Anesthesiology
71:695–703, 1989

Halothane Antagonizes Ouabain Toxicity in Isolated Canine Purkinje Fibers

John D. Gallagher, M.D., John J. Bianchi, Ph.D.,† Lawrence J. Gessman, M.D.‡*

Standard intracellular microelectrode techniques were used to study the effects of halothane on ouabain-induced delayed after depolarizations (DAD) in canine Purkinje fibers. Free running Purkinje fibers were superfused with 2×10^{-7} M ouabain in Krebs-Henseleit buffer for 30–50 min until DAD appeared. Purkinje fibers were then paced for 20 beats at cycle lengths between 1,000 ms and 200 ms, and the amplitude of the DAD and coupling interval between the DAD and last paced beat were determined. Halothane (0.5, 1, and 2%) was then administered and measurements repeated. Halothane produced dose-related decreases in DAD amplitude without changing DAD coupling interval. The ability of calcium to antagonize the effects of halothane was evaluated by doubling buffer calcium concentration to 5 mM in the presence of halothane 2%. Doubling buffer calcium concentration to 5 mM antagonized the reduction of DAD amplitude caused by halothane. In several preparations, dysrhythmias occurred during ouabain superfusion. Halothane reversibly terminated these arrhythmias. Halothane antagonizes DAD and dysrhythmias induced *in vitro* by ouabain toxicity. This effect, in part, may account for the apparent effectiveness of halothane against ouabain-induced dysrhythmias *in vivo*. (Key words: Anesthetics, volatile: halothane. Cardiac glycosides, ouabain. Heart, electrophysiology: delayed after depolarizations; Purkinje fibers; triggered automaticity.)

HALOTHANE increases the lethal dose of digitalis by antagonizing cardiac glycoside induced dysrhythmias.[1,2] The mechanisms for this antagonism are not known, but changes in the rate of Phase 4 depolarization of pacemaker cells in the cardiac Purkinje fibers have been suggested as responsible.[3] Alterations in autonomic outflow may also be important because cyclopropane, which increases sympathetic outflow,[4] decreases the toxic dose of cardiac glycosides[5] whereas halothane, which decreases sympathetic outflow,[6] antagonizes digitalis toxicity.[1,2]

Superfusion of isolated Purkinje fibers with cardiac glycosides produces delayed after depolarizations (DAD), oscillatory membrane depolarizations that occur after full repolarization of the action potential.[7] The amplitude of DAD increases with increasing levels of digitalis toxicity and at faster pacing rates.[7,8] If of sufficient amplitude, DAD can reach threshold potential and trigger repetitive extrasystoles.[7] These triggered rhythms may be a major cause of digitalis-induced dysrhythmias.[9]

We investigated the effects of halothane on DAD induced by cardiac glycosides in canine Purkinje fibers. Our goal was to determine whether the beneficial effects of halothane in digitalis toxicity could be due to a reduction in DAD amplitude and triggered rhythms.

Materials and Methods

This study was reviewed and approved by the Institutional Animal Care and Use Committee. Twenty mongrel dogs of either sex were anesthetized with iv pentobarbital, 30 mg/kg. The heart was rapidly removed through a left thoracotomy and placed in cold, oxygenated Krebs-Henseleit (K-H) buffer. The buffer solution contained 121.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 21.9 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.1 mM glucose and was equilibrated with 95% O_2/5% CO_2. Free running Purkinje fibers (PF) were removed from either ventricle along with a small piece of muscle at each end and placed in a tissue bath with an internal volume of 4 ml, superfused at a rate of 15–25 ml/min with the K-H buffer. Temperature was maintained at 37° C throughout the experiment and buffer *p*H was 7.4. Individual PF cells from the midportion of the PF were impaled with glass microelectrodes filled with 3 M KCl, having tip resistances between 10 and 30 megaohms. These were coupled *via* Ag/$AgCl_2$ junctions to the inputs of a KS700 dual microprobe amplifier (WPI, Inc., New Haven, Connecticut), and action potentials were either photographed from an oscilloscope, printed from a chart recorder, or recorded on magnetic tape. PF were paced using bipolar Teflon-coated silver wire electrodes at cycle lengths described below, a pulse duration of 2 ms, and an amplitude of twice threshold.

Action potentials were photographed and the following data recorded: maximum diastolic potential (MDP); action potential amplitude (AP_{amp}); take-off potential (TOP), the membrane potential from which the action potential arises, which will differ from the MDP whenever spontaneous Phase 4 depolarization or DAD occurs; and action potential duration to 50% and 90% repolarization (APD_{50} and APD_{90}, respectively). V_{max}, the maximum rate of rise of Phase 0 of the action potential was determined by electronic differentiation (fig. 1).

* Associate Professor of Anesthesiology, Dartmouth-Hitchcock Medical Center, Dartmouth Medical School.

† Research Associate, Deborah Research Institute.

‡ Assistant Adjunct Professor of Medicine, University of Pennsylvania School of Medicine; Director of Electrophysiology, Deborah Heart and Lung Center.

Received from the Deborah Research Institute, Browns Mills, New Jersey. Accepted for publication June 6, 1989. Supported by a grant from the New Jersey Affiliate of the American Heart Association. Presented in part at the 1987 International Anesthesia Research Society Annual Meeting, San Diego, California.

Address reprint requests to Dr. Gallagher: Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, Hanover, New Hampshire 03756.

Amplitude and coupling intervals for DAD (DAD_{amp}, DAD CI, respectively) were determined as shown in figure 2. DAD_{amp} was determined from the point of full repolarization from the preceding action potential (MDP) to the peak of the DAD, and DAD CI from the onset of the previous action potential to the peak of the DAD.[8] At faster paced rates, a second DAD often appeared.[8] In these cases DAD_{amp} and DAD CI were also determined for the secondary DAD.

Study Protocol

After a 45-min equilibration period, PF were paced for 19 or 20 beats at cycle lengths (CL) of 1,000, 800, 600, 500, 400, 350, 300, 250, and 200 ms. The period immediately following the last beat of the train of stimuli was observed for the appearance of DAD.

PF were then made ouabain toxic by superfusion with 2×10^{-7} M ouabain in K-H buffer. The protocol of Moak and Rosen[8] was followed. After each 5 min of superfusion, PF were paced for 20 beats at a cycle length of 500 ms. When DAD of a 5 mV amplitude were observed, which required between 30 and 50 min of superfusion, the ouabain was discontinued and superfusion with K-H buffer resumed.

The first series of experiments evaluated the observation of Miura and Rosen[10] that DAD produced in this manner were stable for 1 h. Eight fibers were paced with a train of 20 beats at a 500 ms CL every 5 min for 90 min, and the amplitude and coupling interval of the resultant DAD were recorded. The second group of experiments in another eight fibers evaluated the effects of halothane on DAD induced by ouabain. Fifteen minutes after ouabain was discontinued baseline measurements were obtained and halothane was added to the buffer from a calibrated vaporizer at concentrations of 0.5%, 1%, and 2%. (Three fibers received increasing concentrations of halothane, three fibers received decreasing concentrations, one each received halothane 1%, 0.5%, 2% and 1%, 2%, 0.5%.) Ten minutes were allowed at each concentration and measurements were repeated. The duration of stability of DAD, determined in the first series of experiments, precluded control periods between administration of different halothane concentrations. A third group of experiments in eight additional fibers evaluated the ability of increased concentrations of calcium in the buffer to counteract the effects of halothane.[11] Following induction of ouabain toxicity and obtaining baseline measurements, the calcium concentration of the buffer was doubled from 2.5 to 5 mM and the measurements were repeated. Halothane 2% was administered in both concentrations of calcium and the measurements were repeated. The order of administration was random. In each experiment a single cell impalement was maintained throughout.

In half of the fibers studied, after completion of the protocols described, ouabain 2×10^{7} M was again superfused over the preparation in an effort to induce extreme degrees of ouabain toxicity. When this occurred, halothane was added to the superfusate and the effects were observed. In addition, several fibers developed spontaneous or induced extra beats during the initial superfusion with ouabain. Although not included in the statistical analysis of DAD amplitude and coupling intervals, halothane was administered to each and the effects were observed.

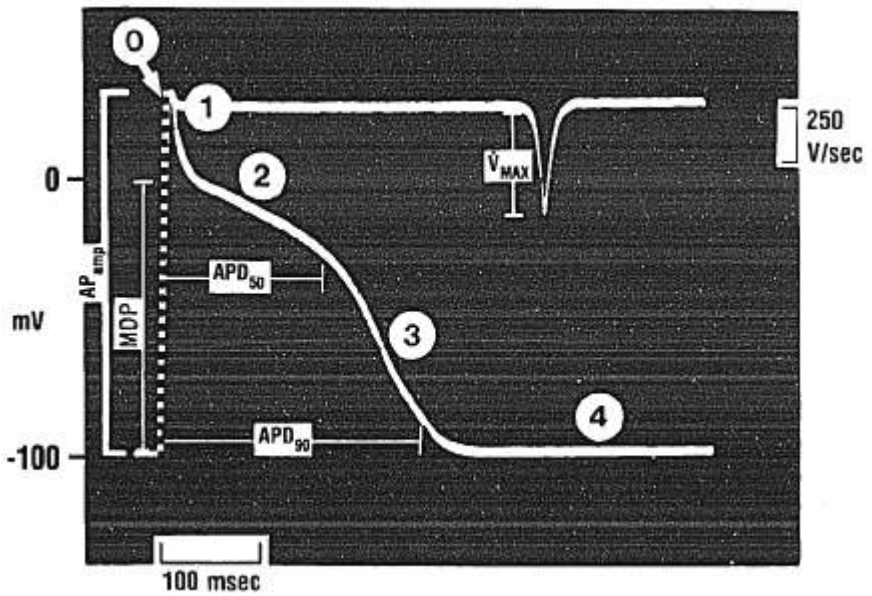

FIG. 1. Control action potential from a canine Purkinje fiber cell paced at a cycle length of 500 ms. Time and voltage calibrations for the action potential are shown. Numbers 0 to 4 represent phases of the action potential. Phase 0, the upstroke, has been accentuated with broken line. Phase 1, early repolarization; Phase 2, the plateau; Phase 3, late repolarization; and Phase 4, the resting potential, are labeled. Action potential amplitude (AP_{amp}), maximum diastolic potential (MDP), which in this example equals take-off potential (TOP), and action potential duration to 50% and 90% repolarization (APD_{50}, APD_{90}) are shown. V_{max}, the maximum rate of rise of Phase 0, is shown on the same figure recorded at a faster oscilloscope sweep speed. The height of the deflection is proportional to V_{max}. The appropriate calibration is shown.

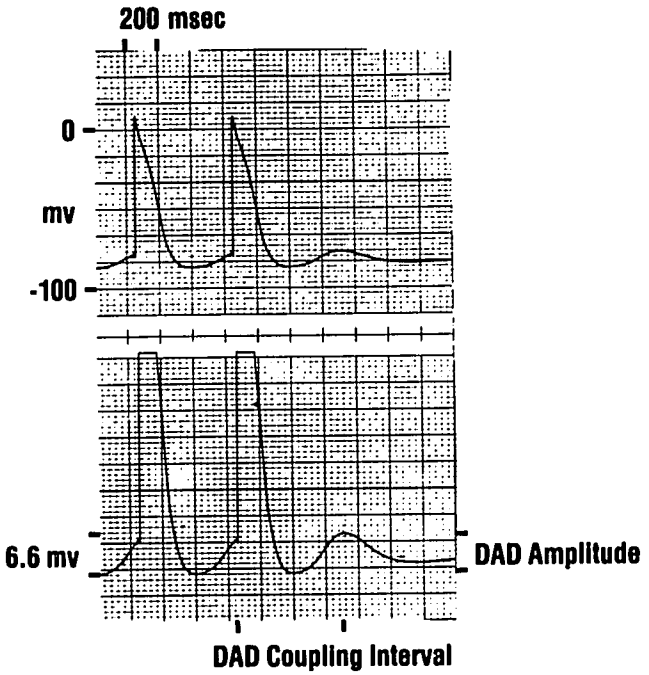

FIG. 2. Characteristics of delayed after depolarizations induced by ouabain toxicity. Upper panel displays the final two action potentials of a train of 20 along with time and amplitude calibrations. A prominent delayed after depolarization (DAD) is seen following repolarization of the final action potential. Lower panel is an amplification of the upper trace (note change in amplitude scale) and shows DAD amplitude as the voltage from full repolarization of the previous action potential to the peak of the DAD, and DAD coupling interval as the time from the onset of the preceding action potential to the peak of the DAD.

Analysis of the duration of ouabain toxicity was performed by using repeated measures one-way analysis of variance (ANOVA) with Bonferroni's modification of the *t* test. In the other experiments data were analyzed by using repeated measures analysis of covariance (ANCOVA) with DAD_{amp} or DAD CI as the response. Primary and secondary DAD were studied separately, and pacing rate was considered as a continuous covariate. Thus, in the halothane dose–response group, we studied a halothane main effect (dose–response) and halothane-by-pacing interaction, all tested within the individual experiment. In the calcium study we measured a halothane main effect, Ca^{2+} main effect, plus all pairwise interactions. All results are presented as mean ± SEM, and a $P < 0.05$ was considered significant.

Results

Duration of Ouabain Toxicity

The effects of ouabain on 8 PF preparations over a 90-min period are shown in figure 3. The zero minute point represents the time when ouabain superfusion was discontinued. Although one-way ANOVA showed that $P = 0.0458$ for the data shown, Bonferroni's test showed that the only significant difference was between the 5 min and 90 min points. The stability of the curve between 15 and 60 min accounts for our use of this time period in subsequent studies. Coupling interval at each measurement point did not vary significantly from control coupling interval (604.1 ± 16.1 ms, mean ± SEM; $P = 0.245$ *vs.* control).

Effects of Halothane on Ouabain Toxicity

Table 1 summarizes the effects of halothane 0.5%, 1%, and 2% on eight PF made ouabain toxic at a paced CL of 500 ms. Control action potentials were normal without spontaneous Phase 4 depolarization or DAD (note that TOP = MDP). Typical ouabain toxic effects,[12] including a significant reduction in AP_{amp} and a depolarization of TOP, which was due to induction of DAD, are shown. Shortening of AP duration and depression of V_{max}, also typical of ouabain toxicity, did not reach statistical significance. Without changing AP amp or MDP *versus* ouabain toxic fibers without halothane, halothane at higher doses shortens APD_{50} and diminishes V_{max} *versus* control in these ouabain toxic fibers.

Figures 4 and 5 display the effects of halothane on DAD_{amp} and DAD CI, respectively. All concentrations of halothane produced significant reductions in primary DAD_{amp} ($P < 0.001$), and the reduction caused by 2% halothane was greater than that of 0.5% halothane ($P < 0.01$). The reduction in secondary DAD_{amp} approached significance ($P = 0.083$), and there was a significant in-

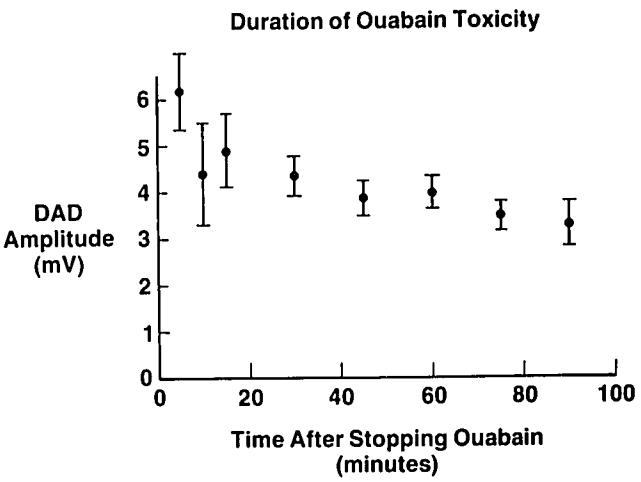

FIG. 3. Duration of ouabain toxicity (n = 8). DAD amplitude following a 20-beat train of stimuli at cycle length 500 ms over a 90-min period are shown. The stability of the DAD amplitude between 15 min and 60 min accounts for the use of this time period in subsequent studies.

TABLE 1. Halothane and Ouabain Toxicity Action Potential Characteristics (n = 8, mean ± SEM); Paced Cycle Length 500 ms

Group	AP_{amp} (mV)	MDP (mV)	TOP (mV)	ADP_{50} (ms)	ADP_{90} (ms)	V_{max} (V/s)
Control	109.7 ± 4.50	85.3 ± 1.67	85.3 ± 1.67	114.2 ± 4.55	185.0 ± 7.64	333.3 ± 44.10
Ouabain toxic	91.7 ± 4.22*	77.7 ± 2.81	72.5 ± 3.02*	96.7 ± 5.58	165.0 ± 2.58	216.7 ± 30.05
Ouabain and halothane 0.5%	96.3 ± 1.05	79.3 ± 1.96	76.0 ± 1.63	91.7 ± 6.91	169.2 ± 4.55	233.3 ± 16.67
Ouabain and halothane 1.0%	90.8 ± 3.23*	77.2 ± 2.90	73.2 ± 2.59*	89.2 ± 7.46	165.8 ± 3.27	191.7 ± 17.87*
Ouabain and halothane 2%	93.2 ± 3.32*	78.0 ± 2.79	74.7 ± 2.42*	81.7 ± 8.23*	164.2 ± 7.12	184.2 ± 22.0*

AP_{amp} = action potential amplitude; MDP = maximum diastolic potential; TOP = take-off potential; $ADP_{50, 90}$ = action potential duration to 50% and 90% repolarization; V_{max} = maximum rate of rise of phase 0 of the action potential.
* $P < 0.05$ *versus* control.

teraction between halothane concentration and pacing ($P < 0.001$), *i.e.*, halothane blunts the rate related rise in DAD_{amp} for secondary DAD. Halothane had no effect on CI, but faster rates of pacing produced significant decreases in DAD CI for both primary and secondary DAD ($P < 0.001$).

Effects of Doubling External Calcium Concentration

Table 2 details changes in action potential characteristics at a paced CL of 500 ms during alterations of Ca^{2+} concentration. Control characteristics in this group of fibers are similar to those shown in table 1. Ouabain-induced action potential changes are similar to those in table 1, but only the change in TOP reached significance. Shortening of APD_{50} was noted in ouabain toxic fibers superfused with 5.0 mM $CaCl_2$ and after administration of halothane 2% to ouabain toxic fibers in 2.5 mM $CaCl_2$. Halothane 2% reduced APD_{90} in ouabain toxic fibers.

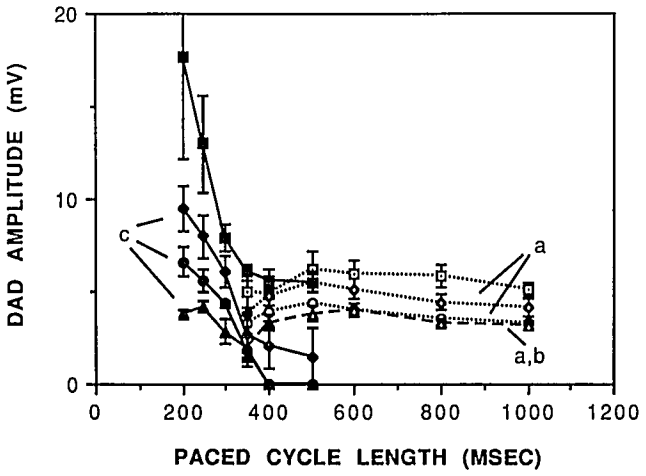

FIG. 4. Effects of halothane on DAD amplitude (n = 8). The amplitude of primary and secondary DAD at paced cycle lengths between 200 and 1,000 ms are shown. Ouabain toxic-DAD1 · · ·□· · ·; DAD2 — ■ —; halothane 0.5%-DAD1 · · ·◇· · ·; DAD2 — ♦ —; halothane 1%-DAD1 · · ·○· · ·; DAD2 — ● —; halothane 2%-DAD1 ---△---; DAD2 — ▲ —. [a]$P < 0.001$ *versus* ouabain toxic-DAD1. [b]$P < 0.001$, halothane 2% *versus* halothane 0.5%-DAD1. [c]$P < 0.001$, interaction between halothane concentration and pacing rate.

Figures 6 and 7 show the effects of doubling extracellular calcium concentration from 2.5 mM to 5 mM in the presence or absence of 2% halothane in eight ouabain toxic PF. In figure 6 effects on DAD_{amp} are shown. Increasing calcium increased the amplitude of primary ($P < 0.001$) and secondary DAD ($P = 0.054$); 2% halothane decreased the amplitude of DAD ($P < 0.001$ for primary and secondary DAD), but this effect was partially reversed by increasing extracellular calcium concentration. Figure 7 shows that neither halothane nor extracellular calcium concentration affects coupling interval, although DAD CI changes with paced CL ($P < 0.001$). Figures 8 and 9 are alternative presentations of data shown in figure 6 and display DAD_{amp} and SEM for primary and secondary DAD during halothane and Ca^{2+} manipulations (figs. 8 and 9, respectively).

Effects of Halothane on Ouabain Toxic Dysrhythmias

Of eight preparations superfused with ouabain after completion of studies above, four became inexcitable

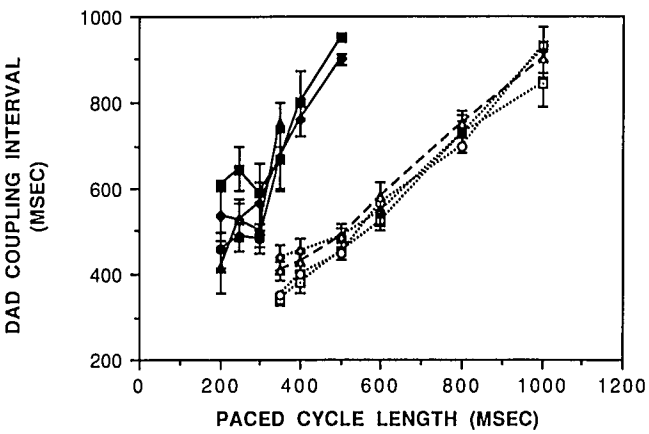

FIG. 5. Effects of halothane on DAD coupling interval (n = 8). Coupling intervals of primary and secondary DAD are shown at paced cycle lengths between 200 and 1,000 ms. Symbols as in figure 4. Halothane produces no significant changes in coupling intervals. Paced CL significantly altered coupling interval for primary and secondary DAD ($P < 0.001$).

TABLE 2. Ouabain Toxicity, Halothane 2%, and Extracellular Calcium Action Potential Characteristics (n = 8, mean ± SEM); Paced Cycle Length 500 ms

	Ouabain	$[Ca^{2+}]$ (mM)	Halothane (%)	AP_{amp} (mV)	MDP (mV)	TOP (mV)	ADP_{50} (ms)	ADP_{90} (ms)	V_{max} (V/s)
Control	0	2.5	0	112.0 ± 2.71	86.3 ± 2.66	86.3 ± 2.66	137.2 ± 7.53	198.3 ± 8.03	333.3 ± 13.94
Ouabain toxic	+	2.5	0	96.7 ± 5.24*	81.1 ± 2.58	76.5 ± 2.92†	117.5 ± 4.96	176.7 ± 5.11	291.7 ± 36.32
Ouabain toxic	+	5.0	0	112.8 ± 4.92	86.3 ± 1.73	81.5 ± 1.95	90.0 ± 13.10†	175.0 ± 1.83	275.0 ± 41.83
Ouabain and halothane	+	2.5	2	117.2 ± 7.24*	89.8 ± 2.50	85.3 ± 3.14	85.0 ± 11.25†	172.2 ± 3.17	295.8 ± 26.64
Ouabain and halothane	+	5.0	2	103.4 ± 2.48	81.5 ± 3.12	76.8 ± 2.87	98.3 ± 10.46	169.2 ± 10.83†	273.3 ± 30.87

* $P < 0.05$ ouabain toxic, Ca^{20} 2.5 mM without *versus* with halothane 2%.

† $P < 0.05$ *versus* control.

during continued ouabain superfusion. In two preparations tachydysrhythmias that were reversibly terminated by 2% halothane were observed, and in two preparations termination of the dysrhythmia by halothane was not reversible, and the fiber became inexcitable. In five additional preparations, excessive toxicity occurred during the initial superfusion with ouabain. In two preparations (fig. 10) extra beats developed during initial superfusion. Halothane reversibly diminished the number of extra beats. In one preparation Phase 4 automaticity ensued, which was eliminated by halothane but not reversible. In two preparations inexcitability occurred.

Figure 10 displays the effects of halothane 0.5%, 1%, and 2% on extrasystoles triggered by a train of 19 paced beats at a CL of 250 ms. Following induction of ouabain toxicity five extra beats (arrows) are triggered (fig. 10*A*). The dysrhythmia terminates with a prominent DAD. After 0.5% halothane (fig. 10*B*) the same pacing sequence results in only two extra beats. Halothane, 1%, reduces the number of extra beats to 1 (fig. 10*C*) and during 2% halothane only a DAD is seen (fig. 10*D*).

Figure 11 was recorded during a triggered dysrhythmia that was induced by pacing at a CL of 400 ms for 20 beats and had been stable for several minutes in this ouabain toxic PF. At the start of figure 11*A*, halothane 2% is added to the buffer; 40 s later (fig. 11*B*) the rhythm terminates with a DAD. The halothane is discontinued, as indicated in figure 11*B*, and soon after (figs. 11*B, C, D,* and *E* are continuous recordings) the tachydysrhythmia recurs. The spontaneous activity during halothane administration represents Phase 4 depolarization-induced automaticity.[13] Whether the second tachydysrhythmia represents accel-

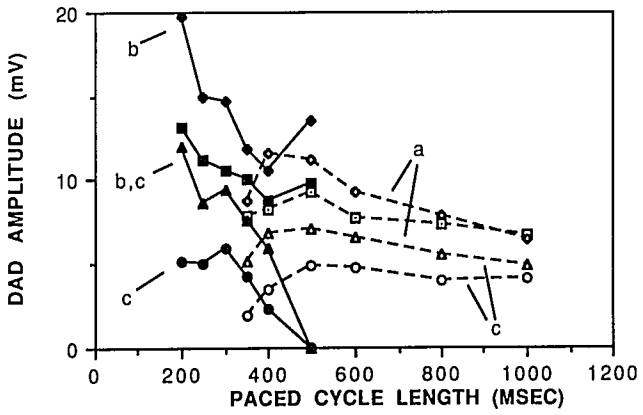

FIG. 6. Effects of doubling calcium concentration on DAD amplitude (n = 8).

Halothane	Calcium	DAD1	DAD2
0%	2.5 mM	- - -□- -	—■—
0%	5 mM	- - -△- -	—◆—
2%	2.5 mM	- - -○- -	—●—
2%	5 mM	- - -△- -	—▲—

[a] $P < 0.001$, calcium 5 mM *versus* calcium 2.5 mM, DAD1.
[b] $P < 0.054$, calcium 5 mM *versus* calcium 2.5 mM, DAD2.
[c] $P < 0.001$, halothane 2% *versus* halothane 0%, DAD1 and DAD2.

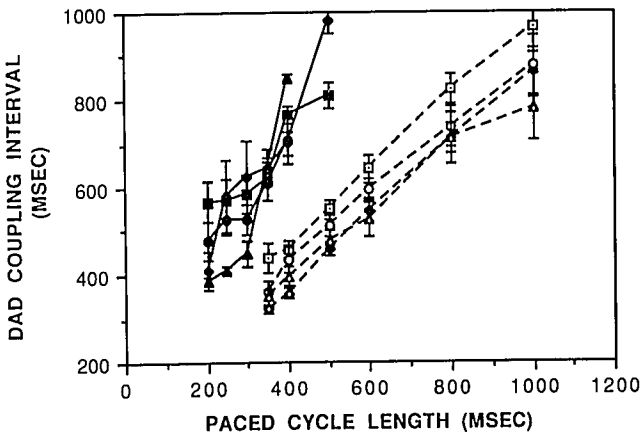

FIG. 7. Effects of doubling calcium concentration on DAD coupling intervals (n = 8). No significant changes on DAD coupling interval were noted when halothane or calcium concentrations were changed, coupling interval changed with paced CL ($P < 0.001$). Same symbols as in figure 6.

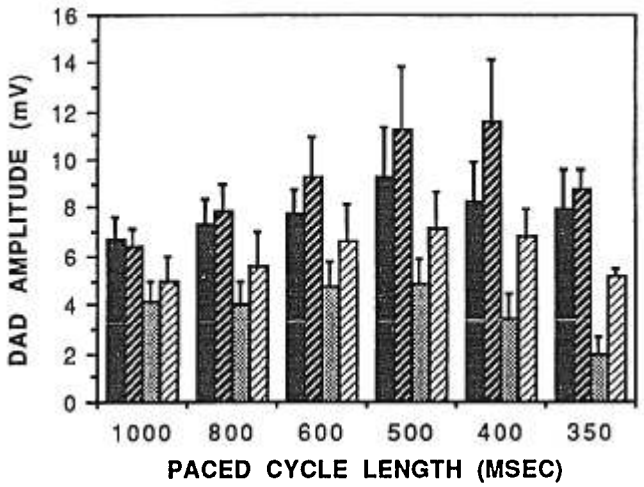

FIG. 8. Alternative presentation of data describing effects of halothane and calcium concentration on primary DAD amplitude, with SEM displayed. Each bar represents DAD amplitude during a different experimental condition. From left to right at each paced cycle length bars represent: halothane 0%, Ca^{2+} 2.5 mM (solid bar); halothane 0%, Ca^{2+} 5 mM (dense diagonal markings); halothane 2%, Ca^{2+} 2.5 mM (crosshatched); and halothane 2%, Ca^{2+} 5 mM (light diagonal markings).

eration of Phase 4 depolarization or triggered automaticity cannot be determined. However, this sequence was reproducible, and following repeated administration of halothane, termination of the dysrhythmia was always associated with a DAD.

Figure 12 shows the effects of halothane on ouabain-induced membrane oscillations.[14] In each panel the lower trace shows a ×2.5 magnification of the upper trace. Figure 12*A* shows spontaneous membrane oscillations that reach threshold and trigger an extra beat. Halothane 1% produced quiescence of the membrane (fig. 12*B*). Three minutes after halothane was discontinued (fig. 12*C*), spontaneous membrane oscillations resumed, culminating in a tachydysrhythmia. These traces are not continuous.

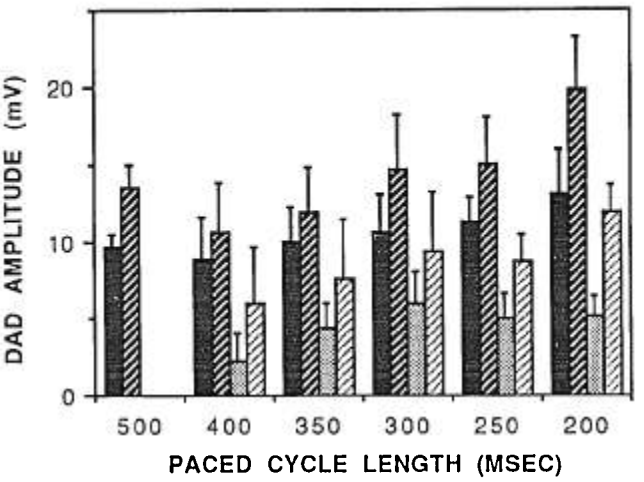

FIG. 9. Alternative presentation of data describing effects of halothane and calcium concentration on secondary DAD amplitude, with SEM displayed. Bars represents DAD amplitude during different experimental conditions. From left to right at each paced cycle length bars represent: halothane 0%, Ca^{2+} 2.5 mM (solid bar); halothane 0%, Ca^{2+} 5 mM (dense diagonal markings); halothane 2%, Ca^{2+} 2.5 mM (crosshatched); and halothane 2%, Ca^{2+} 5 mM (light diagonal markings). Note that at a paced cycle length of 500 ms no secondary DAD were seen when halothane 2% was present.

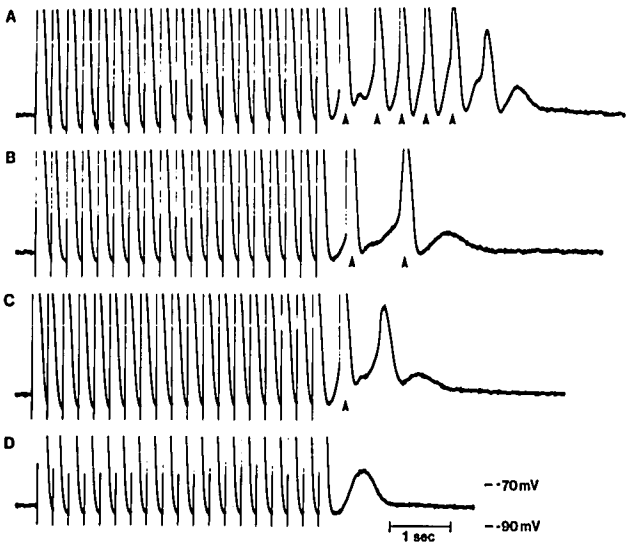

FIG. 10. Effects of halothane on triggered extrasystoles. A Purkinje fiber made ouabain toxic was paced at a cycle length 250 ms for 19 beats in the absence of halothane (*A*). Five extrasystoles were triggered and the dysrhythmia terminates in a DAD. Addition of 0.5% (*B*), 1% (*C*), and 2% (*D*) halothane to the buffer progressively decreased the number of triggered extrasystoles until only a DAD is seen after 2% halothane. These changes were reversible. Amplitude and the calibrations are shown.

Discussion

Ouabain intoxication typically depolarizes resting membrane potential, shortens APD, and reduces V_{max}.[15] These toxic effects of cardiac glycosides depend upon glycosides binding to an inhibitory site on sarcolemmal Na, K-ATPase, inhibiting outward transport of Na^+, and leading to decreased intracellular potassium and increased intracellular Na^+ concentrations.[15] The increased Na^+ concentration accelerates the Na^+–Ca^{2+} exchange mechanism, increasing intracellular calcium ion.[16] Reduction in intracellular potassium from 130 mM to 112.2 mM in a study by Miura and Rosen[10] caused the depolarization of the resting membrane potential, as predicted by the Nernst equation. The shortening of APD has been attributed to a decrease in membrane resistance due to an ouabain-induced increase in potassium conductance during the plateau phase.[17] The reduction in V_{max} is explained by partial inactivation of the sodium current by depolarization[18] and the increase in intracellular Na,

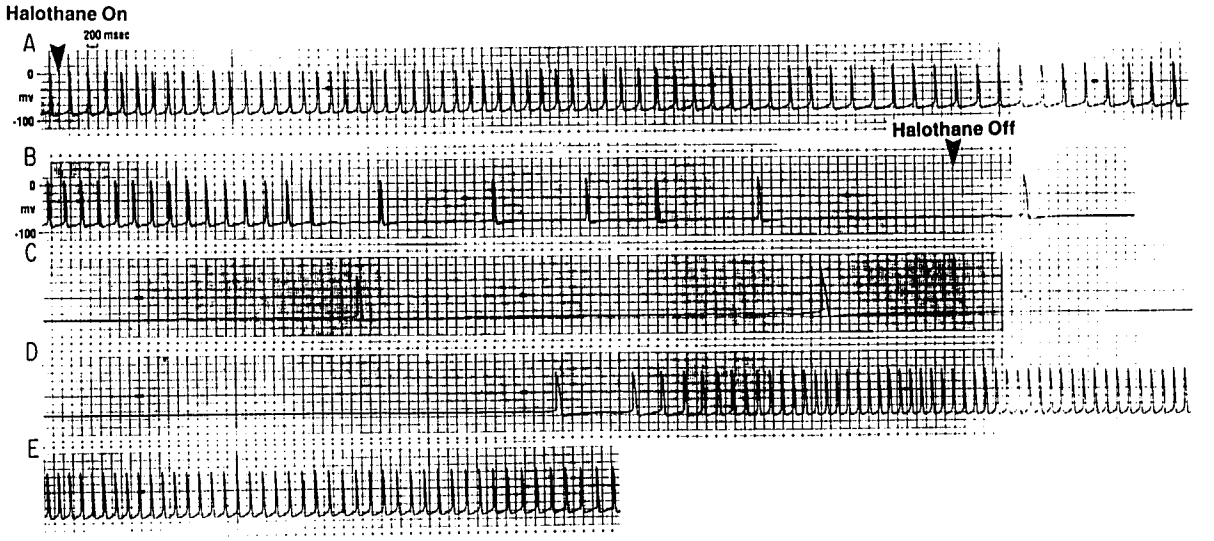

FIG. 11. Effects of halothane on ouabain toxic dysrhythmias. Time and amplitude calibrations are shown. *A.* A stable dysrhythmia that had been induced by pacing at a cycle length of 400 ms for 20 beats. Halothane was introduced during this rhythm, and after 40 s (*B*), the automatic rhythm terminated with a delayed after depolarization implying triggered automaticity. Spontaneous activity due to enhanced Phase 4 depolarization is seen (*B*), which slows as halothane equilibrates. *B, C, D,* and *E.* Continuous recordings. When halothane is discontinued at the arrow toward the end of *B,* within 40 s the arrhythmia occurs (*D*).

which decreases the driving force for the sodium current.[19]

The etiology of DAD is less well understood and they are not limited to digitalis toxic preparations. DAD and triggered automaticity have been produced by catecholamines, noted in the fibers of the mitral valve and coronary sinus and found in infarcted heart.[9] Cranefield[9] suggested that triggered automaticity will prove to be a clinically significant cause of dysrhythmias.

Calcium plays an important role in the genesis of DAD.[11] High concentration of calcium alone can cause DAD,[11] and our own data confirm the observations of others[11] that DAD amplitude is calcium-dependent. Manganese and verapamil, which block the calcium channel, diminish DAD amplitude.[20] However, tetrodotoxin, lidocaine, and decreases in extracellular Na, which diminish the sodium current, also diminish DAD.[15,20,21]

Lederer and Tsien[22] have described an oscillatory inward current induced by ouabain. This transient inward current is a nonspecific cationic current, and it appears

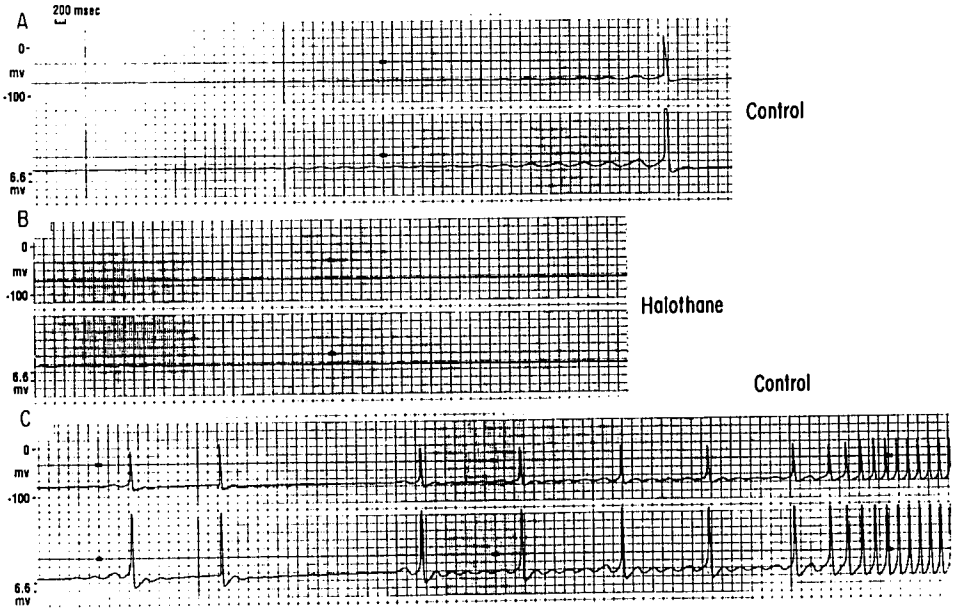

FIG. 12. Effects of halothane on membrane oscillations induced by ouabain. In each panel, the bottom figure is a 2.5 times amplification of the action potential shown in the top panel. *A.* Before administration of halothane, spontaneous membrane oscillations are seen, which increase in amplitude until an action potential is triggered. *B.* Following addition of halothane there is quiescence of the fiber shown by a stable resting membrane potential. *C.* Three minutes after discontinuation of halothane, membrane oscillations are seen to increase in amplitude until spontaneous beats, and finally a tachydysrhythmia occurs.

that DAD are the membrane manifestation of the transient inward current. The increase in intracellular Ca^{2+} caused by digitalis toxicity results in abnormal intracellular calcium handling with oscillatory release of calcium from intracellular stores, such as sarcoplasmic reticulum. This causes fluctuations in membrane permeability allowing sodium and calcium ions into the cell, generating the transient inward current.[23] When the DAD so generated reach threshold, extra beats are triggered.[15] Faster pacing rates, as demonstrated previously[7,8] and confirmed in our study, tend to increase the amplitude of DAD and make triggered automaticity more likely.

In 1970 Reynolds *et al.*[3] stated that halothane depresses the Phase 4 augmentation induced by ouabain by reducing the slope of Phase 4 depolarization. Reinterpretation of their results in light of the present investigation suggests an alternate explanation. Figure 5 from their manuscript shows clear evidence of triggered action potentials, and their tracing showing the effects of 2% halothane reveals a small DAD. Thus, the results of Reynolds *et al.*[3] do not contradict our findings. Turner *et al.*[24] have described the effects of halothane on ischemia-induced DAD in a canine 24-h infarct model studied *in vitro*. In the example presented, pacing produced several seconds of triggered beats terminating in a DAD. Halothane abolished the ability of pacing to produce triggered beats.

Two observations allow us to speculate concerning the mechanism by which halothane reduces cardiac glycoside induced DAD. First, increasing extracellular Ca^{2+} concentration antagonizes the reduction in DAD amplitude caused by halothane. Second, halothane alters the amplitude of DAD without changing coupling interval. We suggest that halothane reduces the amount of intracellular calcium available to trigger the transient inward current, either through slow channel blockade or alterations in intracellular Ca^{2+} handling, without altering the kinetics of the transient inward current.

Reduction of available calcium could occur at several sites. The role of halothane in altering intracellular calcium handling has been extensively reviewed by Rusy and Komai.[25] Halothane depresses calcium-dependent slow action potentials,[26] the slow inward calcium current,[27] and intracellular calcium transients.[28] Recently, Komai and Rusy[29] demonstrated that halothane additionally inhibits release of calcium from sarcoplasmic reticulum, and Ohnishi *et al.*[30] have shown that halothane reduces the amount of La^{3+} releasable calcium. This represents calcium stored by the sarcolemma and released to initiate contraction. Adams and Pruett[31] have demonstrated that the anesthetic enflurane inhibits Na^+–Ca^{2+} exchange. Whether halothane shares this property and the relevance of Na^+–Ca^{2+} exchange inhibition by anesthetics to reduction of DAD amplitude have not been determined.

Halothane has also been shown to reduce the amplitude of the fast inward sodium current, and could reduce DAD amplitude in a manner analogous to that of tetrodotoxin.[21] It has been suggested that tetrodotoxin, by reducing Na^+ influx, reduces intracellular Na^+, thereby causing a decay of intracellular Ca^{2+} *via* the Na^+–Ca^{2+} exchange mechanism.[32] Further experimentation will be necessary to clarify the mechanism by which halothane antagonizes DAD.

The data presented evaluate the effects of halothane on DAD that are responsible for triggered automaticity, a postulated mechanism of glycoside cardiotoxicity, and possible mechanism for clinically significant dysrhythmias in other settings.[9] Phase 4 depolarization leading to abnormal automaticity and alterations in conduction velocity, disposing to reentrant dysrhythmias, are also seen in isolated cardiac tissues made ouabain toxic.[33] Regardless of the mechanism responsible for cardiac glycoside-induced tachydysrhythmias *in vivo*[33] and for the known reduction of digitalis intoxication by halothane in intact animals,[1,2] our results show that halothane antagonizes one major cardiotoxic effect of digitalis glycosides, DAD.

The authors thank Bonnie Haubrich and Christine Donaghy for help with manuscript preparation.

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