A Model for Early Afterdepolarizations: Induction With the Ca^{2+} Channel Agonist Bay K 8644

Craig T. January, Janet M. Riddle, and Joseph J. Salata

Early afterdepolarizations (EADs) are one mechanism proposed to cause certain cardiac arrhythmias. We studied the effect of the Ca^{2+} channel agonist Bay K 8644 (1×10^{-8} to 5×10^{-5} M) on normally polarized sheep and canine cardiac Purkinje fiber short segments. EADs occurred with higher Bay K 8644 concentrations and had an average take-off potential of −34 mV. The initiation of EADs was preceded by lengthening of action potential duration and flattening of the plateau. Induction of EADs with Bay K 8644 was enhanced by low stimulation frequencies, lowering of $[K]_o$, addition of tetraethylammonium chloride, or application of depolarizing constant current pulses during the plateau. EADs were abolished by increasing stimulation frequency, raising $[K]_o$, the addition of tetrodotoxin, lidocaine, ethmozin, verapamil, and nitrendipine, or application of repolarizing constant current pulses. Using current pulses to modify the action potential plateau, a steep inverse relationship was found between the EAD peak voltage and its take-off potential, and EADs could be initiated over only a narrow range of take-off potentials. Thus, interventions that suppressed EADs shortened action potential duration or shifted the plateau away from the voltage range needed to initiate EADs. These observations suggest that mechanisms dependent on both time and voltage underlie EADs, and provide a unifying hypothesis for the induction of the EADs. We propose that induction of EADs requires 1) lengthening of action potential duration within a plateau voltage range where 2) recovery from inactivation and reactivation of an inward current possibly carried through Ca^{2+} channels can occur. (*Circulation Research* 1988;62:563–571)

Afterdepolarizations giving rise to triggered activity have been suggested to cause cardiac arrhythmias (for reviews see Cranefield[1] and Hoffman and Rosen[2]). Afterdepolarizations that develop following complete repolarization of the action potential (delayed afterdepolarizations, DADs) are prototypically induced with cardiac glycoside toxicity, are associated with characteristic changes in excitability,[3] and may give rise to arrhythmias and conduction disturbances.[4] Afterdepolarizations can also develop before repolarization of the action potential is complete. These early afterdepolarizations (EADs) interrupt and delay repolarization[1] of the action potential. In contrast to DADs, which are triggered by rapid stimulation, EADs are more likely to occur at low stimulation frequencies and with lengthening of action potential duration. Although recognized prior to DADs, EADs have received less study, and their underlying mechanism(s) remain unknown. Recently, EADs have assumed increasing importance as a possible arrhythmogenic mechanism. For example, interventions such as Cs^+ or possibly quinidine, which lengthen action potential duration and produce EADs in isolated tissue, have also been shown in intact animal studies to lengthen the QT interval and to induce polymorphic ventricular tachycardia resembling "torsades de pointes."[5–8]

Bay K 8644 is an agonist for Ca^{2+} channel current. In contrast to most dihydropyridines, it has been shown to lengthen the cardiac action potential and to exert a positive inotropic effect.[9,10] We have developed a new model for the induction and study of EADs in cardiac Purkinje fiber short segments using Bay K 8644. EADs were induced in most fibers exposed to higher concentrations of Bay K 8644 and stimulated at low frequencies. Consistent changes in action potential characteristics and plateau voltages preceded the initiation of EADs. Using this model, we systematically studied the effect on EADs of interventions that alter action potential characteristics by different mechanisms. Our results show that several interventions are successful in modifying or abolishing the EADs we studied, permitting a unifying hypothesis for the mechanisms underlying these EADs.

A preliminary report of this work has appeared.[11]

Materials and Methods

Preparations

In most experiments, sheep Purkinje fibers were used.[3] Sheep hearts were obtained from a nearby slaughterhouse, rinsed free of blood, and transported to the laboratory in cooled oxygenated Tyrode's solution. In a few experiments, Purkinje fibers from canine hearts were used. Hearts were rapidly removed from mongrel dogs anesthetized with sodium pentobarbital

From the Cardiac Electrophysiology Laboratories, Department of Medicine, Section of Cardiology, The University of Chicago, Chicago, Illinois.

Supported in part by grants HL 31322 and HL 21788 from the National Institutes of Health, Bethesda, Maryland, and Grant-in-Aid 84 1151 from the American Heart Association, Dallas, Texas.

Address for reprints: Craig T. January, MD, PhD, Department of Medicine, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637.

Received June 1, 1987; accepted October 1, 1987.

(30 mg/kg i.v.). Single free-running unbranched Purkinje fibers having diameters of 60–250 μm were used. Fine wire ties were placed around fibers to isolate short segments of <2.0 mm in length. One end of the preparation was pinned distal to the wire tie to the wax floor of a lucite chamber (volume 1.5 ml). The other end of the preparation was attached by its wire tie to a tension transducer (Cambridge Technology, Cambridge, Massachusetts). Fibers were mechanically loaded by stretching them 20% to 30% beyond slack length. Preparations were superfused with Tyrode's solution at a rate of 3–5 ml/min, and the bath temperature was maintained at 37±0.5°C.

Electrical Recording

Membrane potential was measured with glass microelectrodes (1B150F, World Precision Instruments, New Haven, Connecticut) that were filled with 3 M KCl, had resistances of 5–12 MΩ and had tip potentials <5 mV. Similar microelectrodes were used for current injection. The current-passing microelectrode was inserted near the center of the preparation and was within 200 μm of the voltage recording microelectrode. The signal from each microelectrode was fed to the input of a high-input impedance amplifier before being displayed on a digital voltmeter. The current-passing electrode also could be connected to a multichannel current source (World Precision Instruments). Stimulating current pulses usually were 2 msec in duration with amplitudes of 1.5 times the threshold. The bath was held at ground potential by a current-to-voltage circuit. Signals were displayed on a storage oscilloscope (Tektronix, Beaverton, Oregon) and a stripchart recorder (Gould, Cleveland, Ohio). The signals also were stored on FM tape (A.R. Vetter, Redersburg, Pennsylvania; recording speed 3¾ or 7½ ips) and replayed (15/16 ips) on the stripchart recorder giving an effective bandwidth (−3 db) of 0.5 or 1.0 kHz. Because of limitations in frequency response of the stripchart recorder, this method led to attenuation of rapid transients (action potential overshoot, stimulus pulse amplitude) but otherwise did not distort the records.

Solutions

Tyrode's solution contained (mM) 127 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 2.4 NaH_2PO_4, 22 $NaHCO_3$, and 5.5 glucose, was gassed continuously with a mixture of 95% O_2-5% CO_2, and had a pH of 7.4.

Bay K 8644 [methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate] was supplied by Miles Pharmaceuticals (West Haven, Connecticut) as a racemic mixture. Bay K 8644 was dissolved in absolute ethanol to form a 1×10^{-2} M stock and diluted for each experiment with Tyrode's solution usually to a concentration of 1×10^{-6} M, from which further dilutions were prepared. The final ethanol concentration was less than 0.01%, and had no electrophysiological or mechanical effects ($n=3$). In a few experiments, Bay K 8644 was diluted in Tyrode's solution to a concentration of 1×10^{-5} M to obtain higher test concentrations. In all experiments, room and chamber lighting was extinguished during drug superfusion to avoid photoinactivation of Bay K 8644.

Tetrodotoxin (TTX, Calbiochem, San Diego, California, and Sigma, St. Louis, Missouri), lidocaine hydrochloride (Astra, Santa Clara, California), verapamil hydrochloride (Knoll Pharmaceutical, Whippany, New Jersey), and ethmozin (a gift from Dr. A. Undrovinas, All-Union Cardiology Research Center, Moscow, USSR) were dissolved in distilled water to form separate stock solutions. Nitrendipine (Miles Pharmaceuticals) was dissolved in absolute ethanol to form a stock solution (1×10^{-2} M). Each drug stock was added to Tyrode's solution containing 1×10^{-6} M Bay K 8644 to final drug concentrations of 4×10^{-6} M TTX, 1.7×10^{-5} M lidocaine, $1–2\times10^{-6}$ M verapamil, $1–5\times10^{-6}$ M nitrendipine, and 4×10^{-6} M ethmozin. The addition of drug stock solution did not alter the ionic concentration of Tyrode's solution by more than 0.1%. In other experiments, tetraethylammonium chloride (TEA, Sigma) was directly added at a concentration of 10 mM or the KCl concentration was reduced to 3.5 mM without osmotic correction.

Experimental Protocols

Fibers were stimulated at 1 Hz usually for a 1-hour equilibration period before experiments were begun. Because EADs can occur in freshly isolated fibers, our experimental protocols required that every fiber for study have normal resting and action potentials and be free of automaticity and afterdepolarizations. To characterize the control Purkinje fiber preparation and the concentration- and frequency-dependent effects of Bay K 8644, the following protocol was used. At the end of the equilibration period, control measurements were made of the following parameters: maximum diastolic potential; action potential overshoot; action potential duration at 90% of repolarization (APD_{90}); the action potential plateau voltage at 100, 150, and 200 msec following upstroke; and twitch tension amplitude. The stimulation frequency then was reduced from 1 Hz to 0.2 Hz for 4–5 minutes, and the measurements were repeated. Measurement of APD_{90} was made at the same membrane voltage used at the 1-Hz stimulation frequency. The stimulation frequency was returned to 1 Hz and Bay K 8644 was introduced into the chamber. We applied concentrations of 1×10^{-8} M, 5×10^{-8} M, 1×10^{-7} M, 5×10^{-7} M, 1×10^{-6} M, and 5×10^{-6} M. Because the effect of Bay K 8644 is not easily removed with washout of the drug from the chamber, the drug was always applied in increasing concentrations. All preparations were exposed to 1×10^{-6} M Bay K 8644 but not always to every other concentration. At each drug concentration the preparation was stimulated at 1 Hz for 5 minutes before action potential characteristics and twitch tension amplitude were measured. The stimulation frequency was then reduced to 0.2 Hz for 4 minutes, and the measurements were repeated. These time periods permitted action potential characteristics and twitch tension amplitude to reach steady state (see also Thomas et al[9]), yet usually allowed us to complete

the protocol within 1 hour. In two fibers, the stimulation frequency was kept constant (one fiber each at 1 and 0.2 Hz) with the Bay K 8644 concentration incremented as described above. Fibers that did not develop EADs before completion of the protocol were exposed to 1×10^{-6} M Bay K 8644, were continuously stimulated (0.2–0.5 Hz), and were observed for up to one additional hour for development of EADs.

A second protocol was used to test the effect on EADs of several interventions that modify action potential duration. These included interventions directed toward Na^+ channels (TTX, lidocaine, and ethmozin), Ca^{2+} channels (verapamil and nitrendipine), repolarizing K^+ current (lowered $[K]_o$, TEA), and the passage of polarizing constant current pulses. For this protocol, fibers were continuously exposed to 1×10^{-6} M Bay K 8644 Tyrode's solution and stimulated at a constant frequency sufficiently low to give a single EAD with each action potential (0.2 to 1 Hz), or at a frequency slightly higher so that no EADs were present. One of the above interventions then was introduced, and in most fibers, the return to the initial condition also was studied.

In these experiments, we established conditions to study a single EAD initiated with each action potential. We did not focus on conditions where multiple EADs or sustained rhythmic activity were present. Conditions which favored these included very low stimulation frequencies, lowered $[K]_o$, and TEA. Where appropriate, data are given as mean ± SEM. For each fiber, twitch tension amplitude was normalized as percent of the control value at the same stimulation frequency. Data were analyzed using a paired t test and least-squares regression analysis. A p value of <0.05 was considered statistically significant.

Results

Induction of EADs: Concentration- and Frequency-Response to Bay K 8644

In the initial series of Purkinje fibers ($n = 10$) under control conditions and at a stimulation frequency of 1 Hz, the maximum diastolic potential was -85.1 ± 1.5 mV, action potential overshoot was 22.4 ± 3.2 mV, and the APD_{90} was 280.0 ± 32.4 msec. Figure 1 shows typical concentration- and frequency-dependent effects of Bay K 8644 on action potentials and twitch tension. At 1 Hz (Panel A), 1×10^{-8} M Bay K 8644 decreased action potential duration slightly. Higher concentrations of Bay K 8644 produced a concentration-dependent increase in action potential duration and a positive shift in the voltage level of the plateau. At the 0.2-Hz stimulation frequency the maximum diastolic

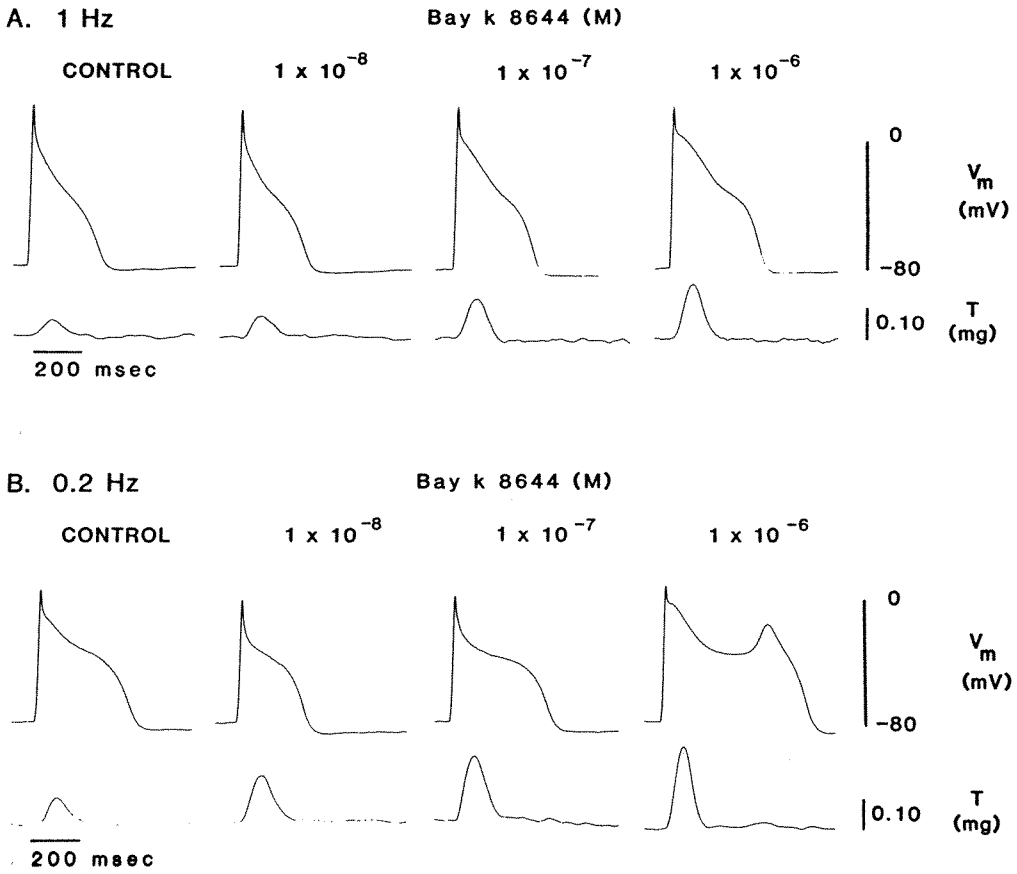

FIGURE 1. *Frequency- and concentration-dependent effects of Bay K 8644 in a sheep Purkinje fiber short segment. Representative action potentials and twitches are shown at stimulation frequencies of 1 Hz* (A) *and 0.2 Hz* (B). *Bay K 8644 concentration-dependent lengthening of action potential duration was present at both stimulation frequencies. At the 0.2-Hz stimulation frequency, flattening of the action potential plateau preceded the development of early afterdepolarizations.*

otential was unchanged; however, diastolic depolarization caused the take-off potential and action potential overshoot to be reduced. With 1×10^{-8} M Bay K 8644 there was a slight decrease in action potential duration. At 1×10^{-7} M Bay K 8644, action potential duration was similar to control, although the plateau was flattened reflecting slowing of repolarization. At 1×10^{-6} M Bay K 8644, action potential duration increased further and was followed by the abrupt development of a single EAD with each action potential. At both frequencies of stimulation, twitch tension amplitude increased with increasing concentrations of Bay K 8644.

As shown in Figure 1, EADs could be induced in fibers exposed to higher concentrations of Bay K 8644 and stimulated at low frequencies. In four out of nine fibers stimulated at 0.2 Hz, EADs developed before completion of the protocol. In four additional fibers, EADs developed after completion of the protocol with continued exposure (up to 45 minutes) to 1×10^{-6} M Bay K 8644. Thus, EADs developed in 89% of this initial series of fibers. The lowest concentration of Bay K 8644 that induced EADs was 1×10^{-7} M ($n=1$). We arbitrarily chose three EADs from each fiber for analysis to quantify EAD characteristics (24 measurements in 8 fibers). These EADs were initiated from a take-off potential (the most negative plateau voltage reached before the depolarization of the EAD) of -33.9 ± 1.2 mV (range -44 to -15 mV) and reached a peak voltage of -23.2 ± 1.6 mV (range -34 to -6 mV). The average amplitude was 10.6 ± 1.6 mV, and the coupling interval between the action potential upstroke and the peak voltage of the EADs was 532.4 ± 59.0 msec (range 320 to 872 msec).

We also analyzed the effect of Bay K 8644 on several action potential characteristics in these fibers. Electrophysiological measurements were not made after EADs had developed in a fiber. The effects of Bay K 8644 on APD_{90} are summarized in Table 1. The most obvious effect was a concentration-dependent lengthening of APD_{90} at both stimulation frequencies. At 1 Hz, the average control APD_{90} was 280.0 msec, and this increased to 364.9 msec after exposure to 1×10^{-6} M Bay K 8644 ($p<0.002$). At 0.2 Hz, the average control APD_{90} was 300.0 msec. With 1×10^{-6} M Bay K 8644, APD_{90} in the fibers without EADs averaged 342.5 msec ($p<0.038$). There was a trend toward shortening of APD_{90} at the lowest Bay K 8644 concentrations compared with the respective control values; however, this was not statistically significant at 1 Hz ($p=0.21$) or at 0.2 Hz ($p=0.16$). Although the average APD_{90} was slightly longer at 1 Hz, EADs developed only at 0.2 Hz. Table 1 also shows that Bay K 8644 produced a concentration-dependent augmentation of twitch tension amplitude even at the lowest concentration tested.

As shown in Figure 2, Bay K 8644 also produced changes in the action potential plateau voltages. A concentration-dependent shift in the plateau voltages to less negative potentials was present at both stimulation frequencies, and plateau voltages were more negative at 0.2 Hz. At 1 Hz, the difference between these voltages remained relatively constant for all Bay K 8644 concentrations. In contrast, at 0.2 Hz, there was a marked concentration-dependent decrease in the difference between the plateau voltages, which reflects slowing of repolarization. This confirms the observation shown in Figure 1 that with low stimulation frequencies and higher Bay K 8644 concentrations, progressive flattening of the action potential preceded development of EADs.

Induction and Block of EADs with Interventions That Modify Outward and Inward Membrane Currents

Enhancement of induction of EADs with lowered $[K]_o$ and TEA. Reduction in bath $[K]_o$[12] or the addition of TEA[13] is known to lengthen action potential duration, and these interventions enhanced the induction of EADs by Bay K 8644. In Figure 3A, $[K]_o$ initially was 5.4 mM and EADs had not developed (left trace) despite 30 minutes of exposure to Bay K 8644. After switching to a $[K]_o$ of 3.5 mM (dashed line), the

Table 1. Concentration- and Frequency-Dependent Effects of Bay K 8644 on Action Potential Duration and Twitch Tension Amplitude

		Bay K 8644 (M)					
	Control	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}
1 Hz							
APD_{90}	280.0±32.4	256.7±15.6	273.1±11.1	291.3±15.6	309.8±11.0	364.9±21.6	372.0±108.0
T	100.0	135.2±16.4	168.6±25.1	204.4±35.2	264.9±38.8	316.9±44.9	379.0±57.2
n	9	7	7	9	9	9	2
0.2 Hz							
APD_{90}	300.9±53.4	219.3±14.1	248.7±25.5	247.4±13.9	282.0±16.2	342.4±57.1	492.0±200.0
n	9	6	6	7	6	5	2
T	100.0	125.8±23.4	120.4±15.2	153.5±32.5	186.6±29.7	212.0±27.3	283.0±49.6
n	9	6	6	8	8	9	2

Results are from the initial group of fibers studied. Fibers developing early afterdepolarizations (EADs) at 0.2 Hz during performance of the protocol were excluded from further analysis of action potential duration. This accounts for the different *n* at 0.2 Hz. APD_{90}, action potential duration in milliseconds at 90% of repolarization; T, normalized tension (%). Control twitch tension amplitude at 1 and 0.2 Hz was 0.61±0.18 and 0.76±0.28 mg, respectively.

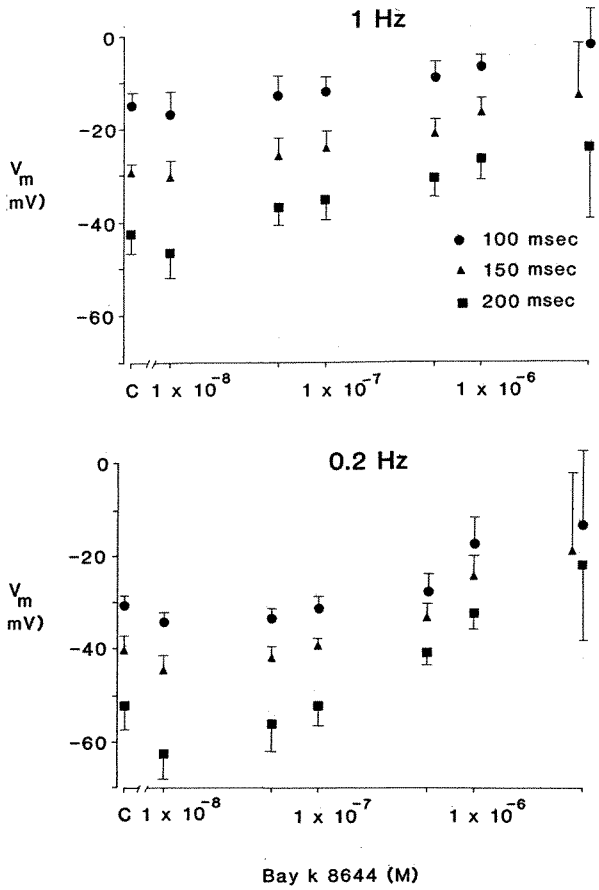

FIGURE 2. *Frequency- and concentration-dependent changes in the action potential plateau voltages with Bay K 8644. In each fiber, the action potential plateau voltage was measured 100, 150, and 200 msec following the upstroke. Mean values ± SEM are shown with the n's identical to those given for the action potential duration measurements in Table 1. Plateau voltages were more negative at the lower stimulation frequency of 0.2 Hz. With higher Bay K 8644 concentrations and at the 0.2-Hz stimulation frequency the differences between the voltages measured at different times during the plateau decreased, reflecting slowing of repolarization and flattening of the plateau.*

maximum diastolic potential hyperpolarized by 4 mV and action potential duration lengthened (second trace). An EAD then developed with each action potential (third trace). Returning $[K]_o$ to 5.4 mM rapidly abolished the EADs (right trace). Similar results occurred in five fibers. The effect of 10 mM TEA is shown in Figure 3B. The fiber had not developed EADs (left trace) after 30 minutes of exposure to Bay K 8644. Following the addition of TEA to the bath, action potential duration increased (second trace) and was followed by the development of an EAD with each action potential (right trace). Although the effect of TEA had a rapid onset ($n=3$), it was not reversed during washout periods of up to 30 minutes.

Block of EADs with TTX, lidocaine, ethmozin, nitrendipine, and verapamil. TTX is a specific blocker of Na^+ channels, and in intermediate concentrations, decreases the duration of cardiac action potentials.[14] Lidocaine and ethmozin also have been shown to suppress Na^+ current and decrease action potential duration.[15-17] Representative results for TTX and lidocaine are shown in Figures 4A and 4B. The left traces show EADs triggered with each action potential. When added to the superfusate, each drug rapidly abolished the EADs (second traces) and action potential duration decreased to a steady state within a few minutes (third traces). These effects were rapidly reversed with washout (right traces). Similar effects were found with TTX (4×10^{-6} M, $n=4$), lidocaine (1.7×10^{-5} M, $n=3$), and ethmozin (4×10^{-6} M, $n=2$).

Nitrendipine and verapamil are selective Ca^{2+} channel blocking drugs. Representative results for nitrendipine are shown in Figure 4C. An EAD initially was present with each action potential (left trace). Following the addition of nitrendipine to the bath, the EAD was rapidly abolished (second trace), and over a few minutes, action potential duration decreased to a steady state (third trace). Washout of nitrendipine required nearly 20 minutes to reverse the sequence of changes (right trace). Similar results were obtained with nitrendipine ($1\text{–}5\times10^{-6}$ M, $n=4$) and verapamil ($1\text{–}2\times10^{-6}$ M, $n=3$).

Induction and Block of EADs With Polarizing Constant-Current Steps

We also studied the effect on EADs of modifying the action potential with polarizing constant-current steps. In Figure 5A, an EAD was present with each action potential, was initiated from a take-off potential of −40 mV, and reached a peak voltage of −18 mV (left trace). In the second panel, a repolarizing current step

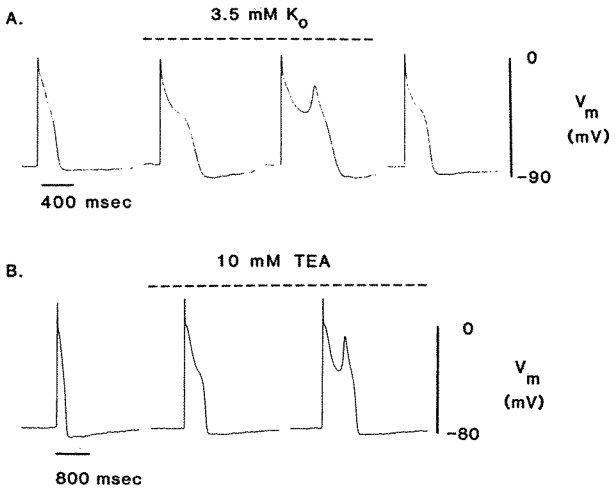

FIGURE 3. *Effect of lowering $[K]_o$ and of tetraethylammonium chloride (TEA) on the induction of early afterdepolarizations (EADs). Fibers were continuously superfused with 1×10^{-6} M Bay K 8644.* A: *Lowering $[K]_o$ from 5.4 to 3.5 mM (dashed line) resulted in lengthening of action potential duration (second trace) and was followed by the appearance of EADs (third trace). Restoring $[K]_o$ to 5.4 mM resulted in suppression of EADs and shortening of action potential duration.* B: *The addition of TEA (dashed line) to the bath resulted in lengthening of action potential duration (second trace) and induction of EADs (third trace).*

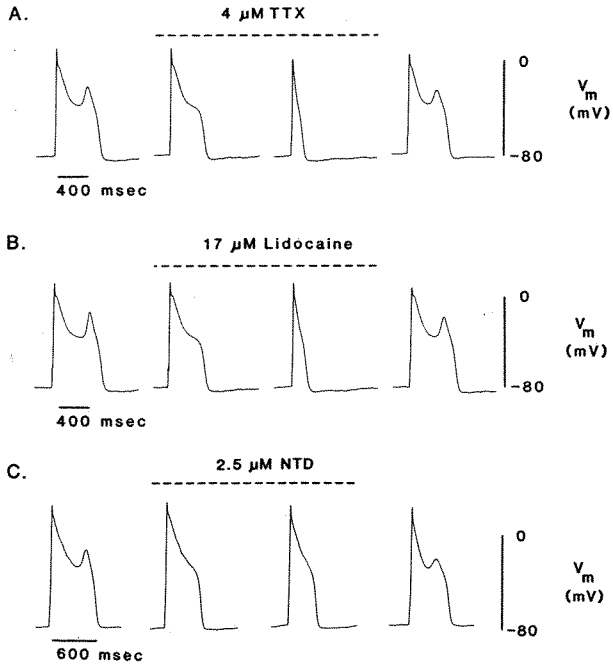

FIGURE 4. *Suppression of early afterdepolarizations (EADs) with tetrodotoxin (TTX), lidocaine, and nitrendipine (NTD). Fibers were continuously superfused with 1×10^{-6} M Bay K 8644 and an EAD initially was present with each action potential in each fiber. TTX* (A), *lidocaine* (B), *and nitrendipine* (C) *were applied (dashed lines). Each drug rapidly abolished the EAD (second trace in each panel), which was followed by shortening of action potential duration to a steady state (third trace in each panel). The return to the initial conditions resulted in reversal of the sequence with lengthening of action potential duration and redevelopment of EADs.*

of −20 nA amplitude and 600 msec duration followed the stimulus. An EAD was again initiated but from a more negative take-off potential of −43 mV and reached a peak voltage of −17 mV. In the third panel, increasing the amplitude of the current step to −30 nA resulted in repolarization of the action potential with no EAD elicited. Return to initial conditions (no current step, fourth panel) resulted in redevelopment of EADs.

Passage of depolarizing current steps could induce EADs as shown in Figure 5B. In the presence of Bay K 8644, APD_{90} had increased to 680 msec but EADs were not present (first panel). With the application of a depolarizing current step of 6 nA amplitude and 1,200 msec duration (second panel), an EAD was initiated from a take-off potential of −43 mV, and its peak voltage was −26 mV. Increasing the amplitude of the current step to 8 nA again initiated an EAD (third panel). However, the EAD was initiated from a less negative take-off potential of −39 mV, and the peak voltage was reduced to −33 mV. Eliminating the current step (fourth panel) resulted in the disappearance of the EADs.

Figure 5 also shows that following the twitch, a small contraction was present coincident with EADs. While the amplitude of the twitch was constant, the amplitude of the EAD-associated contraction appeared to be graded with the amplitude of the EAD.

The relation between EAD peak voltage and its take-off potential was examined using polarizing constant current pulses to systematically vary the take-off potential. Current steps were terminated after the initiation of the EAD to avoid polarizing the peak voltage. Results from two fibers are plotted in Figure 6. Polarizing the action potential plateau to less negative take-off potentials resulted in EADs with decreased peak voltages. With sufficient depolarization, the take-off potential and peak voltage for EADs approached the same value and no EAD was elicited. This voltage was approximately −21 and −30 mV for the fibers shown in Panels A (sheep) and B (dog), respectively. Conversely, polarizing the action potential plateau to more negative take-off potentials increased EAD peak voltage. At take-off potentials of −32 mV (Panel A) or −34 mV (Panel B), the EAD reached a maximum peak voltage of −6 and −21 mV, respectively. Increasing the current amplitude further repolarized the action potential and abolished the EADs as was shown in Figure 5A. Thus, a range of voltages was identified over which EADs were initiated. Comparison of results obtained in six fibers showed a steep inverse relation between the EAD peak voltage and its take-off potential, however, the relation was not identical among fibers. Differences included 1) the range of take-off potentials over which EADs were elicited, 2) the maximum EAD peak voltage, 3) the slope of the relation between EAD peak voltage and take-off potential, and 4) the voltage at which EAD peak voltage and take-off potential became equal. We performed regression analysis on the relation between take-off potential and peak voltage of the EADs for each fiber. Linear analysis was used because the voltage range over which EADs occurred was relatively limited and good correlations ($r = 0.84$–0.98, $p < 0.001$ for each regression) were obtained. These data were used to calculate the voltage at which the EAD peak voltage equaled the take-off potential. For these fibers, the mean slope of the EAD peak voltage–take-off potential relation was -1.87 ± 0.26 mV/mV, and the voltage at which the EAD peak voltage and take-off potential became identical was -25.8 ± 2.3 mV.

Discussion

We present a new model for induction of EADs. The EADs were secondary depolarizations initiated at action potential plateau voltages, and their induction was preceded by lengthening of action potential duration. Induction of EADs required low stimulation frequencies (1 Hz or less), and raising the stimulation frequency abolished them. Where the results can be compared with other models (see below), their sensitivity to drug and ionic interventions was similar. We used the Ca^{2+} channel agonist Bay K 8644, which produces a voltage- and use-dependent increase in macroscopic Ca^{2+} current primarily by increasing the mean open time of L-type Ca^{2+} channels.[9,18–21] Most previously studied models for inducing EADs have

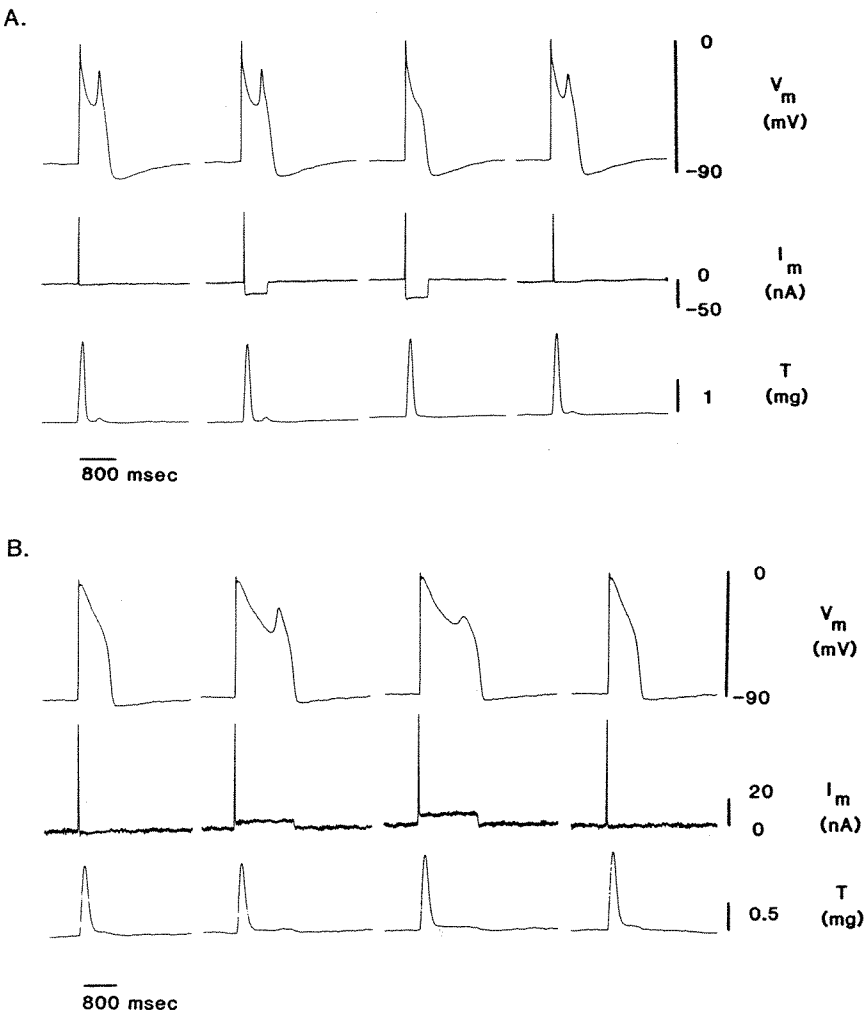

FIGURE 5. *Effect of hyperpolarizing (A) and depolarizing (B) constant-current steps. Action potential (V_m, top), current (I_m, middle), and tension (T, bottom) traces are reproduced in each panel. Fibers were continuously superfused with 1×10^{-6} M Bay K 8644.* A: *An early afterdepolarization (EAD) was triggered with each action potential, and following the twitch there wasan EAD-associated contraction (left traces). Application of a repolarizing −20-nA, constant-current step during the action potential plateau failed to abolish the EAD or its associated contraction (second set of traces). Application of a repolarizing −30-nA, constant-current step abolished the EAD and its associated contraction (third set of traces), which was reversible (right traces).* B: *Results from a different fiber which had not developed EADs are shown (left traces). Application of a depolarizing 6-nA, constant-current step resulted in the initiation of an EAD and a small EAD-associated contraction (second set of traces). Increasing the amplitude of the constant-current step (third set of traces) resulted in an EAD initiated from a more positive take-off potential and reaching a less positive peak voltage. Turning off of the constant-current step resulted in the return to initial conditions (righthand traces). See text for additional details.*

used drug or ionic interventions that suppress repolarizing currents, and most of these models required lowering of $[K]_o$.

EADs can be induced in isolated tissue with a variety of interventions that also frequently lengthen the action potential. This has led to the suggestion that conditions that diminish repolarizing current relative to depolarizing current at action potential plateau voltages induce EADs.[2,4,6] Interventions reported to promote the induction of EADs include a reduction in stimulation frequency,[6] lowered $[K]_o$,[22] Cs^{+}[5,6], quinidine,[7] *n*-acetyl procainamide,[23] sotalol,[24] batrachotoxin,[25] acidosis,[26] and catecholamines.[27,28] Interventions that act through different mechanisms also abolish EADs and include increasing stimulation frequency, raising $[K]_o$, TTX,[7] lidocaine,[7] verapamil,[29] D-600,[30] and nitrendipine.[31] This has led to the implication of several mechanisms in the generation of EADs including Na^+ "window" or slowly inactivating current,[26] Ca^{2+} current,[31,32] I_{x1},[33] the transient inward current activated by elevated intracellular Ca^{2+} (I_{ti}),[34] the Na-Ca exchange mechanism,[35] and intercellular K^+ accumulation.[26] Interpretation of these results remains complex. Added to this is the finding that EADs can be initiated from more than one voltage range with Cs^+.[6] The EADs induced with Bay K 8644 appeared more like low-membrane potential EADs.[6]

Electrotonic interactions between differently polarized regions cannot be excluded as a cause of EADs in large preparations. In our Purkinje fiber short segments, space clamp conditions should exist, making electrotonic interactions unlikely. This is consistent with preliminary reports of EADs in isolated single cells (J.J. Salata, unpublished observations; see also Damiano and Rosen[6]). Another mechanism postulated to cause EADs is I_{ti}.[34] This current is the charge carrier for DADs and is driven by intracellular Ca^{2+} (see Kass and Tsien[36]). The finding of an EAD-associated contraction is consistent with this possibility since aftercontractions occur with DADs. However, several arguments exist against a common mechanism for EADs and DADs. First, we never observed DADs with Bay K 8644. Second, when DADs are initiated from less negative voltages, their amplitude is increased which gives a positive slope to the relation between initiating voltage and DAD amplitude[37] (see also Kass et al[38]). In contrast, the slope of the relation between the EAD peak voltage and its take-off potential was negative. Finally, we recorded EAD peak voltages up to +4 mV, which may exceed the reversal potential of I_{ti}.[38] Our conclusion

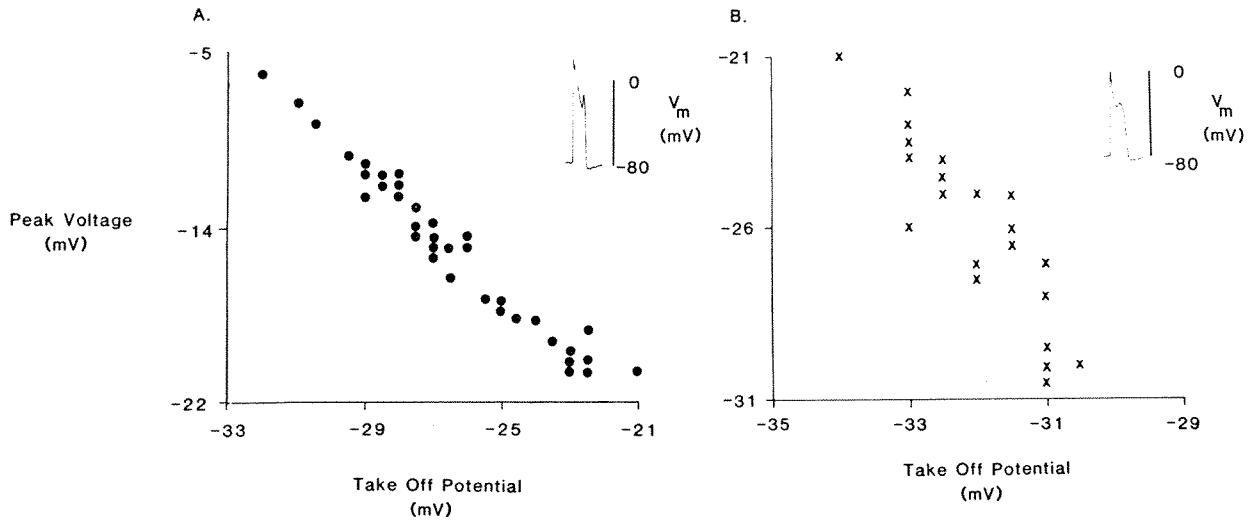

FIGURE 6. *Relation between early afterdepolarization (EAD) peak voltage and take-off potential. Results from two Purkinje fibers (A, sheep; B, canine) are shown. Constant-current steps were applied to polarize the EAD take-off potential. Representative action potentials are shown in the insets. Each plot shows a steeply negative relation between the EAD peak voltage and take-off potential. At approximately −21 mV (A) and −30.5 mV (B), EADs could no longer be elicited. See text for additional details.*

is in agreement with recent findings by Marban and coworkers.[31]

Our findings support roles for time- and voltage-dependent mechanisms for EADs. Our results are consistent with previous reports[6,7] that have suggested an important role for lengthening of action potential duration in the initiation of EADs. While lengthening of action potential duration preceded their initiation, it failed to distinguish fibers stimulated at 1 Hz that did not develop EADs from fibers stimulated at 0.2 Hz that did develop EADs. Shortening of action potential duration at stimulation frequencies below 1 Hz has been previously reported[39] although not universally.[7] Differences in action potential plateau voltages also were shown. The action potential plateau voltages were more negative at the 0.2-Hz stimulation frequency, and at both stimulation frequencies, these voltages were dependent on the Bay K 8644 concentration. A striking difference was the flattening of the action potential plateau at the low stimulation frequency. This preceded the development of EADs and was similar to that shown qualitatively by others.[7] These results suggest that lengthening of action potential duration (present at both stimulation frequencies) and flattening of the action potential within a range of plateau voltages (present mainly at the 0.2-Hz stimulation frequency) may be prerequisites for the initiation of EADs. Further evidence for a time-dependent mechanism was shown when interventions that alter the induction of EADs with Bay K 8644 were studied; enhanced induction of EADs was associated with lengthening of action potential duration, whereas suppression of EADs was associated with shortening of action potential duration. Additional evidence for a voltage-dependent mechanism was shown by the findings that once EADs were initiated, a steep relation existed between the peak voltage and take-off potential, that EADs could be elicited over only a narrow range of take-off potentials, and that with sufficient polarization EADs were eliminated. While our results are consistent with roles for several membrane currents in the initiation and depolarization of EADs, they were obtained with action potentials and do not unambiguously reveal the mechanism that underlies EADs.

We interpret these findings in the context of a hypothesis that the development of EADs requires two phases: an initiation phase (i.e., lengthening and flattening of the action potential within a certain plateau voltage range) followed by an EAD depolarization phase (i.e., a net depolarizing current). The development of the conditions needed to initiate EADs should reflect the sum of the currents that regulate the action potential plateau. These are of small magnitude and include Na^+ "window" current or slowly inactivating current, Ca^{2+} current, K^+ currents, and possibly metabolic pump or exchange currents (Na-K pump or Na-Ca exchange). At low stimulation frequencies, or following pauses in stimulation, the transient outward current (I_{to})[40] also may increasingly contribute to modifying the action potential plateau. For the depolarization of the EAD, a net inward current is required. The nature of the relation between the EAD peak voltage and its take-off potential may suggest the voltage- (and time-) dependent turn-on of a current having a positive reversal potential. Because Bay K 8644 has Ca^{2+} channel agonist properties, it is tempting to suggest that the current is carried through L-type Ca^{2+} channels. Recovery from inactivation and activation of Ca^{2+} channels may occur[18,41] at the voltages where EADs are induced, whereas Na^+ channels are likely to remain voltage inactivated[42] during EADs such as these. This hypothesis can account for the effects of the various interventions we studied. Since several distinct membrane currents (each with its own voltage and time dependence) may underlie the initiating phase, it follows that an intervention that modifies any one of

membrane currents, or simply polarizing the ibrane, may alter the initiating conditions so that ction of EADs is blocked (indirect block of EADs). ntervention also may directly modify the current the EAD depolarization phase (direct block of)s). Combinations of different interventions could additive effects. Clearly, alternative explanations , such as a different charge carrier underlying)larization of the EADs (modification of a repo- ing current, Na^+ channel current, electrogenic Ca exchange current), and are not excluded by the ent results.

References

Cranefield PF: *The Conduction of the Cardiac Impulse*. Mt Kisco, NY, Futura Publishing Co, Inc, 1975

Hoffman BF, Rosen MR: Cellular mechanisms for cardiac arrhythmias. *Circ Res* 1981;49:1–15

Ferek RM, January CT: Excitability and oscillatory afterpotentials in isolated sheep cardiac Purkinje fibers. *Am J Physiol* 1987;252:H645–H652

Rosen MR: Is the response to programmed electrical stimulation diagnostic of mechanisms for arrhythmias? *Circulation* 1986;73(suppl II):II-18–II-27

Brachmann J, Scherlag BJ, Rosenshtraukh LV, Lazzara R: Bradycardia-dependent triggered activity: Relevance to drug-induced multiform ventricular tachycardia. *Circulation* 1983; 68:846–856

Damiano BP, Rosen MR: Effect of pacing on triggered activity induced by early afterdepolarizations. *Circulation* 1984;69: 1013–1025

Roden DM, Hoffman BF: Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibers. Relationship to potassium and cycle length. *Circ Res* 1985;56:857–867

Roden DM, Thompson KA, Hoffman BF, Woosley RL: Clinical features and basic mechanisms of quinidine-induced arrhythmias. *J Am Coll Cardiol* 1986;8:73A–78A

Thomas G, Chung M, Cohen CJ: A dihydropyridine (Bay k 8644) that enhances calcium currents in guinea pig and calf myocardial cells. A new type of positive inotropic agent. *Circ Res* 1985;56:87–96

Tsuji Y, Tajima T, Yuen J, Pappano AJ: Positive inotropic effects of acetylcholine and BAY K 8644 in embryonic chick ventricle. *Am J Physiol* 1987;252:H807–H815

January CT, Riddle JM, Salata JJ: Early afterdepolarizations: Mechanism of induction with Ca^{++} channel agonist Bay k 8644 (abstract). *Circulation* 1986;74(suppl II):II-420

Weidmann S: Shortening of the cardiac action potential due to a brief injection of KCl following the onset of activity. *J Physiol* (Lond) 1956;132:157–163

Kenyon JL, Gibbons WR: Influence of chloride, potassium, and tetraethylammonium on the early outward current in sheep cardiac Purkinje fibers. *J Gen Physiol* 1979;73:117–138

Coraboeuf E, Deroubaix E, Coulombe A: Effect of tetrodotoxin on action potentials of the conducting system in the dog heart. *Am J Physiol* 1979;236:H561–H567

Colatsky TJ: Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibers. An effect on steady state sodium currents? *Circ Res* 1982; 50:17–27

Bean BP, Cohen CJ, Tsien RW: Lidocaine block of cardiac sodium channels. *J Gen Physiol* 1983;81:613–642

Rosenshtraukh LV, Anyukhovsky EP, Nesterenko VV, Undrovinas AI, Shugushev KhKh, Portnoy VF, Burnashev NA: Electrophysiological aspects of moricizine. *Am J Cardiol* 1987;60:27F–34F

Sanguinetti MC, Krafte DS, Kass RS: Bay K 8644: Voltage-dependent modulation of Ca channel current in heart cells. *J Gen Physiol* 1986;88:369–392

Hess P, Lansman JB, Tsien RW: Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature* 1984;311:538–544

20. Kokubun S, Reuter H: Dihydropyridine derivatives prolong the open state of Ca channels in cultured cardiac cells. *Proc Natl Acad Sci USA* 1984;81:4824–4827
21. Bean BP: Two kinds of calcium channels in canine atrial cells. *J Gen Physiol* 1985;86:1–30
22. Roden DM, Iansmith DHS: Effects of low potassium or magnesium concentrations on isolated cardiac tissue. *Am J Med* 1987;82(suppl 3A):18–23
23. Dangman KH, Hoffman BF: In vivo and in vitro antiarrhythmic and arrhythmogenic effects of n-acetyl procainamide. *J Pharmacol Exp Ther* 1981;217:851–862
24. Strauss HC, Bigger JT, Hoffman BF: Electrophysiological and beta-receptor blocking effects of MJ 1999 on dog and rabbit cardiac tissue. *Circ Res* 1970;26:661–678
25. Brown BS: Early afterdepolarizations induced by batrachotoxin: Possible involvement of a sodium current (abstract). *Fed Proc* 1983;42:1692
26. Coulombe A, Coraboeuf E, Malecot C, Deroubaix E: Role of the "Na window" current and other ionic currents in triggering early afterdepolarization and resulting re-excitation in Purkinje fibers, in Zipes DP, Jalife J (eds): *Cardiac Electrophysiology and Arrhythmias*. Orlando, Fla, Grune & Stratton, Inc, 1985, pp 43–49
27. Hoffman BF, Cranefield PF: *Electrophysiology of the Heart*. New York, McGraw-Hill Book Co, 1960, pp 183–184
28. Kupersmith J, Hoff P, Duo GS: In vitro characteristics of repolarization abnormality—A possible role of arrhythmias. *J Electrocardiol* 1986;19:361–370
29. Quantz M, Nattel S: The ionic mechanism for quinidine-induced early afterdepolarizations (abstract). *Circulation* 1986; 74(suppl II):II-420
30. Aliot E, Szabo B, Sweidan R, Lazzara R: Prevention of torsades de pointes with calcium channel blockade in an animal model (abstract). *J Am Coll Cardiol* 1985;5:492
31. Marban E, Robinson SW, Wier WG: Mechanism of arrhythmogenic delayed and early afterdepolarizations in ferret muscle. *J Clin Invest* 1986;78:1185–1192
32. Katzung BG: Effects of extracellular calcium and sodium on depolarization-induced automaticity in guinea pig papillary muscle. *Circ Res* 1975;37:118–127
33. Hauswirth O, Noble D, Tsien RW: The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J Physiol* (Lond) 1969;200:255–265
34. Capogrossi M, Lakatta EG: Frequency modulation and synchronization of spontaneous oscillations in cardiac cells. *Am J Physiol* 1985;248:H412–H418
35. Szabo B, Sweidan R, Patterson E, Scherlag BJ, Lazzara R: Increased intracellular Ca^{++} may be important also for early afterdepolarizations (abstract). *J Am Coll Cardiol* 1987;9 (suppl A):210A
36. Kass RS, Tsien RW: Fluctuations in membrane current driven by intracellular calcium in cardiac Purkinje fibers. *Biophys J* 1982;38:259–269
37. Wasserstrom JA, Ferrier GR: Voltage dependence of digitalis afterpotentials, aftercontractions, and inotropy. *Am J Physiol* 1981;241:H646–H653
38. Kass RS, Lederer WJ, Tsien RW, Weingart R: Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibers. *J Physiol* (Lond) 1978;281:187–208
39. Fedida D, Boyett MR: Mechanisms underlying the shortening of the action potential at high and low stimulus rates in sheep Purkinje fibres. *Proc R Soc Lond* (Biol) 1985;225:481–502
40. Fozzard HA, Hiraoka M: The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. *J Physiol* (Lond) 1973;234:569–586
41. Isenberg G, Klockner U: Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude. *Pflugers Arch* 1982;395:30–41
42. Weidmann S: The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J Physiol* (Lond) 1955;127:213–224

KEY WORDS • Purkinje fibers • early afterdepolarizations • Bay K 8644 • contraction • arrhythmia