticulum[28] could also be involved, since cyclic fluctuations of intracellular Ca^{++} are associated with the transient inward current and generation of afterdepolarizations.[29] Recently, Lynch[30] reported that halothane inhibits the generation of aftercontractions in depolarized ventricular fibers exhibiting slow action potentials. The finding that halothane tended to reduce the amplitude of afterdepolarizations in ischemic Purkinje fibers (fig. 7) supports their hypothesis that halothane may have an important influence on the occurrence of triggered arrhythmias.

The results are limited in their extrapolation to the *in vivo* state, in which the electrophysiologic actions of halothane may depend on important neural,[9] metabolic, and hemodynamic factors not present *in vitro*. While the findings indicate that this agent has several actions on isolated preparations which influence the generation of arrhythmias due to abnormal conduction and impulse initiation, the effects of halothane on the cardiac rhythm in the intact infarcted animal are not known. Recently, Kroll and Knight reported that both halothane and verapamil decrease the incidence of ventricular fibrillation in a canine model of acute occlusion/reperfusion arrhythmias.[31] Their findings and the observation that halothane decreases the rate of abnormal impulse initiation in superfused infarcted preparations may both be related to its Ca^{++} channel blocking actions. However, comparison of these anti-arrhythmic actions is difficult, due to the substantial differences between the two models of reversible and irreversible myocardial ischemia. It may be possible to separately evaluate the effects of halothane on arrhythmias due to abnormal conduction and impulse initiation in the intact 24-h infarcted dog, since it has been shown that re-entrant arrhythmias are associated with continuous diastolic electrical activity in the ischemic region, while spontaneous activity originating in the infarct does not show this phenomenon.[32] Combined *in vitro* and *in vivo* study of the actions of anesthetic agents on ischemic cardiac tissues may permit development of specific therapeutic approaches for the perioperative management of arrhythmias in patients with acute myocardial infarction.

In conclusion, the present study demonstrates that 0.39 mM halothane has highly specific regional effects on the action potentials of canine left ventricular Purkinje fibers. In non-infarcted hearts, the regional actions of halothane are similar to those reported for lidocaine, while, in infarcted hearts, its actions on fibers surviving within the ischemic region more closely resemble those reported for verapamil. The actions of halothane abbreviating repolarization in non-ischemic fibers and slowing the conduction of premature impulses into the ischemic region of infarcted hearts facilitate the induction of a type of repetitive activity that may be due to reentry. On the other hand, halothane inhibits the generation of abnormal impulses in ischemic fibers in a manner suggesting that it may have an anti-arrhythmic influence on triggered arrhythmias and abnormal automaticity. The findings may provide some insight into the mechanisms underlying the potential variable responses of the cardiac rhythm to halothane in the setting of clinical myocardial infarction. The *in vitro* canine infarction model may be particularly useful in determining the influence of the volatile anesthetics on the abnormal electrical activity of ischemic cardiac tissues.

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