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Effects of Halothane on Delayed Afterdepolarization and Calcium Transients in Dog Ventricular Myocytes Exposed to Isoproterenol

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The effects of halothane on isoproterenol-induced delayed afterdepolarizations (DADs) were investigated in canine ventricular myocytes. In addition, the effects of halothane on the intracellular free calcium transient were determined in fura-2–loaded myocytes exposed to isoproterenol to explore the mechanisms underlying halothane effects on DADs. Isoproterenol (100 nM) induced DADs and/or undriven action potentials in myocytes stimulated electrically with the use of trains of 10 stimuli delivered at basic drive cycle lengths of 200–1,000 ms. Isoproterenol (100 nM) increased the peak ratio (350/380 nm excitation) of stimulated myocyte calcium transients; furthermore, isoproterenol induced a second spontaneous component in the calcium transients of 62% of treated myocytes (n = 72). Halothane (1.5%, 0.53 mM) significantly decreased the amplitude of isoproterenol-induced DADs ($P < 0.01$). Halothane not only reduced the peak ratio of the stimulated calcium transient, but also eliminated the second spontaneous component in myocytes previously exposed to isoproterenol (n = 14). Elevated extracellular calcium concentrations (5 mM) restored the amplitudes of DADs and the second components of the calcium transients in myocytes exposed to isoproterenol and halothane. These data suggest that halothane opposes isoproterenol-induced DADs by altering intracellular calcium stores. The authors' findings do not support a role for DAD-induced triggered activity in the genesis of anesthetic–catecholamine dysrhythmias. (Key words: Anesthetics, volatile: halothane. Ions, calcium: calcium transient; fura-2. Heart: arrhythmias. Heart, myocytes: delayed afterdepolarizations. Sympathetic nervous system, beta-adrenergic agonists: isoproterenol.)

HALOTHANE is known for its ability to reduce the dose of exogenously administered catecholamines required to induce ventricular dysrhythmia.[1,2] Despite extensive investigation, the mechanisms underlying anesthetic–catecholamine dysrhythmias are poorly understood.[2] It has been suggested that triggered activity (TA) associated with delayed afterdepolarizations (DADs) may play a role in the genesis of dysrhythmias that result from the interaction of halothane with catecholamines[3]; however, the electrophysiologic effects of halothane have not been evaluated with the use of an experimental model of catecholamine-induced DADs in ventricular tissue.

DADs are oscillations in membrane potential that occur after repolarization of an action potential and are caused by that preceding action potential.[4] DADs can precipitate cardiac dysrhythmias by evoking excitation in otherwise nonautomatic cells.[4–6] DADs are most easily induced under conditions that favor excess concentrations of intracellular free calcium ($[Ca^{2+}]_i$): increased extracellular calcium,[7] catecholamine stimulation,[7–10] ischemia and infarction,[8,11] and cardiac glycoside toxicity.[10,12,13] DADs are caused by a transient inward current (I_{ti}) that is distinct from those responsible for the normal action potential.[13] The cellular basis for I_{ti} is controversial.[5,6] One hypothesis favors current through a nonselective membrane cation channel with conductance regulated by intracellular calcium.[14–16] Electrogenic Na^+–Ca^{2+} exchange is an alternate mechanism suggested for I_{ti}.[17,18] Evidence exists for both mechanisms, and both may be involved.[19,20]

Pharmacologic agents can influence DADs through action at several different sites (reviewed by Wit and Rosen[5]). Drugs that change the time course of repolarization, by modification of inward sodium or calcium currents or outward potassium currents, can affect DADs by indirectly affecting the intracellular concentration of calcium. Drugs that act intracellularly at the level of the sarcoplasmic reticulum (SR) can influence DADs either by suppressing or enhancing oscillatory movements of calcium between SR and myoplasm. In addition, drugs that affect Na^+–Ca^{2+} exchange or the membrane channels associated with I_{ti} may modify DADs. The effects of halothane on DADs and TA are difficult to predict. The anesthetic could potentially affect the development of DADs not only at the sarcolemma but also at the SR. Halothane would be expected to suppress DADs and TA, on the basis of its inhibition of transsarcolemmal calcium flux[21–23] and its ability to decrease SR capacity for calcium (reviewed by Rusy and Komai[24]). However, halothane depletes the SR of calcium not only by decreasing calcium uptake, but also by enhancing calcium release.[24–26] On the basis of its ability to promote SR calcium release, halothane may potentiate the development of DADs and TA. Indeed, the effects of halothane on intracellular calcium have been compared to those of caffeine.[27,28] Caffeine is also known

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to release calcium from and to impair its re-uptake into the SR of cardiac muscle; caffeine is capable of promoting and suppressing DADs and TA.[29,30]

Halothane has been shown to decrease the amplitude of DADs induced by cardiac glycosides in isolated atrial muscle[31] and Purkinje fibers[32] and to oppose formation of triggered dysrhythmias associated with digitalis intoxication.[33] It may not be assumed, however, that the effects of halothane on DADs and TA induced by catecholamines will be similar to its effects on DADs and TA associated with cardiac glycoside toxicity. Prominent differences can be found between properties of DADs and TA caused by digitalis and those caused by calcium overload unrelated to blockade of the Na^+/K^+ pump.[5,34] The current study was designed to document the effects of halothane on DADs and TA induced in isolated myocytes by isoproterenol. In addition, the effects of halothane on the intracellular free calcium transient were determined in the presence and absence of isoproterenol to explore the cellular mechanisms responsible for halothane effects on DADs.

Materials and Methods

Cell Isolation

As specified in a protocol approved by the Institutional Laboratory Animal Care Committee of The Ohio State University, mongrel dogs weighing 15–25 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). Their hearts were rapidly removed by a right lateral thoracotomy and placed in iced-cold physiologic saline. Myocytes were isolated by the collagenase perfusion technique described in detail by Hohl *et al.*[35] Briefly, an excised segment of canine left ventricle was perfused through the left anterior descending coronary artery at 20 ml/min with the following (in millimolar concentrations): 60 NaCl; 16 KCl; 3.25 $MgSO_4$; 1.2 KH_2PO_4; 25 $NaHCO_3$; 10 taurine; 11 glucose; 5 pyruvate; 80 mannitol; 0.05 ethyleneglycol-bis(β-aminoethyl ether)N,N,N′N′-tetraacetic acid (EGTA); 0.005 adenosine; amino acids (minimum essential medium [MEM] and basal Eagle's medium [BME; GIBCO]); collagenase (1 mg/ml, Types I and IV, Worthington Biochemical); and bovine serum albumin (BSA; 1 mg/ml), *p*H 7.4, 37° C. As the tissue began to soften, the perfusate was switched to Krebs-Henseleit buffer containing the following (in millimolar concentrations): 118 NaCl, 4.8 KCl, 25 $NaHCO_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 glucose, 5 pyruvate, amino acids, BSA, and collagenase. Calcium (1 mM) was added incrementally. When digestion was complete, the piece was abraded and the tissue slurry incubated in the perfusate. The dispersed myocytes were filtered through cheesecloth, sedimented, and resuspended in enzyme-free Krebs-Henseleit solution containing 11 mM glucose, 5 mM pyruvate, 2% BSA, and 25 mM HEPES.

Calcium Transients

Isolated myocytes were loaded with the acetoxymethyl ester of fura-2 (fura-2/AM) by a 5–10-min incubation in HEPES-buffered Krebs-Henseleit solution containing 2 μM fura-2/AM, followed by at least 2 h postincubation in fura-2–free solution, at room temperature. The brief exposure of the cells to fura-2/AM was necessary to eliminate excess intracellular dye accumulation. Satisfactory signal to noise ratios were obtained by the dye trapped inside the myocytes after 5–10 min exposure. The prolonged postincubation was used to facilitate hydrolysis of the ester form of fura-2.

Fura-2 fluorescence measurements were made as previously described.[36,37] Briefly, fura-2–loaded cells were placed in a perfusion chamber mounted on the stage of an inverted microscope (IM, Zeiss) and perfused continuously with Krebs-Henseleit bicarbonate buffer (2 mM Ca^{2+}) that was aerated with 95% O_2/5% CO_2 and maintained at 37° C, *p*H 7.3. Experimental compounds were added to the Krebs-Henseleit buffer, and exchange of solutions was accomplished by a pressurized valving system. Cells were stimulated by passing currents through two platinum electrodes positioned in parallel at either side of the perfusion chamber. A single quiescent, rod-shaped myocyte was selected and stimulated continuously at 0.5 Hz.

Fluorescence from the cell was recorded with the use of a PTI Deltascan system (South Brunswick, NJ). Briefly, the PTI Deltascan system was used to provide ultraviolet excitation by a 75-W xenon lamp. Two excitation wavelengths (350 and 380 nM) were switched at 80–100 Hz. Emission (420–620 nm) from the cell at the two excitation wavelengths was collected by a photomultiplier tube (Hammamatsu 928 PMT) and stored in the IBM/AT-Deltascan system. Each myocyte served as its own control. Sixteen to 20 triggered fluorescence transients were recorded for each experimental condition. Eight to 16 transients at steady state were curve-averaged with the use of the residue program provided for off-line data analysis. Because uncertainty exists regarding the accuracy of fluorescence calibration, calculation of intracellular calcium concentrations was avoided by comparing peak fluorescence ratios (350/380 nm excitation) of treated and control myocytes with the use of arbitrary units of fluorescence.

Electrophysiology

Myocytes free of fura-2 were transferred to a Plexiglass acrylic plastic perfusion chamber on the stage of an inverted microscope and perfused with bicarbonate-buffered Krebs-Henseleit (2 mM Ca^{2+}) that was aerated with 95% O_2/5% CO_2 and maintained at 37 °C, *p*H 7.3. Action potentials were recorded with the use of standard micro-

electrode techniques, as described in detail by Li *et al.*[37] Briefly, myocytes were impaled intracellularly with the use of glass microelectrodes filled with 1 M KCl (tip resistance 120–200 MΩ). Electrical stimulation of isolated cells was achieved by passing intracellular current pulses through the impaling electrode.

After a 10-min equilibration period, quiescent rod-shaped cells with resting membrane potentials negative to −75 mV were driven for 5, 10, or 20 beats at basic cycle lengths (BCLs) of 200–1,000 ms. The period immediately after the last beat of the train of stimuli was observed for the appearance of DADs. If no DADs were observed under control conditions, the stimulation protocol was repeated while the cells were perfused with buffer, containing first isoproterenol alone, and then containing isoproterenol and halothane. In a limited number of experiments, the drugs were washed out and the stimulation protocol was repeated, first in the presence of isoproterenol and halothane, and then in the presence of isoproterenol and halothane and elevated calcium levels (5 mM). When DADs were present, the amplitudes of the first DAD following the train was measured from maximum diastolic potential to the peak of the DADs.[13] Coupling intervals were measured from the upstroke of the preceding action potential to the peak of the DAD.[13]

Drug Protocols

1. Halothane: Halothane was added to the perfusate with the use of a calibrated vaporizer. The carrier gas was 95% O_2/5% CO_2. Halothane concentrations were verified with the use of gas chromatography (Varian); a vaporizer setting of 1.5% yielded on average a chamber concentration of 0.53 mM halothane. Intracellular calcium transients were measured in cells exposed to halothane (0.75–3%), in the presence and absence of isoproterenol (100 nM). The effects of halothane (1.5%) on the amplitude of isoproterenol-induced DADs were examined in a separate set of experiments.
2. Isoproterenol: Isoproterenol solutions were freshly prepared before each experimental period. The heavy metal chelator ethylenediaminetetraacetic acid disodium dihydrate (Na_2EDTA) (50 μM) was added to all solutions containing isoproterenol to retard oxidation. DADs and/or TA were induced in canine myocytes exposed to isoproterenol concentrations in the range 10 nM to 1 μM. In preliminary experiments, isoproterenol concentrations less than 100 nM did not induce DADs consistently, whereas isoproterenol concentrations greater than 100 nM often induced early afterdepolarizations and TA in addition to DADs. Therefore, isoproterenol (100 nM) was used in subsequent experiments.

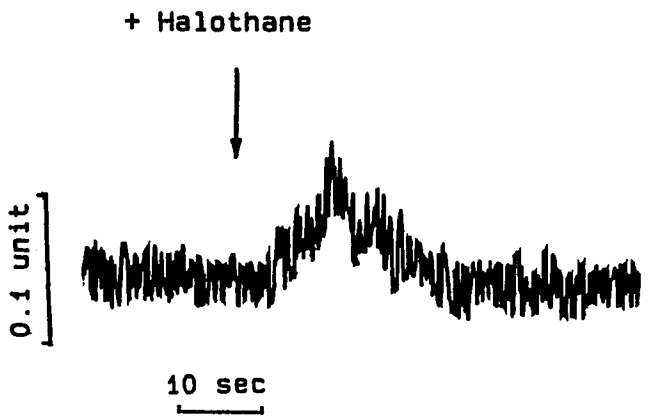

FIG. 1. Calcium response from quiescent canine myocyte during acute introduction of halothane (1.5 vol% = 0.52 mM, 37° C). The response shown was observed in 50% of 14 fura-2-loaded myocytes acutely exposed to halothane.

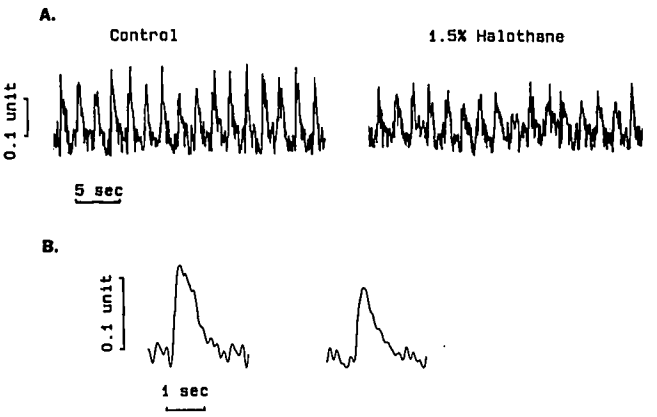

FIG. 2. Representative recordings of Ca^{2+} transients from a single canine myocyte presented on the same absolute scale. (*A*) Fura-2-loaded myocytes were perfused with Krebs–Henseleit bicarbonate buffer at 37° C and pH 7.3 and excited by electrical field stimulation (0.5 Hz). The elicited Ca^{2+} transients (fluorescence ratios 350/380-nm excitation) were recorded from a single myocyte in the absence and presence of 1.5% halothane. (*B*) Averaged Ca^{2+} transients from 16 consecutive beats in (*A*) demonstrate the depressant effect of halothane on the Ca^{2+} transients.

Statistical Analysis

Delayed afterdepolarization amplitudes and peak heights of calcium transients were compared with the use of either the *t* test for paired data or repeated measures analysis of variance with Bonferroni's modification of the *t* test. Results are presented as mean ± SEM. A probability level of 0.05 was used to detect significant differences.

Results

Calcium Transients

Acute exposure of canine myocytes to perfusate containing halothane (1.5%) caused a transitory increase in

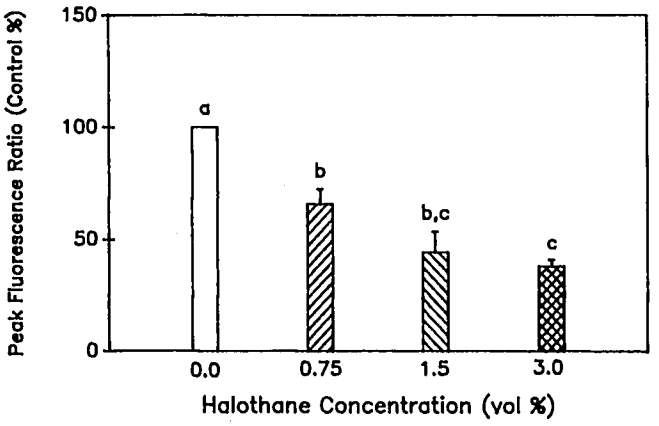

FIG. 3. Concentration-dependent depression by halothane of Ca^{2+} transients from electrically stimulated (0.5 Hz) myocytes. Summarized data from myocytes exposed to varying concentrations of halothane for approximately 1 min (0.0% [n = 14]; 0.75% [n = 5]; 1.5% [n = 7]; 3% [n = 7]). To facilitate comparisons, data are expressed as percentage of the peak fluorescence ratio measured under control conditions. Bars without a common superscript are significantly different at $P < 0.05$.

free intracellular calcium in 50% of myocytes studied (n = 14). Fluorescence from a quiescent myocyte acutely exposed to halothane is shown in figure 1. This calcium transient produced during the introduction of halothane was not an artifact of changing solutions; similar calcium transients were not produced by changing from control solution to control solution. Acute introduction of halothane (1.5%) to electrically stimulated myocytes also resulted in brief enhancement of calcium transients in 50% of cells studied (n = 9); however, within 1 min of the introduction of halothane, the amplitude of stimulated calcium transients was decreased consistently (fig. 2). Halothane decreased the peak ratio of calcium transients in a concentration-dependent manner (fig. 3).

The beta-adrenoceptor agonist isoproterenol (100 nM) increased the peak ratio of stimulated myocyte calcium transients; furthermore, in 62% of myocytes treated (n = 72), 100 nM isoproterenol induced a second spontaneous component in the calcium transient (figs. 4 and 5). Halothane antagonized isoproterenol enhancement of the calcium transient in all cells. In cells previously exposed to 100 nM isoproterenol (n = 15), halothane (1.5%) not only reduced the peak ratio of the stimulated calcium transient, but also eliminated the second component (figs. 4 and 5). Increasing the extracellular calcium concentration ($[Ca^{2+}]_o$) from 2 to 5 mM restored the second component of the calcium transient in myocytes previously exposed to isoproterenol (100 nM) and halothane (1.5%) (figs. 4 and 5); 5 mM $[Ca^{2+}]_o$ alone did not induce a second component in the calcium transient (n = 10).

ELECTROPHYSIOLOGY

When canine ventricular myocytes were stimulated with trains of 5, 10, or 15 impulses delivered at a BCL of 200–1,000 ms, neither TA nor DADS were recorded under control conditions from any of 30 myocytes isolated from six hearts. Identical stimulation protocols did induce DADs and TA in cells exposed to isoproterenol (100 nM). The amplitudes of the DADs induced by isoproterenol

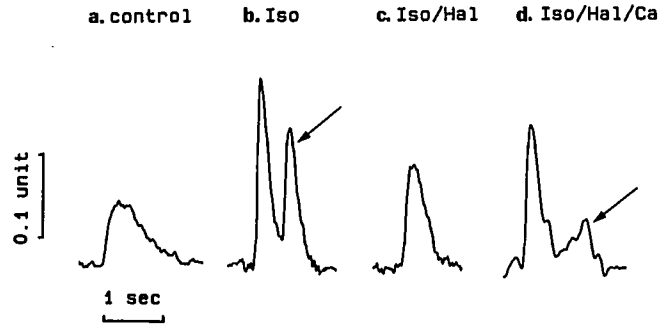

FIG. 4. Effects of isoproterenol, halothane, and high $[Ca^{2+}]_o$ on Ca^{2+} transients from electrically stimulated (0.5 Hz) canine myocytes. Fluorescence data (ratio 350/380-nm excitation) from a single myocyte under four different conditions are presented on the same absolute scale. Each trace represents the average of 16 consecutive beats at steady state. (*A*) Control. (*B*) Isoproterenol (100 nM) increased the magnitude and shortened the duration of the stimulated Ca^{2+} transient. A second spontaneous component of the Ca^{2+} transient (*arrow*) was also evident in the presence of isoproterenol. (*C*) Halothane (1.5%) not only depressed the peak of the Ca^{2+} transient but also eliminated the second component. (*D*) A second spontaneous component of the calcium transient was again evident when $[Ca^{2+}]_o$ was increased from 2 to 5 mM.

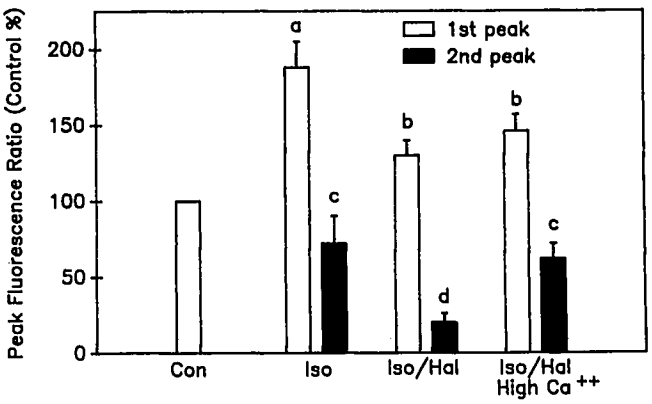

FIG. 5. Effects of isoproterenol, halothane, and high $[Ca^{2+}]_o$ on Ca^{2+} transients from electrically stimulated (0.5 Hz) myocytes. In untreated cells, only Ca^{2+} transients with a single stimulated peak were seen. Isoproterenol (100 nM) caused the appearance of Ca^{2+} transients with a second spontaneous component. Halothane depressed both the first and second peaks of Ca^{2+} transients in cells previously exposed to isoproterenol. Increasing $[Ca^{2+}]_o$ from 2 to 5 mM restored the second but not the first peak of Ca^{2+} transients in cells exposed to both isoproterenol and halothane. Bars without a common superscript are significantly different at $P < 0.05$ (n = 15).

were influenced both by the number and BCL of the applied stimuli; DAD amplitudes tended to increase as the number of preceding beats increased (fig. 6) or the BCL shortened (fig. 7). With the use of trains of 10 stimuli, DADs were first induced at BCLs of 900–1,000 ms in 54% of myocytes; 700–800 ms in 18% of myocytes; 500–600 ms in 0% of myocytes; and 300–400 ms in 8% of myocytes (n = 24). TA, consisting of either one to three undriven action potentials or sustained runs of electrical activity, was observed in 46% of the 24 myocytes studied. In myocytes exhibiting TA, undriven action potentials were first recorded at BCLs of 900–1,000 ms in 18%; 700–800 ms in 55%; 500–600 ms in 18%; and 300–400 ms in 9%. The coupling interval of TA to the last driven action potential coincided approximately with that of the preceding DADs (fig. 7).

Triggered activity observed in the presence of isoproterenol was eliminated during introduction of 1.5% halothane (n = 10) and returned on washout of halothane. Acute exposure to halothane consistently suppressed DADs, even though 50% of fura-2–loaded cells showed a transient increase in free intracellular calcium during similar introduction of halothane (see above). In myocytes exposed sequentially to isoproterenol, then isoproterenol

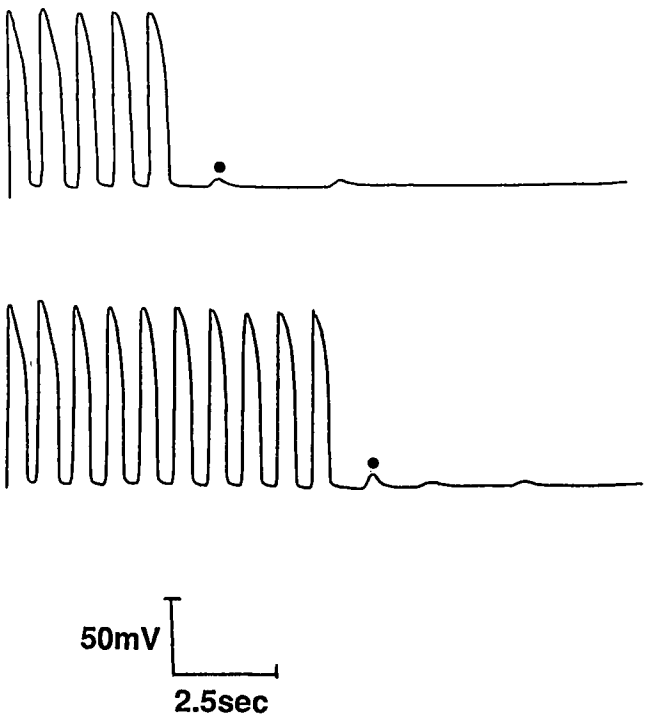

FIG. 6. The amplitude of isoproterenol-induced delayed afterdepolarizations (DAD) increased as the number of preceding driven action potentials increased. Representative records from an isolated myocyte stimulated at a drive cycle length of 800 ms with trains of five (*top*) and ten (*bottom*) stimuli, in the presence of 100 nM isoproterenol. The first DAD after each train of stimuli is indicated by a dot.

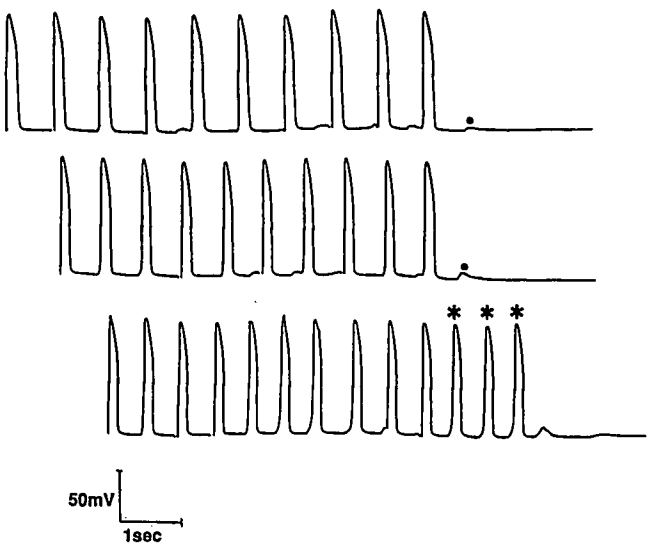

FIG. 7. The amplitude of isoproterenol-induced delayed afterdepolarizations (DAD) increased as the drive cycle length decreased. Representative records from an isolated myocyte stimulated using trains ten stimuli at basic cycle lengths of 800 (*top*), 700 (*middle*), and 600 (*bottom*) ms, in the presence of 100 nM isoproterenol. The first DAD after each train of stimuli is indicated by a dot. Undriven action potentials are indicated by asterisks.

with halothane, the amplitude of DADs observed in the presence of isoproterenol was 5.9 ± 0.6 mV, whereas the amplitude of DADs observed during the acute introduction of halothane was 2.4 ± 0.7 mV (n = 11, $P < 0.01$). Halothane significantly decreased the amplitude of DADs induced over the range of BCLs studied (fig. 8); DAD amplitudes diminished by halothane were restored during washout of the anesthetic.

In the presence of halothane, the amplitudes of iso-

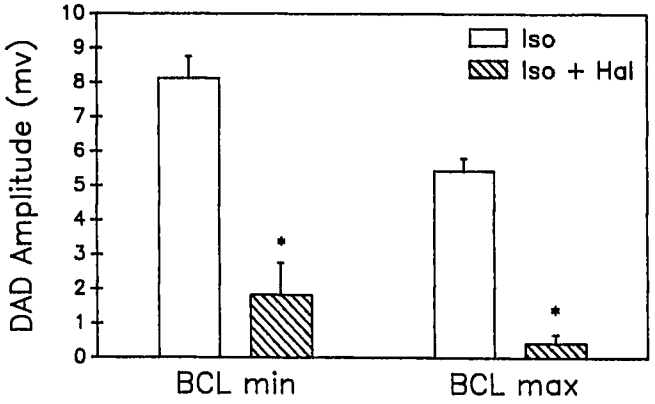

FIG. 8. Effect of halothane (1.5%, 60–90 s) on the amplitude of delayed afterdepolarizations (DAD) induced by isoproterenol (100 nM) with trains of ten stimuli. Data shown for the shortest (BCL min) and longest (BCL max) basic drive cycle lengths at which stable DAD were induced (n = 24). Amplitudes depicted for the first DAD after the stimulus train. Asterisks indicate a significant difference due to the addition of halothane ($P < 0.01$).

proterenol-induced DADs were restored by increasing $[Ca^{2+}]_o$ from 2 to 5 mM (fig. 9). The effect of high calcium concentrations on DAD amplitude was reversed on return to physiologic concentrations (2 mM); 5 mM calcium alone did not induce DADs (n = 5).

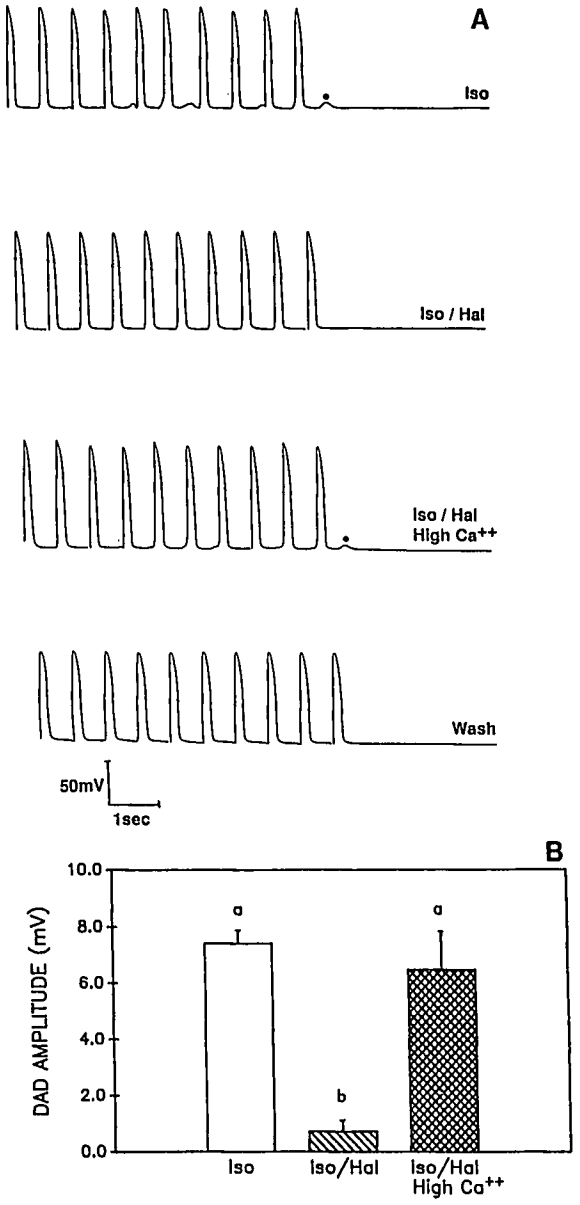

FIG. 9. Restoration by calcium of delayed afterdepolarizations (DAD) suppressed by halothane. (*A*) Representative data from a single myocyte. Where present, the first DAD after each train of stimuli is indicated by a dot. DAD were induced by exposure to isoproterenol (100 nM) in the presence of a physiologic $[Ca^{2+}]_o$ (2 mM) (*first trace*). Halothane (1.5%) significantly decreased the amplitude of such DAD (*second trace*). Subsequent exposure of halothane-treated myocytes to a higher $[Ca^{2+}]_o$ (5 mM) restored DAD amplitude (*third trace*). DAD were abolished upon return to control conditions (*fourth trace*). BCL = 650. (*B*) Summary data from eight myocytes. Bars without a common superscript are significantly different at $P < 0.01$.

Discussion

In the current study, we developed and used a myocyte model to study the effects of halothane on isoproterenol-induced DADs. In addition, halothane effects on free intracellular calcium transients were determined in fura-2–loaded myocytes exposed to isoproterenol, and the results were correlated with the electrophysiologic data. Halothane depressed the amplitudes of both isoproterenol-induced DADs and isoproterenol-enhanced calcium transients in canine myocytes. These data suggest that halothane opposes formation of catecholamine-induced DADs and TA by altering intracellular calcium stores.

Delayed afterdepolarizations have been observed previously in cardiac myocytes exposed to isoproterenol.[38] Preliminary reports from our laboratory and others'[39–42] have suggested that isolated heart cells can be used to study catecholamine-induced TA. In these experiments, we used isoproterenol to induce DADs in canine myocytes and found that the characteristic properties of the DADs were similar in many respects to those of DADs induced by catecholamines in multicellular preparations. When DADs were induced, the amplitude of the first afterdepolarization increased as the drive cycle length decreased, resulting, in some cases, in TA (fig. 7). DAD amplitude also was dependent on the number of preceding action potentials (fig. 6). Thus, the dependence of DAD amplitude on rate and rhythm was similar in isolated myocytes and multicellular preparations.[8,9] Similarities in DAD behavior between isolated cells and tissues strongly support similarities in underlying mechanisms. Activation of I_{ti} by an increase in intracellular calcium concentration is believed to be responsible for generating DADs and TA in myocytes as well as in multicellular preparations.[39,41,43,44]

A transitory increase in free intracellular calcium during acute exposure of canine myocytes to halothane was observed inconsistently when intracellular calcium was monitored with the use of fura-2 fluorescence of individual cells (fig. 1). Transient increases in fluorescence during introduction of halothane were previously described in suspensions of rat heart cells loaded with either quin-2[27] or fura-2.[28] This difference between our data and those of the other groups[27,28] may reflect species differences in myocyte calcium handling,[45] differences in methods,[46] or both. Our finding that halothane decreased the peak height of the intracellular free calcium transient in electrically stimulated myocytes (figs. 2 and 3) is similar to published observations from isolated ventricular myocytes[28] and multicellular preparations.[47,48] Bosnjak *et al.*[48] reported a 55% decrease in intracellular calcium, induced by 0.55 mM halothane; our data demonstrate a 56% decrease in intracellular calcium, in the presence of 0.53 mM halothane (fig. 3).

We have shown for the first time depression of isopro-

terenol-enhanced calcium transients by halothane. In cells previously exposed to isoproterenol, halothane not only reduced the peak ratio of the stimulated calcium transient, but also eliminated the second spontaneous component (figs. 4 and 5). Such secondary fluctuations in fluorescence have been associated with activation of I_{ti} and induction of DADs and aftercontractions in myocytes.[40,44] Halothane consistently depressed isoproterenol-induced DADs and TA in cardiac myocytes (fig. 8). Depression of DADs by halothane was probably related to similar effects on the intracellular calcium transients.

Proposed mechanisms for induction of DADs by isoproterenol are shown in figure 10. Our observation that increased extracellular calcium can restore DADs suppressed by halothane (fig. 9) suggests that halothane opposes DAD formation by reducing the amount of intracellular calcium available to activate I_{ti}. Reduction of available calcium may involve halothane action at several sites. Halothane may interfere with isoproterenol stimulation of inward calcium current by decreasing sarcolemmal beta-adrenoceptor density,[49,50] by interfering with the generation of the second messenger cyclic adenosine monophosphate (cAMP),[51,52] or by decreasing the magnitude of the slow inward calcium current (I_{si}) by some direct action at the L-type calcium channel.[21-23,53,54] In addition, halothane may decrease the total calcium content of myocytes by interfering with SR calcium storage.[24-28] Halothane depression of intracellular calcium transients, in the presence and absence of isoproterenol, is consistent with these proposed mechanisms for suppression of DADs.

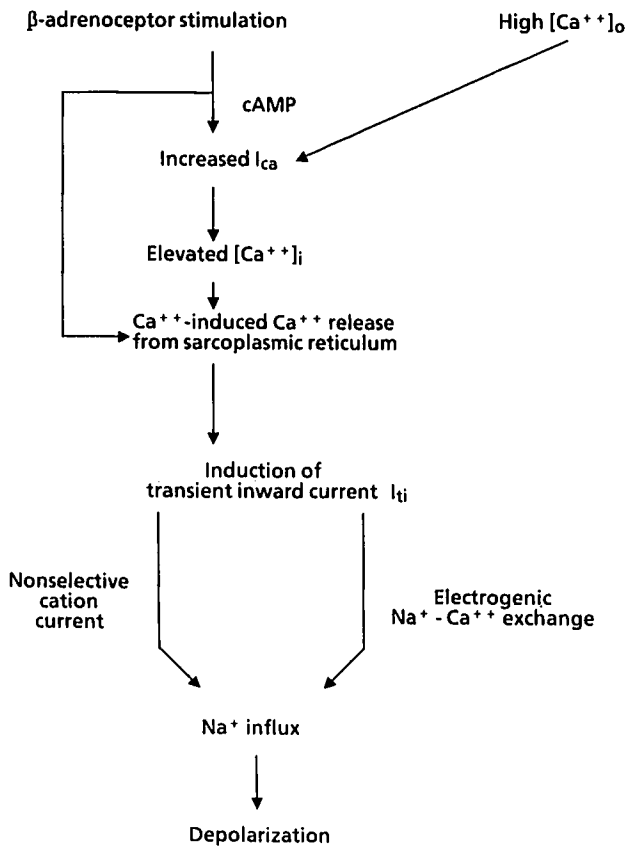

FIG. 10. Mechanisms proposed for induction of delayed afterdepolarizations by β-adrenergic agonists such as isoproterenol.

Spontaneous "aftertransients" similar to those shown in figure 4 have been associated with activation of I_{ti} by calcium-induced calcium release from SR.[19,44] In all mammalian species, calcium released from the SR dominates the calcium transient; however, calcium entering the cytoplasm through L-type calcium channels does make a direct contribution to the calcium transient.[55] Halothane-induced decreases in the peak height of calcium transients have been associated with both depletion of SR Ca^{2+}[28] and reduction of I_{si}.[2,47,48] Volatile anesthetics have also been shown to inhibit Na^+–Ca^{2+} exchange[56,57] and to decrease the magnitude of other transsarcolemmal ionic currents.[22] Direct effects of halothane on I_{ti} cannot be ruled out on the basis of the data presented and may exist, in addition to halothane effects on inward calcium current and intracellular calcium storage.

The mechanisms responsible for halothane suppression of isoproterenol-induced DADs may be similar to those responsible for halothane suppression of ouabain-induced DADs; in both settings DADs are restored by increased $[Ca^{2+}]_o$.[32] Additional experimentation will be necessary to clarify the mechanisms by which halothane antagonizes isoproterenol-induced DADs and TA.

We investigated the effects of halothane on catecholamine-induced DADs and TA, a potential mechanism for clinically significant dysrhythmias. In isolated canine myocytes, halothane depressed DADs and TA induced by the beta-adrenergic agonist isoproterenol. Both alpha- and beta-adrenergic stimulation are believed to play a role in the genesis of anesthetic–catecholamine arrhythmias.[2] In view of recent findings that alpha-adrenoceptor stimulation neither induces DADs[39] nor potentiates DADs induced by beta-adrenoceptor stimulation,[42] our data suggest that DADs and associated TA are unlikely to play an important role in the dysrhythmogenic interaction between halothane and adrenergic amines. However, additional experiments are needed to determine the combined effects of halothane and alpha- and beta-adrenergic stimulation.

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