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The electrophysiologic effects of ersentilide on canine hearts

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Abstract

Ersentilide is a benzamide derivative that has been found effective in several intact animal models of arrhythmia. Cellular studies have indicated it blocks the delayed rectifier, I_K, and is a β_1-adrenoceptor antagonist. We used standard electrophysiologic techniques to study ersentilide's actions on canine Purkinje fiber and atrial and ventricular myocardium. Ersentilide had no effect on maximum diastolic potential, action potential amplitude or $\dot{V}_{max}$ of any of those tissues. Neither did it affect normal Purkinje fiber automaticity. Ersentilide prolonged action potential duration and effective refractory period, and prolonged the duration of Ca^{2+}-dependent 'slow response' action potentials. It also suppressed abnormal automaticity in Ba^{2+} superfused fibers and attenuated isoproterenol-induced automaticity. Although ersentilide increased the magnitude of digitalis-induced delayed afterdepolarizations it neither increased nor suppressed the incidence of digitalis-induced arrhythmias in intact dogs. Because it selectively prolongs action potential duration and refractoriness and induces β_1-adrenoceptor blockade, ersentilide warrants further consideration as an antiarrhythmic agent.

Keywords: Cardiac electropharmacology; Mechanism of arrhythmia; Antiarrhythmic drug

1. Introduction

The benzamide derivative, ersentilide, ((*s*)-*N*-[4-[2 hydroxy-3-[[2-[4-(1*H*-imidazol-1-yl) phenoxy] ethyl] amino] propoxy] phenyl] methanesulfamide) is an antiarrhythmic drug whose actions have been reported on intact animals and isolated cardiac tissues (Argentieri et al., 1992). In intact dogs ersentilide was effective against epinephrine-induced ventricular arrhythmias during halothane anesthesia and against ventricular arrhythmias induced by programmed electrical stimulation 3–8 days post myocardial infarction (Argentieri et al., 1992). Cellular electrophysiologic studies have demonstrated that ersentilide prolongs repolarization of canine Purkinje fibers and ventricular trabeculae, and voltage clamp experiments indicate that it blocks the delayed rectifier, I_K, of feline ventricular myocytes (Argentieri et al., 1992). In addition, ersentilide induces β_1-adrenoceptor blockade.

The purpose of the present study was to determine the cellular electrophysiologic effects of ersentilide on canine atrial muscle and ventricular endocardial and epicardial muscle, as well as on Purkinje fibers, to determine its actions on conduction and on mechanisms for abnormal impulse initiation and propagation, and to further evaluate its β_1-adrenoceptor blocking properties.

2. Materials and methods

2.1. Isolated tissue studies

Mongrel dogs weighing 10–20 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Their hearts were removed through a left lateral thoracotomy and immersed in cold Tyrode's solution equilibrated with 95% O_2-5% CO_2 and containing (mM); NaCl, 131; $NaHCO_3$, 18; KCl, 4; $CaCl_2$, 2.7; $MgCl_2$, 0.5; NaH_2PO_4, 1.8; and dextrose, 5.5. Free-running Purkinje fibers, or ventricular myocardial preparations or atrial preparations were dissected, placed in a tissue bath, and superfused with Tyrode's solution warmed to

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37°C. Epicardial strips (approximately $1.0 \times 1.5 \times 0.2$ cm) were filleted with a surgical blade from the right and left ventricular free walls. Endocardial strips were obtained from the surface of the papillary muscles, per Litovsky and Antzelevitch (1988). Atrial strips (1.0×1.5 cm $\times < 1$ mm) were removed from left and right atria and placed in a tissue bath, endocardial side up. The right atrial preparations did not include tissue from the sinus node area.

The pH of the Tyrode's solution was 7.3 ± 0.05. Solutions were pumped through the tissue bath at a flow rate of 12 ml/min, changing chamber content 3 times per minute. The bath was connected to ground using a 3 M KCl/Ag/AgCl junction.

All preparations were impaled with 3 M KCl-filled glass capillary microelectrodes having tip resistances of 10–20 MΩ. The maximum rate of rise of phase 0 of the action potential ($\dot{V}_{max}$) was obtained by electronic differentiation with an operational amplifier (Rosen et al., 1973). The electrodes were coupled by an Ag/AgCl junction to an amplifier with high input impedance and input capacity neutralization (model Duo 773, World Precision Instruments, New Haven, CT, USA). The transmembrane action potentials and $\dot{V}_{max}$ were displayed on an oscilloscope (model 4074, Gould, Cleveland, OH, USA) and recorded on a plotter (model 7470A, Hewlett-Packard, CA, USA). The system was calibrated as previously described (Rosen et al., 1973,1977).

For stimulation of preparations, standard techniques were used to deliver square-wave pulses 1.0 ms in duration and 2.0 times threshold via bipolar Teflon-coated silver electrodes (Rosen et al., 1973, 1977).

In studies of canine Purkinje fibers, preparations were driven at cycle lengths of 2000, 1000, 500, and 300 ms in sequence to investigate the frequency-dependent effects of ersentilide. After obtaining control records (following a 60 min stabilization period in control Tyrode's solution), the fibers were superfused with Tyrode's solution containing graded concentrations (1×10^{-7} to 5×10^{-6} M) of ersentilide. The transmembrane potential characteristics recorded were the maximum diastolic potential, action potential amplitude, $\dot{V}_{max}$ and duration to 50% (APD_{50}) and 90% (APD_{90}) repolarization (Rosen et al., 1973,1977). We determined conduction velocity by measuring the time required for the signal to propagate along an unbranched Purkinje fiber bundle from a microelectrode positioned at least 2 mm distal to the stimulating electrode to a bipolar surface recording electrode positioned at least 4 mm distal to the microelectrode. The bipolar electrode recordings were channeled through preamplifiers (model DAM 50, World Precision Instruments) to an oscilloscope. This permitted display of the electrogram recordings at rapid sweep speeds and accurate measurement of conduction time. Distance between the microelectrode and bipolar surface electrode was measured using a reticle mounted in a microscope (model StereoZoom 5, Bausch and Lomb, Rochester, NY, USA). To study the effective refractory period, Purkinje fibers were stimulated at basic cycle lengths of 1000 and 500 ms. The effective refractory period was measured by delivering premature stimuli of 2 ms duration and 2.5 times threshold in amplitude progressively earlier during repolarization, at intervals of 8 cycles. The earliest premature response which propagated along an unbranched Purkinje fiber was assumed to represent the end of the effective refractory period (Zaza et al., 1989).

The effects of ersentilide on action potential characteristics and effective refractory period at different K^+ concentrations were studied in the same fiber. Each fiber was sequentially superfused with Tyrode's solution containing ($[K^+]_o$ = 2, 4, 6, and 8 mM, first in the absence and then in the presence of 5×10^{-7} M ersentilide. The preparations were allowed to equilibrate for 20 min at each $[K^+]_o$, and for 45–50 min after the transition to solutions containing ersentilide. The action potential parameters and effective refractory period were recorded at the end of each step.

To study normal automaticity Purkinje fibers were superfused with Tyrode's solution containing $[K^+]_o$ = 2.7 mM, and were permitted to beat spontaneously. After equilibration, fibers were exposed to ersentilide in graded concentrations. To study isoproterenol-induced automaticity, Purkinje fibers were superfused with Tyrode's solution containing $[K^+]_o$ = 2.7 mM, and were permitted to beat spontaneously. One group of fibers was superfused with isoproterenol, alone, 10^{-9}–10^{-6} M, to establish a concentration-response relationship. The fibers were allowed to equilibrate for 20 min at each isoproterenol concentration. Another group was subjected to the same protocol as the first, but all Tyrode's solutions contained 5×10^{-7} M ersentilide (a concentration that had no effect on normal Purkinje fiber automaticity: see Results). EDTA, 5×10^{-7} M, was added to all solutions to retard oxidation of isoproterenol. This concentration of EDTA has no effect on electrophysiological properties of Purkinje fibers (Moak et al., 1986).

2.2. Studies of arrhythmogenic mechanisms

Slow response action potentials

Slow responses were obtained by superfusing Purkinje fibers with Tyrode's solution containing $[K^+]_o$ = 22 mM and 1×10^{-5} M isoproterenol (Damiano et al., 1985). To maintain osmolarity of Tyrode's the sodium concentration was reduced to 113 mM. EDTA, 5×10^{-5} M, was included to retard oxidation. Action potential parameters were evaluated during stimulation at a stimulus cycle length of 2000 ms.

Abnormal automaticity

To study Ba^{2+}-induced abnormal automaticity, we used Tyrode's solution containing $[K^+]_o = 4.0$ mM. After a period of equilibration in control solution (60 min) fibers driven at a cycle length of 2000 ms were superfused for 20 min with solutions containing 0.15 mM $BaCl_2$. If abnormal automaticity did not appear the concentration of $BaCl_2$ was gradually increased in steps of 0.05 mM and intervals of 20 min until the appearance of stable abnormal automaticity. This is characterized by a low maximal diastolic potential, phase 4 depolarization and action potentials with slow upstrokes and low overshoots (Dangman and Hoffman, 1980). When spontaneous activity commenced, the stimulus was discontinued. Then fibers were sequentially superfused for 40 min with Tyrode's containing $BaCl_2$ and ersentilide, 5×10^{-7} M, 1×10^{-6} M and 5×10^{-6} M. It has been shown that if superfusion with barium-containing Tyrode's, alone, is continued, abnormal automaticity is stable for several hours (Dangman and Hoffman, 1983). Moreover, this type of automaticity is identical to that seen at low membrane potentials in subendocardial Purkinje fibers in the setting of myocardial infarction (LeMarec et al., 1985).

Afterdepolarizations

Delayed afterdepolarizations are oscillations in membrane potential occurring after full repolarization and which are capable of inducing tachyarrhythmias referred to as triggered (Wit and Rosen, 1991). We studied two types of delayed afterdepolarizations: Tyrode's solution containing $[K^+]_o = 4$ mM was used for all experiments. After stabilization in control solution for 60 min, one set of fibers was superfused with ouabain 2×10^{-7} M for 20–30 min. The end of superfusion was signalled by the occurrence of delayed afterdepolarizations having an amplitude of 5 mV. When the ouabain was discontinued, fibers were exposed to ersentilide, 5×10^{-7} M. It has been shown that on cessation of ouabain superfusion after attainment of toxicity with this protocol, the maximum diastolic potential remains at a consistent, depolarized level for about 1 h (Miura and Rosen, 1978). The amplitude of delayed depolarizations was measured from the point of maximum hyperpolarization of the membrane before the afterdepolarization to its peak amplitude (Moak and Rosen, 1984).

In another set of experiments, fibers were superfused with Tyrode's containing Ca^{2+}, 10.8 mM, and isoproterenol, 1×10^{-5} M. The time course for development of delayed afterdepolarizations was determined, as was their magnitude. For another group of fibers, the same superfusate plus ersentilide, 5×10^{-7} M, was used.

Early afterdepolarizations are oscillations in membrane potential occurring during phases 2 and 3 of repolarization. They are most prominent when APD is long and/or stimulus rate is low, and they are capable of reaching threshold potential and inducing triggered activity (Wit and Rosen, 1991). Early afterdepolarizations were induced in Purkinje fibers by superfusing them with Tyrode's solution containing $[K^+]_o = 2.7$ mM and $CsCl_2 = 5$ mM (Damiano and Rosen, 1984). Fibers were stimulated at cycle lengths of 1–4 s.

Intact animal studies

Studies of intact animals were designed to evaluate the proarrhythmic potential of ersentilide. Here, 18 dogs were anesthetized with ketamine (12 mg/kg) and pentobarbital (35 mg/kg i.v.). We did three groups of experiments, using 6 animals in each group while continuously recording the ECG.

Protocol A: control ouabain-induced arrhythmias

Following a 30 min control period; we infused ouabain, 40 μg/kg i.v. over 2 min. We observed and recorded the time of onset of the first ventricular ectopic beats. If no arrhythmia occurred in 20 min, we gave an additional 10 μg/kg ouabain and observed for 10 min. We continued this sequence until the first arrhythmia occurred. We then observed the animal for 2 h from the time of onset of the arrhythmia and quantified the ventricular ectopic beats that occurred.

Protocol B: effect of preadministration of ersentilide on the course of ouabain-induced arrhythmias

Following a 30 min control period, we infused ersentilide, 1 mg/kg i.v. over 10 min. Ten minutes after completing ersentilide infusion we gave ouabain as in protocol A and continued the protocol as in A.

Protocol C: effect of ersentilide administration on ongoing ouabain-induced arrhythmias

After a 30 min control period we infused ouabain as in protocol A. We carried out the protocol precisely as listed in A through the first 30 min after the onset of arrhythmia. We then gave ersentilide as in B and observed the course of the arrhythmias for the next 90 min.

Statistical analysis

Microelectrode data were analyzed only from impalements that were maintained throughout the course of each experimental protocol. Automaticity is reported only for those experiments in which the control automatic rates showed a variance not greater than 10%. Data are expressed as mean $\pm$ S.E.M.

The statistical technique used was analysis of variance, with Scheffé's test when the F value permitted this (Snedecor and Cochran, 1980). For experiments on the incidence of early afterdepolarizations and the incidence of triggered activity induced by early or de-

layed afterdepolarizations, Fisher's exact test was used. For experiments in which one concentration of drug was examined and compared to control a paired t-test was used to analyze changes in variables between control conditions and maximal drug effect. Significance was determined at $P < 0.05$.

3. Results

3.1. Studies of normal transmembrane action potentials

Effects of ersentilide on the normal transmembrane action potentials of Purkinje fibers

Ersentilide achieved a steady-state effect in 30–40 min. Hence, in all experiments, each drug concentration was superfused for 45–50 min. Fig. 1 illustrates representative transmembrane potentials recorded from Purkinje fibers stimulated at cycle lengths of 2000–300 ms. The mean data demonstrating the effects of varying concentrations of ersentilide on the action potential duration at basic cycle length = 2 s are summarized in Table 1. No effect was seen on maximum diastolic potential, action potential overshoot, or $\dot{V}_{max}$. Conduction velocity was unaffected by ersentilide (control = 1.6 ± 0.1 M/s; 5×10^{-6} M = 1.6 ± 0.1 M/s) at any basic cycle length. There was a concentration-dependent prolongation of repolarization, which attained significance for APD_{50} and APD_{90} at the 5×10^{-7} M

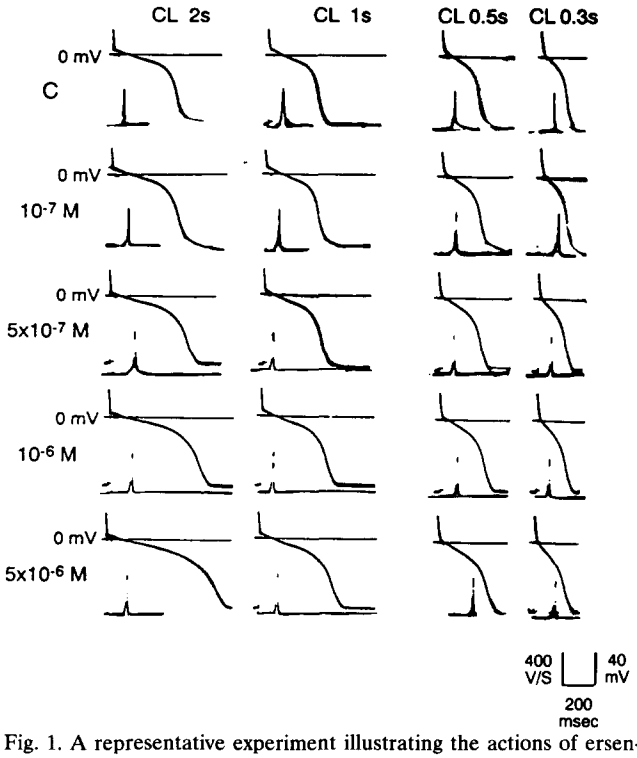

Fig. 1. A representative experiment illustrating the actions of ersentilide on the transmembrane potential of a Purkinje fiber driven at cycle lengths of 2000, 1000, 500 and 300 ms. In each panel, the two upper traces show the transmembrane action potential displayed at slow and rapid sweep speeds; the lower trace, $\dot{V}_{max}$. Vertical calibrations are for the action potential and $\dot{V}_{max}$, respectively; horizontal for the action potential.

Table 1
Effects of ersentilide on action potential duration at basic cycle length = 2 s ($n = 5$)

	Control	10^{-7} M	5×10^{-7} M	10^{-6} M	5×10^{-6} M
A: Purkinje fibers ($n = 5$)					
Maximum diastolic potential (−mV)	91 ± 1	91 ± 1	91 ± 1	91 ± 1	91 ± 1
Overshoot (mV)	38 ± 2	39 ± 1	39 ± 1	38 ± 2	38 ± 2
$\dot{V}_{max}$ (V/s)	610 ± 22	615 ± 24	612 ± 18	609 ± 25	614 ± 22
APD_{50} (ms)	322 ± 22	340 ± 25	390 ± 25 [a]	454 ± 31 [a]	526 ± 35 [a]
APD_{90} (ms)	395 ± 28	419 ± 31	484 ± 38 [a]	568 ± 44 [a]	674 ± 57 [a]
B: Ventricular subepicardium ($n = 5$)					
Maximum diastolic potential (−mV)	86 ± 2	87 ± 2	87 ± 2	86 ± 2	87 ± 2
Overshoot (mV)	30 ± 2	31 ± 3	31 ± 2	31 ± 2	31 ± 3
$\dot{V}_{max}$ (V/s)	225 ± 39	235 ± 47	245 ± 56	240 ± 56	235 ± 57
APD_{50} (ms)	134 ± 10	160 ± 8 [a]	171 ± 10	190 ± 12 [a]	213 ± 15 [a]
APD_{90} (ms)	175 ± 12	203 ± 10 [a]	218 ± 12 [a]	240 ± 12 [a]	268 ± 17 [a]
C: Ventricular subendocardium ($n = 5$)					
Maximum diastolic potential (−mV)	84 ± 1	85 ± 2	86 ± 1	86 ± 1	86 ± 1
Overshoot (mV)	22 ± 2	22 ± 1	23 ± 2	22 ± 2	22 ± 1
$\dot{V}_{max}$ (V/s)	156 ± 5	156 ± 8	156 ± 7	154 ± 7	156 ± 10
APD_{50} (ms)	175 ± 8	194 ± 9 [a]	205 ± 9 [a]	221 ± 11 [a]	233 ± 13 [a]
APD_{90} (ms)	204 ± 9	226 ± 10 [a]	237 ± 6 [a]	255 ± 6 [a]	272 ± 5 [a]
D: Atrium ($n = 4$)					
Maximum diastolic potential (−mV)	76 ± 1	76 ± 1	75 ± 1	76 ± 1	76 ± 1
Overshoot (mV)	36 ± 1	37 ± 1	37 ± 1	38 ± 1	38 ± 2
$\dot{V}_{max}$ (V/s)	285 ± 17	292 ± 14	302 ± 9	302 ± 9	297 ± 13
APD_{50} (ms)	131 ± 10	147 ± 10	159 ± 5 [a]	171 ± 7 [a]	177 ± 10 [a]
APD_{90} (ms)	206 ± 6	217 ± 6	237 ± 4 [a]	260 ± 7 [a]	287 ± 10 [a]

[a] $P < 0.05$ vs. control.

Table 2
Effects of ersentilide, 5×10^{-7} M, on Purkinje fiber action potentials and effective refractory periods (ERP) at various $[K^+]_o$; $n = 5$

	Cycle length 1 s				Cycle length 0.5 s				
	Maximum diastolic potential (−mV)	$\dot{V}_{max}$ (V/s)	ERP (ms)	ERP/APD_{90}	Maximum diastolic potential (−mV)	$\dot{V}_{max}$ (V/s)	APD_{90} (ms)	ERP (ms)	ERP/APD_{90}
K = 2 mM									
Control	101 ± 2	622 ± 16	276 ± 5	0.83 ± 0.03	102 ± 1	610 ± 22	260 ± 9	216 ± 4	0.83 ± 0.02
Ersentilide	100 ± 1	588 ± 10	374 ± 23 [a]	0.90 ± 0.01 [a]	101 ± 1	615 ± 18	303 ± 13 [a]	261 ± 14 [a]	0.86 ± 0.05
K = 4 mM									
Control	89 ± 1	596 ± 13	292 ± 5	0.86 ± 0.03	89 ± 2	605 ± 18	257 ± 15	223 ± 3	0.87 ± 0.02
Ersentilide	90 ± 1	593 ± 14	378 ± 19 [a]	0.91 ± 0.03	91 ± 2	612 ± 21	296 ± 14 [a]	260 ± 9 [a]	0.90 ± 0.03
K = 6 mM									
Control	81 ± 1	522 ± 27	265 ± 6	0.95 ± 0.03	82 ± 2	510 ± 25	218 ± 17	197 ± 5	0.90 ± 0.04
Ersentilide	81 ± 1	514 ± 17	352 ± 16 [a]	0.96 ± 0.03	82 ± 1	505 ± 21	257 ± 10 [a]	245 ± 9 [a]	0.97 ± 0.03
K = 8 mM									
Control	72 ± 1	412 ± 15	231 ± 11	1.0 ± 0.04	73 ± 1	430 ± 27	182 ± 13	182 ± 4	1.01 ± 0.05
Ersentilide	72 ± 1	426 ± 15	283 ± 7 [a]	0.99 ± 0.03	73 ± 1	426 ± 19	214 ± 6 [a]	224 ± 7 [a]	1.05 ± 0.04

[a] $P < 0.05$ vs. control.

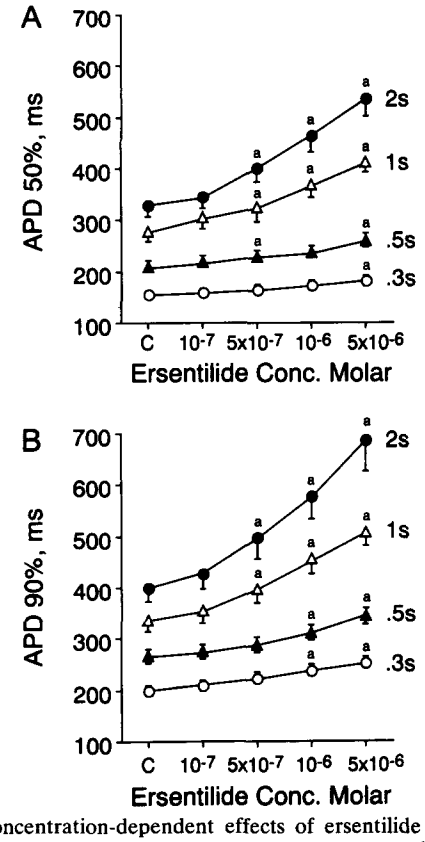

Fig. 2. Concentration-dependent effects of ersentilide on Purkinje fiber APD_{50} (A) and APD_{90} (B) at all cycle lengths. [a] $P < 0.05$ vs. control.

concentration when basic cycle length = 2 s. At shorter cycle lengths, higher concentrations were needed before an effect on APD was noted (see Fig. 2A and B). The change in APD was associated with a significant decrease in the slopes of phases 2 and 3 of repolarization, but no change in the phase 1 notch (data not shown).

Effects of ersentilide on ventricular subepicardial and subendocardial muscle and atrial muscle

After placement in the tissue bath all preparations were equilibrated in control Tyrode's solution at basic cycle length = 1000 ms. Action potential duration reached a steady state after 2–3 h of superfusion with Tyrode's solution. As for Purkinje fibers, ersentilide had no effect on maximum diastolic potential, overshoot or $\dot{V}_{max}$ (Table 1). However, there was a profound prolongation of APD_{50} and APD_{90}, which, as for Purkinje fibers in Fig. 2, showed 'reverse use dependence' (data not shown) and a lower threshold concentration than for Purkinje fibers.

The actions of ersentilide on atrial muscle are demonstrated in Table 1D. Again, no effect on maximum diastolic potential, overshoot or $\dot{V}_{max}$ occurred. APD_{50} and APD_{90} were prolonged in a reverse use-dependent fashion (data not shown), and higher concentrations of drug were needed to prolong APD as drive cycle length decreased.

Effects of ersentilide on the Purkinje fiber effective refractory period

The goal of this protocol was to analyze the effects of ersentilide on the effective refractory period and the dependence of ersentilide's effects on Purkinje fiber action potentials and effective refractory period on the K^+ concentration ($[K^+]_o$) of the superfusate. The mean data demonstrating the effects of ersentilide on maximum diastolic potential, action potential amplitude and $\dot{V}_{max}$ at different $[K^+]_o$ are summarized in Table 2. The effects of changing $[K^+]_o$, alone, on maximum diastolic potential, amplitude and $\dot{V}_{max}$ were not tested statistically, as these are standard and expected changes consistent with past work, as summarized in Reder et al., 1980. Ersentilide had no effect on maximum diastolic potential, action potential overshoot or $\dot{V}_{max}$ at all $[K^+]_o$. Its effects to prolong APD persisted at all $[K^+]_o$ and it prolonged effective refractory period at all $[K^+]_o$, as well.

As shown in Table 2, at basic cycle length = 1000 ms, ersentilide increased the effective refractory period/APD ratio when $[K^+]_o = 2$ mM. At higher $[K^+]_o$, the ratio did not change. In contrast, at basic cycle length = 500 ms, there was no change in effective refractory period/APD ratio at any $[K^+]_o$.

Effects of ersentilide on normal and isoproterenol-induced automaticity

At all concentrations ersentilide had no effect on normal automaticity. Control rate = 25 ± 5 bpm; at 5×10^{-6} M ersentilide, it was 24 ± 5 bpm ($n = 6$, $P > 0.05$). At all concentrations tested neither maximum diastolic potential, activation voltage nor the slope of phase 4 depolarization was altered (see Fig. 3). Maximum diastolic potential during control was −96 ± 2 mV and at ersentilide, 5×10^{-7} M, it was −95 ± 2 mV. Comparable activation voltages were −84 ± 5 and −85 ± 5 mV respectively.

Mean values reflecting isoproterenol's effects on maximum diastolic potential and spontaneous rate are presented in Table 3. In the absence of ersentilide, isoproterenol induced a marked concentration-dependent increase in spontaneous rate, with a threshold concentration of 5×10^{-9} M. There was marked competitive blockade of the rate increase by ersentilide, and the threshold concentration was shifted to 10^{-7} M. Hence, ersentilide manifested a profound β-blocking action.

3.2. Studies of arrhythmogenic mechanisms

Effects of ersentilide on slow response action potentials

Mean data from these experiments are summarized in Table 4. Ersentilide had no effect on the maximum

Table 3
Effects of ersentilide on isoproterenol-induced automaticity ([K^+] = 2.7 mM)

	Control	Isoproterenol			
		10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
Tyrode's ($n = 6$)					
Maximum diastolic potential (−mV)	94 ± 2	95 ± 3	95 ± 2	95 ± 3	95 ± 2
Rate (bpm)	15 ± 2	25 ± 4 [a]	30 ± 45 [a,b]	35 ± 5 [a,b]	38 ± 4 [a,b]
Tyrode's + ersentilide (5×10^{-7} M; $n = 6$)					
Maximum diastolic potential (−mV)	95 ± 2	94 ± 3	95 ± 2	95 ± 2	94 ± 3
Rate (bpm)	16 ± 4	17 ± 5	19 ± 6	22 ± 8 [a]	29 ± 6 [a]

[a] $P < 0.05$ vs. control, [b] $P < 0.05$ vs. ersentilide.

diastolic potential. However, it reduced $\dot{V}_{max}$ and prolonged action potential duration in a concentration-dependent fashion.

Effects of ersentilide on barium-induced abnormal automaticity

The effects of ersentilide were studied in five Purkinje fibers. The concentration of barium in the superfusate in the different experiments varied between 0.15 and 0.45 mM. Ersentilide, 1×10^{-6} M, significantly suppressed barium-induced automaticity from a control of 65 ± 5 bpm to 49 ± 4 bpm at 10^{-6} M ($P < 0.05$) and 39 ± 4 bpm at 5×10^{-6} M ($P < 0.05$). There was no effect on maximum diastolic potential, (60 ± 2 mV in control and 60 ± 3 mV at 5×10^{-6} M).

Effects of ersentilide on delayed afterdepolarizations

As shown in Table 5, in the presence of ouabain, ersentilide, 5×10^{-7} M, increased action potential duration and delayed afterdepolarizations amplitude. In seven experiments, maximum diastolic potential in ouabain, alone, was −82 ± 2 mV, and delayed afterdepolarizations amplitude, 5.8 ± 1.3 mV. With ersentilide, there was no significant change in maximum diastolic potential (−81 ± 2 mV) but delayed afterdepolarizations amplitude increased to 11.5 ± 2.7 mV ($P < 0.05$) and the incidence of triggered activity increased, as well ($P < 0.05$). That the result was unique to ouabain-induced delayed afterdepolarizations is shown in our experiments on Ca^{2+}-and isoproterenol-induced delayed afterdepolarizations, in which ersentilide had no effect on delayed afterdepolarization amplitude. Here, 10 experiments were done. Maximum diastolic potential in high $[Ca^{2+}]_o$ and isoproterenol alone was −96 ± 2 mV and delayed afterdepolarizations amplitude was 12 ± 2 mV. In the presence of ersentilide, 5×10^{-7} M, there was no significant change in either variable (−97 ± 2 mV and 13 ± 3 mV, respectively). The incidence of triggered activity was 2/10 in the absence of ersentilide and 4/10 in its presence ($P > 0.05$).

Table 4
Effects of ersentilide on slow response action potentials ($n = 6$)

	Control	5×10^{-7} M	10^{-6} M	5×10^{-6} M
Maximum diastolic potential (−mV)	53 ± 2	54 ± 2	55 ± 2	54 ± 2
Amplitude (mV)	64 ± 3	63 ± 3	62 ± 2	60 ± 2 [a]
$\dot{V}_{max}$ (V/s)	8.0 ± 0.2	7.8 ± 0.2	7.4 ± 0.2 [a]	6.8 ± 0.3 [a]
APD_{50} (ms)	114 ± 6	125 ± 7	132 ± 7 [a]	145 ± 6 [a]
APD_{90} (ms)	145 ± 7	159 ± 7 [a]	167 ± 6 [a]	182 ± 8 [a]

[a] $P < 0.05$ vs. control.

Table 5
Effects of ersentilide on delayed afterdepolarizations induced by ouabain ($n = 7$)

CL 500	Control	Ouabain 2×10^{-7} M	Ersentilide 5×10^{-7} M
Maximum diastolic potential (−mV)	90 ± 1	82 ± 2 [a]	81 ± 2 [a]
$\dot{V}_{max}$ (V/s)	618 ± 25	412 ± 30 [a]	390 ± 27 [a]
APD_{50} (ms)	200 ± 20	152 ± 15 [a]	164 ± 18
APD_{90} (ms)	245 ± 15	210 ± 20 [a]	230 ± 23
Slope of phase 2 (mV/s)	152 ± 10	220 ± 12 [a]	173 ± 13
Slope of phase 3 (V/s)	1.30 ± 0.08	0.57 ± 0.05 [a]	0.53 ± 0.06 [a]
Delayed afterdepolarizations amplitude (mV)	0	5.8 ± 1.3	11.5 ± 2.7 [a]
Incidence of triggered activity	0/7	3/7	5/7 [a]

[a] $P < 0.05$ vs. control.

Table 6
Studies of ventricular ectopic beats (VEB) in intact animals (mean ± S.E.)

Total dose of ouabain (μg/kg)	Time to onset VEB (min)	0–30 min		30–60 min		60–90 min		90–120 min		Total	
		VEB	VEB/min	VEB	VEB/min	VEB	VEB/min	VEB	VEB/min	VEB	VEB/min
Protocol A:											
58 ± 5	32 ± 5	5412 ± 327	180 ± 11	5653 ± 368	188 ± 12	5591 ± 266	186 ± 9	4366 ± 705	145 ± 23	21022 ± 1047	176 ± 9
Protocol B:											
53 ± 3	27 ± 3	4818 ± 462	161 ± 15	5215 ± 830	175 ± 27	5411 ± 563	180 ± 19	5264 ± 611	190 ± 14	23135 ± 1622	195 ± 8
Protocol C:											
57 ± 3	33 ± 5	588 ± 247	196 ± 8	5703 ± 612	190 ± 20	5286 ± 510	176 ± 17	5588 ± 576	186 ± 19	22622 ± 2083	188 ± 17

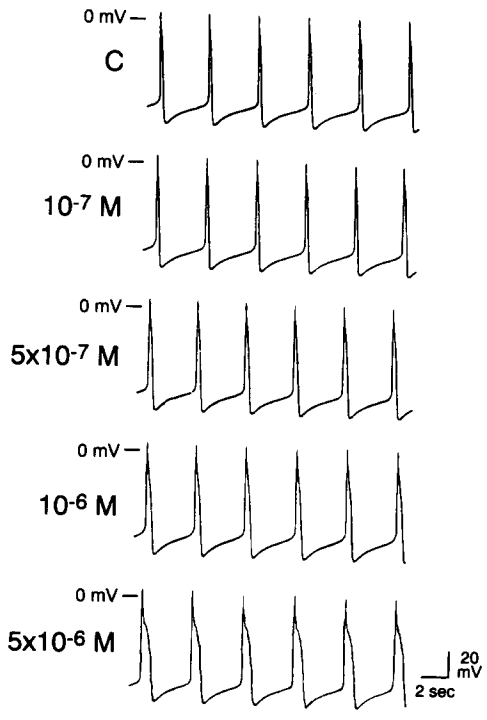

Fig. 3. Effects of ersentilide on normal automaticity of a Purkinje fiber. There is a prominent prolongation of action potential duration, but no change in rate, maximum diastolic potential or activation voltage.

Effects of ersentilide on early afterdepolarizations

Early afterdepolarizations at low or at high membrane potentials appeared in 2 of 6 preparations after exposure to cesium. Two of six also showed triggered activity. All fibers were then superfused sequentially with Tyrode's solution containing 5 mM cesium and 5×10^{-7} M–5×10^{-6} M ersentilide. Early afterdepolarizations increased in magnitude at higher concentrations of ersentilide and at long drive cycle lengths such that at 5×10^{-6} M and basic cycle length = 4 s, 6 of 6 fibers showed early afterdepolarizations ($P < 0.05$), and 4 of 6 showed triggered activity.

3.3. Studies of intact animals

The results of experiments in protocols A–C are summarized in Table 6. One animal from protocol A (ouabain, alone) returned to sinus rhythm at 100 min. Two animals from protocol B did the same (at 60 and 112 min). In addition, one animal from protocol B went into cardiac arrest at 98 min. With respect to protocol C, one animal had a cardiac arrest at 30 min, and another fibrillated at almost the same time. One returned to sinus rhythm at 90 min. In sum, 3 of the 12 animals that received ersentilide returned to sinus rhythm, and 3 died. One control animal (protocol A) returned to sinus rhythm and none died. None of the values for control and ersentilide treatment differed significantly.

4. Discussion

In this study we have systematically evaluated the actions of ersentilide on normal electrophysiologic characteristics of Purkinje fiber and myocardial fiber action potentials and on specific arrhythmogenic mechanisms. It is clear that in normal fibers, ersentilide is completely lacking in local anesthetic effects. It manifests no actions on the amplitude, $\dot{V}_{max}$ or maximum diastolic potential of atrial or ventricular myocardium or Purkinje fibers. Neither does it alter Purkinje fiber conduction velocity. Rather, its major effect is to prolong action potential duration in such a way that the longer the drive cycle length, the greater the magnitude of drug effect. This is an action characteristic of many so-called 'Class 3' drugs (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991).

As is the case for drugs such as sotalol, the occurrence of so-called reverse use dependence (i.e., greater action potential prolongation at longer cycle lengths) may have a potential drawback (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991). We state this because the ability of the drug to prolong action potential duration diminishes as drive cycle length is shortened. One would ideally want to see a drug whose ability to prolong action potential duration did not diminish in the face of a tachyarrhythmia.

The prolongation of action potential duration is mirrored by a prolongation of the effective refractory period. At longer cycle lengths and at low extracellular K^+ concentrations, the prolongation in refractoriness exceeds that of action potential duration, conferring an additional 'protective' effect against the propagation of premature depolarizations. However, as drive cycle length shortens and/or as extracellular K^+ concentration is increased, the ratio of action potential duration prolongation to that of the effective refractory period is never diminished. Hence, even in the least favorable case, there is a net increase in the refractory period.

Biophysical studies previously reported (Argentieri et al., 1992) suggest ersentilide prolongs action potential duration via an effect to block the delayed rectifier. Information concerning whether this action comes about via effects on the slow (I_{Ks}) or rapid (I_{Kr}) components of the delayed rectifier is not yet available.

A potentially negative aspect of ersentilide's effect on action potential duration is the occurrence of early afterdepolarizations. It must be noted that we never saw these at cycle lengths usually considered to be in the physiologic range. They only occurred at very long cycle lengths in three settings: either during slow idioventricular rhythms where we had lowered extracellular K^+ concentration (which would reduce membrane K^+ conductance in its own right) or during cesium or

barium superfusion (both of which also reduce K^+ conductance). Hence, the ability to induce early afterdepolarizations, although viewed as a characteristic proarrhythmic action of drugs that prolong repolarization (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991), occurs only under highly artificial conditions with ersentilide.

We evaluated the effects of ersentilide on several arrhythmogenic mechanisms: the first was the slow response action potential that is not only thought responsible for certain reentrant arrhythmias, but that has a Ca^{2+}-dependent action potential, not unlike that of the normal atrioventricular node (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991). The effect of ersentilide to prolong the duration of this action potential would suggest an ability to increase AV nodal refractoriness as well as to prolong refractoriness in reentrant loops. The reduction in $\dot{V}_{max}$ of the slow response, while small in magnitude, could also depress prolongation of abnormally conducted beats. It should be emphasized, however, that the effect of ersentilide on the slow response may not be via Ca channel blockade, but rather, via block of β_1-adrenoceptors.

Although having no effect on normal automaticity, ersentilide did slow abnormal automaticity in barium superfused fibers. Whether this was secondary to an effect on the pacemaker mechanism itself, or due to profound prolongation of the action potential duration which occurred here is impossible to say. Given that the drug is reported to have no effect on arrhythmias in the Harris dog (Helopharm, in-house documentation) in which abnormal automaticity is the mechanism (LeMarec et al., 1985), one would expect this to be a minor aspect of ersentilide's action.

Ersentilide significantly increased the amplitude of ouabain-induced delayed afterdepolarizations. This action is not unexpected of a drug that prolongs action potential duration. We state this because the plateau prolongation is associated with a net increase in Ca and Na entry, both of which have been shown to potentiate digitalis-induced delayed afterdepolarizations (Wit and Rosen, 1991). The studies of digitalis toxic intact dogs are consistent with our cellular electrophysiologic observations. That is, neither the incidence of return to sinus rhythm or of death differed from the control group. Hence, ersentilide would not be expected to be antiarrhythmic in the setting of digitalis toxicity. The occurrence of cardiac death in the ersentilide-treated group, while not statistically different from control, would lead one to suggest the need for evaluation of a digitalis-ersentilide interaction. Such interactions are well known for drugs such as quinidine (Gessman and Rosen, 1983) and verapamil (Pedersen et al., 1981). If such an interaction were shown with ersentilide, it would not preclude the use of the drug, but would suggest appropriate cautionary measures in patients receiving digitalis therapy.

Ersentilide neither increased nor decreased the amplitude of delayed afterdepolarizations induced by high extracellular calcium and isoproterenol. One might have anticipated an increase, based on the experiments on digitalis-induced afterdepolarizations. That this did not occur may have been due to the β-blocking action of ersentilide, offsetting the effects of prolongation of the action potential plateau. That the drug does potently β block was convincingly shown in the experiments on isoproterenol-induced automaticity, in which the threshold concentration of isoproterenol was shifted by more than two orders of magnitude.

Hence, in ersentilide we see a spectrum of effects quite similar to that of sotalol. Given recent data about the clinical utility and antiarrhythmic efficacy of sotalol (e.g., Mason, 1993), this is an encouraging finding. However, additional information is needed if we are to fully comprehend the similarities and differences between the two drugs. For example, it is uncertain whether the action potential prolonging effects of ersentilide might in some instances predispose to torsades de pointes, as can occur with sotalol. Moreover, ersentilide's β-blocking actions which are reportedly β_1 selective (Argentieri et al., 1992) are important in that, in vivo, the action of catecholamines would tend to accelerate repolarization and offset the action potential prolonging effects of ersentilide. To the extent that ersentilide induces β_1-adrenoceptor blockade, its ability to prolong refractoriness would be protected in the presence of β_1-adrenoceptor agonists.

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