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Electrophysiologic Effects of Ranolazine. A Novel Anti-Anginal Agent with Antiarrhythmic Properties

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Abstract

—Ranolazine is a novel anti-anginal agent capable of producing anti-ischemic effects at plasma concentrations of 2–6 μM without reducing heart rate or blood pressure. The present study examines its electrophysiologic effects in isolated canine ventricular myocytes, tissues and arterially-perfused left ventricular wedge preparations.

Methods—Transmembrane action potentials (AP) from epicardial and midmyocardial (M) regions and a pseudo-electrocardiogram were simultaneously recorded from wedge preparations. APs were also recorded from epicardial and M tissues. Whole cell currents were recorded from epicardial and M myocytes.

Results—Ranolazine inhibited I_{Kr} (IC_{50}=11.5μM), late I_{Na}, late I_{Ca}, peak I_{Ca}, and $I_{Na\text{-}Ca}$ (IC_{50}=5.9, 50, 296 and 91 μ M, respectively) and I_{Ks} (17% at 30 μM), but caused little or no inhibition of I_{to} or I_{K1}. In tissues and wedge preparations, ranolazine produced a concentration-dependent prolongation of AP duration of epicardial, but abbreviation of that of M cells, leading to reduction or no change in transmural dispersion of repolarization (TDR). At $[K^+]_o$=4 mM, 10μ M ranolazine prolonged QT interval by 20 ms but did not increase TDR. Extrasystolic activity or spontaneous Torsade de Pointes (TdP) were never observed and stimulation-induced TdP could not be induced at any concentration of ranolazine, either in normal or low $[K^+]_o$. Ranolazine (5–20 μ M) suppressed early afterdepolarizations (EADs) and reduced the increase in TDR induced by selective I_{Kr} blocker, d-sotalol.

Conclusion—Ranolazine produces ion channel effects similar to those observed following chronic amiodarone (reduced I_{Kr}, I_{Ks}, late I_{Na} and I_{Ca}). Ranolazine's actions to suppress EADs and reduce TDR suggest that, in addition to its anti-anginal actions, the drug may possess antiarrhythmic activity.

Abridged Abstract—Ranolazine is a novel anti-anginal agent capable of producing anti-ischemic effects without reducing heart rate or blood pressure. The present study examines its electrophysiologic effects in isolated canine ventricular myocytes, tissues and arterially-perfused left ventricular wedge preparations. Ranolazine produces ion channel effects similar to those observed following chronic amiodarone (reduced I_{Kr}, I_{Ks}, late I_{Na} and I_{Ca}). Preferential prolongation of epicardial vs M cell action potentials results in modest QT interval prolongation but reduction in transmural dispersion of repolarization (TDR). Ranolazine's effects to reduce TDR and suppress EADs suggest that, in addition to its anti-anginal actions, the drug may possess antiarrhythmic activity.

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Keywords

Anti-ischemic; I_{Kr} blocker; Transmural dispersion of repolarization; QT prolongation; Early afterdepolarizations

Ranolazine is the first of a new class of compounds shown to exert anti-anginal effects without causing significant bradycardia and/or lowering systemic blood pressure.[1–3] The drug has been shown to increase left ventricular function in animals with chronic heart failure[4] and two clinical trials (MARISA and CARISA) have established its effectiveness as an anti-anginal agent. [1–3] Ranolazine is known to produce a modest prolongation of the QT interval, yet little is known about the electrophysiologic actions of the drug.[1] The present study was designed to assess the electrophysiological effects of ranolazine in myocytes, tissues and arterially-perfused wedge preparations isolated from the canine left ventricle.

Methods

Dogs weighing 20–35 kg were anticoagulated with heparin (180 IU/kg) and anesthetized with pentobarbital (35 mg/kg, i.v.). The chest was opened via a left thoracotomy, the heart excised and placed in a cold cardioplegic solution ($[K^+]_0$ = 8 mmol/L, 4°C). All protocols were in conformance with guidelines established by the Institutional Animal Care and Use Committee.

Voltage Clamp Studies in Isolated Canine Ventricular Myocytes

Myocytes were isolated by enzymatic dissociation from a wedge-shaped section of the left ventricular free wall supplied by the left circumflex coronary artery[5]. We used epicardial and M cells for most ion channel studies, the exception being measurement of I_{Ks}, which was done exclusively in epicardial cells, because of the low density of this current in M cells. We did not note any differences between the two cell types in response to ranolazine. Tyrode's solution used in the dissociation contained (in mM): 135 NaCl, 5.4 KCl, 1 $MgCl_2$, 0 or 0.5 $CaCl_2$, 10 glucose, 0.33 NaH_2PO_4, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and the pH was adjusted to 7.4 with NaOH.

External solutions

$I_{Kr}$and I_{K1}(mM)	**I_{Ks}(mM)**	**I_{Na-Ca}, Late $I_{Na}$and I_{Ca}Whole cell/Perf-patch (mM)**	**I_{to}Whole cell (mM)**
11 glucose	11 glucose	10 glucose	10 glucose
4 KCl	4 KCl	-	4 KCl
1.2 $MgSO_4$	1.8 $MgCl_2$	1 $MgCl_2$	1 $MgCl_2$
2 $CaCl_2$	1.8 $CaCl_2$	2 $CaCl_2$	2 $CaCl_2$
132 NaCl	145 NaCl	140 Na-methanesulfonate	140 N-methyl-D-glucamine-Cl
	1 NaH_2PO_4		
20 HEPES	10 HEPES	10 HEPES	10 HEPES
pH 7.4 with NaOH	pH 7.4 with NaOH	pH 7.4 with methane sulfonic acid	pH 7.4 with HCl

Internal solutions

I_{Ca}, Late I_{Na}, (mM)	**I_{Na-Ca} (mM)**	**I_{to} (mM)**	**I_{Ks}, $I_{Kr}$and I_{K1} (mM)**
140 Cs-aspartate	140 Cs-aspartate	130 K-aspartate	20 KCl
-	-	20 KCl	125 K-Aspartate
10 NaOH	10 NaOH	-	1 $MgCl_2$
1 $MgCl_2$	1 $MgCl_2$	1 $MgCl_2$	10 EGTA
5 MgATP	5 MgATP	5 MgATP	5 MgATP
10 HEPES	10 HEPES	10 HEPES	5 HEPES
10 EGTA	0.1 EGTA	5 EGTA	pH 7.1 with KOH
pH 7.1 with CsOH	pH 7.1 with CsOH	pH 7.1 with KOH	

L-type calcium current (I_{Ca}), late sodium current (Late I_{Na}), transient outward current (I_{to}), inward rectifier potassium current (I_{K1}), slowly (I_{Ks}) and rapidly (I_{Kr}) activating delayed rectifier potassium current, and sodium-calcium exchange current ($I_{Na\text{-}Ca}$) were recorded at 37°C using standard patch electrodes. The composition of the external and pipette solutions is shown in the Tables above. I_{K1} was measured in presence of 3 μM ouabain and 5 μM nifedipine to block the sodium-potassium pump and L-type calcium current ($I_{Ca,L}$), respectively. I_{Ks} was measured in the presence of 5 μM E-4031 and 300 μM cadmium to block I_{Kr} and I_{Ca}. I_{Kr} was measured in the presence of 5 μM nifedipine.

Isolated myocytes were placed in a temperature controlled 0.5 ml chamber (Medical Systems, Greenvale, NY) on the stage of an inverted microscope and superfused at a rate of 2 ml/min. An eight-barrel quartz micromanifold (ALA Scientific Instruments Inc., Westbury, NY) placed 100 μm from the cell was used to apply ranolazine at concentrations of (in μM): 0.1, 0.5, 1.0, 5.0, 10 and 100 and in some cases 300 and 900. An Axopatch 1D amplifier (Axon Instruments, Foster City, CA) was operated in voltage clamp mode to record currents. Whole cell currents were filtered with a 3-pole low-pass Bessel filter at 5 kHz, digitized between 2 – 5 kHz (Digidata 1200A, Axon Instruments) and stored on a computer. Clampex 7 acquisition and analysis software (Axon Instruments) was used to record and analyze ionic currents. Pipette tip resistance was 1.0– 2.0 MΩ and seal resistance was greater than 5 GΩ. Electronic compensation of series resistance averaged 76 %. Voltages reported in the text were corrected for patch electrode tip potentials[6]. A 3 M KCl-agar bridge was used between the Ag/AgCl ground electrode and external solution to avoid development of a ground potential when switching to experimental solution.

I_{Ks} was elicited by depolarization to +40 mV for 2 sec from a holding potential of −50 mV followed by a repolarization step to −30 mV. The time-dependent tail current elicited by the repolarization was termed I_{Ks}. I_{to} was not blocked, but it had little influence on our measurement of I_{Ks} because of its fast and complete inactivation. All measurements were obtained 5–12 min after patch rupture because no significant run-down of I_{Ks} is generally observed during this interval. I_{Kr} was measured as the time-dependent tail current elicited at a potential of − 40 mV following a short 250 ms depolarizing pulse to 30 mV. I_{K1} was recorded during 900 ms of 10 mV voltage steps applied from a holding potential of − 40 mV to test potentials ranging from −100 mV to 0 mV, and was characterized as the 5 ms average of the steady state current at the end of the test pulse.

Peak I_{Ca} was defined as maximum inward current minus the current at the end of the test pulse. External solution contained 10 μM TTX to block the steady state component of late I_{Na}. Cells were rested for 20 seconds at −90 mV before evoking an 800 ms ramp to −60 mV and a 15 ms step to −50 mV to inactivate sodium channels and maintain voltage control immediately followed by a 500 ms step to 0 mV to record I_{Ca} in control solutions. This protocol was repeated 5 times at a rate of 0.5 Hz for each of the drug concentrations. Late I_{Ca} was defined as the sum of the slow and fast components calculated 300 ms after the start of the test pulse.

To trigger $I_{Na\text{-}Ca}$ by means of the normal calcium transient, a 5 ms step to 0 mV to activate I_{Ca} and a calcium transient was immediately followed by a pulse to −80 mV to record $I_{Na\text{-}Ca}$. $I_{Na\text{-}Ca}$ was quantified as total charge transported (pA × ms). Voltage clamp protocols were preceded by a train of ten pulses to 20 mV delivered at a rate of 0.5 Hz followed by a rest of 6 sec to maintain calcium loading of the SR.

Late I_{Na} was measured during a train of 30 pulses at repetition rates of 300 and 2000 ms. Currents during the last 5 pulses of the trains were averaged to reduce noise, and late I_{Na} was defined as the TTX-sensitive current. Protocols were repeated in drug-free solution, 2–4 min after adding ranolazine, and immediately after 10 μM TTX was added to completely block late

I_{Na}. Action potentials, rather than square pulses were used to voltage clamp late I_{Na}. At a basic cycle length (BCL) of 300 ms, measurements were made midway through the plateau at a voltage of 13 mV and during phase 3 repolarization at a voltage of −28 mV. At a BCL of 2000 ms, measurements were made at voltages of 20 mV and −28 mV.

Action Potential Studies in Isolated Canine Ventricular Epicardial and M region Tissues

Epicardial and midmyocardial (M) cell preparations (tissue slices approximately 1 × 0.5 × 0.15 cm) were isolated from the left ventricle. The tissue slices were placed in a tissue bath (5 ml volume with flow rate of 12 ml/min) and allowed to equilibrate for at least 4 hours while superfused with an oxygenated Tyrode's solution (pH=7.35, t^0 =37±0.5°C) and paced at a basic cycle length (BCL) of 500 ms using field stimulation. The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 2 or 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5. Transmembrane potentials were recorded using standard glass microelectrodes filled with 2.7 M KCl (10–20 MΩDC resistance). Action potentials were recorded from isolate free-running Purkinje fibers as well as epicardial and M cell preparations. In the case of ventricular myocardial slices, control recordings were obtained after a 4–6 hour equilibrium period. The effects of ranolazine were determined at concentrations of 1, 5, 10, 50, and 100 μM, with recordings started 30 minutes after the addition of each concentration of the drug. Rate-dependence of ranolazine's actions were determined by recording transmembrane action potentials at basic pacing cycle lengths (ec) of 300, 500, 800, 1000, 2000, 5000 ms. Time controls demonstrated the stability of the preparations over a period of 6 hours. The rate of rise of the action potential (V_{max}) was recorded as a differentiated signal or measured from the digitally recorded signal (at 50 KHz).

Action Potential Studies in Arterially-Perfused Canine Left Ventricular Wedge Preparations

Transmural left ventricular wedges with dimensions of approximately 12 mm × 35 mm × 12 mm were dissected from the mid-to-basal anterior region of the left ventricular wall and a diagonal branch of the left anterior descending coronary artery was cannulated to deliver the perfusate (Tyrode's solution as defined above). Transmembrane action potentials were recorded from epicardial and subendocardial M cell regions using floating microelectrodes. A transmural pseudo-electrocardiogram (ECG) was recorded using two Ag/AgCl half cells (2 mm diam. × 4 mm) placed approx. 1 cm. from the epicardial(+) and endocardial(−) surfaces of the preparation and along the same axis as the transmembrane recordings. Ventricular wedges were allowed to equilibrate in the chamber for 2 hrs while paced at BCL of 2000 ms using silver bipolar electrodes contacting the endocardial surface. Perfusion pressure was maintained at 40–50 mmHg and temperature at 35 or 37.5 °C. The preparations were fully immersed in the extracellular solution throughout the course of the experiment.

Programmed electrical stimulation (PES)—Premature stimulation was applied to the epicardial surface before and after each concentration of drug in an attempt to induce arrhythmias. Single pulses (S2) were delivered once after every tenth basic beat (S1) at cycle lengths of 2000 ms. The S1–S2 coupling interval was progressively reduced until refractoriness was encountered (S2 stimuli were of 2–3 ms duration with an intensity equal to 3–5 times the diastolic threshold).

DRUGS—Ranolazine, E-4031 and d-sotalol were all dissolved in distilled water.

STATISTICS—Statistical analysis was performed using one way repeated measures analysis of variance (ANOVA) followed by Bonferroni's test, as appropriate. Mean values were considered to be different when $p < 0.05$.

RESULTS

Effect of Ranolazine on I_{Kr}, I_{Ks}, I_{K1}, I_{to}, late I_{Na}, I_{Ca}, late I_{Ca} and I_{Na-Ca}

Figures 1–3 show the effect of ranolazine to inhibit I_{Kr}, late I_{Na}, I_{Ca}, late I_{Ca} and I_{Na-Ca} in canine LV myocytes (midmyocardial and epicardial cells). Ranolazine inhibited both inward depolarizing and outward repolarizing currents. The potency (IC_{50}) of drug inhibition of late I_{Na} ranged between 5 and 21 μM, with a greater potency at more positive voltage-clamp test potentials and higher frequency of depolarization. Although ranolazine inhibited late $I_{Ca,L}$ with an IC_{50} of 50 μM, significant inhibition (25–30 %) occurred within the therapeutic range (2–6 μM). The drug weakly inhibited I_{Na-Ca} (inward sodium-calcium exchange current), peak $I_{Ca,L}$, and the slow component of the delayed rectifier potassium current (I_{Ks}-17% inhibition at 30 μM and 20% at 900 μM), but produced no effect on the outward rectifier potassium current (I_{K1}), or the transient outward potassium current (I_{to}).

Figure 3 shows the superimposed concentration-response curves for all of the currents for which such a relationship could be obtained. The plot highlights the overlap between the inhibition by ranolazine of I_{Kr}, late I_{Na} and late I_{Ca}, at clinically relevant therapeutic concentrations (2–6 μM), suggesting that the effect of the drug to block outward repolarizing current, which prolongs APD, is substantially counterbalanced by its effect to inhibit the inward current, which is expected to abbreviate the action potential.

Effect of Ranolazine in Isolated Canine Ventricular Tissues

Ranolazine caused no change in resting membrane potential, action potential amplitude or overshoot in either M or epicardial cells, except at very high concentrations (100 μM) considerably above the plasma concentrations encountered in clinical use (Table 1).

Figure 4 illustrates the effect of ranolazine on the maximal rate of rise of the upstroke (V_{max}) of the action potential recorded from free-running canine Purkinje fibers. Significant inhibition, presumably due to the effect of ranolazine to block peak I_{Na}, was observed only at concentrations ≥50 μM. Similar inhibition was observed in canine ventricular M cells (not shown). Shown in the bottom panel of Figure 4 is the effect of ranolazine on the APD of canine Purkinje fibers. At a BCL of 2000 ms, ranolazine produced a concentration dependent abbreviation of APD which was greater at 50% than at 90% repolarization. At a BCL of 500 ms, the drug produced little change in APD_{90}, but a concentration-dependent depression of the action potential plateau.

The effects of ranolazine on the action potential of canine ventricular M cell and epicardial tissues are illustrated in Figure 5. The drug caused a small concentration-dependent prolongation of APD in epicardium, but an abbreviation or biphasic effect in M cell preparations. Thus, ranolazine caused a concentration-dependent reduction of transmural dispersion of action potential duration. Higher concentrations of the drug produced a depression of the plateau of the action potential, likely due to the effect of ranolazine to inhibit I_{Ca} and late I_{Ca}. The preferential prolongation of epicardial APD was more apparent at a $[K^+]_o$ of 2 mM than at 4 mM (Figure 5, right panels). Early afterdepolarizations (EADs) were not observed at any concentration of ranolazine, not even under bradycardic and hypokalemic conditions known to potentiate the effect of I_{Kr} blocker to induce EADs.

These concentration and rate-dependent characteristics of ranolazine distinguish it from other agents that block I_{Kr}. Whereas selective I_{Kr} blockers invariably produce a concentration - dependent prolongation of APD that is greater in Purkinje fibers and M cells than in epicardial cells, the multi-ion channel block produced by ranolazine causes a concentration dependent abbreviation of Purkinje and M cell APD, but a slight prolongation of APD of epicardial cells.

Figure 6 illustrates another important distinction between typical I_{Kr} blockers and ranolazine. E-4031, a highly selective I_{Kr} blocker, caused a reverse rate-dependent prolongation of APD that is much greater in M cells than in epicardial cells, leading to a bradycardia-dependent accentuation of transmural dispersion of APD (TD-APD), typical of the actions of I_{Kr} blockers. In contrast, ranolazine produced a largely rate-independent prolongation of APD in epicardium and abbreviation in M cells, leading to a rate-independent ***reduction*** of transmural dispersion of APD. The reverse rate-dependent prolongation of APD and accentuation of TD-APD by E-4031 were still more dramatic at a $[K^+]_o$ of 2 mM (Fig 6, right panel). In contrast, ranolazine persisted in causing a rate-independent reduction of TD-APD at the lower $[K^+]_o$. These results are consistent with the clinical finding that prolongation of QTc by ranolazine, unlike that of pure I_{Kr} blockers, is not reverse-rate-dependent.[7] Thus, although ranolazine modestly prolongs APD and QTc, it is fundamentally different from other I_{Kr} blockers, which are typically associated with proarrhythmic actions.

Effect of Ranolazine in Canine Left Ventricular Wedge Preparation

Table 2 shows the effect of ranolazine in isolated canine left ventricular wedge preparations. In the presence of 4 mM $[K^+]_o$ and at a BCL of 2000 ms, ranolazine caused a small concentration dependent prolongation of epicardial APD and a small biphasic effect in the M region, resulting in a 30 ms QT prolongation at the highest concentration tested (100 μM). It is noteworthy that much of the QT interval prolongation observed at the higher concentrations of ranolazine is due a slowing of conduction secondary to sodium channel inhibition. Transmural dispersion of repolarization (TDR) showed a tendency to diminish, although the decrease was not statistically significant. Under hypokalemic conditions ($[K^+]_o$=3 mM), ranolazine produced a much greater prolongation of the QT interval, but TDR showed a similar tendency to decrease.

Ranolazine did not induce either spontaneous or programmed electrical stimulation-mediated TdP during endocardial or epicardial pacing of the canine left ventricular wedge preparation at any concentration up to 100 μM. TdP did not develop nor could it be induced, even in the presence extremely low $[K^+]_o$ (2 or 3 mM) and high concentrations of ranolazine. In contrast, both spontaneous and stimulation induced TdP have been shown to develop in the perfused wedge preparation in response to a wide variety of I_{Kr} blockers.[8]

Neither early or delayed afterdepolarizations were observed in either tissue or wedge preparations pretreated with any concentration of ranolazine. On the contrary, as illustrated in Figure 7, ranolazine proved to be effective in suppressing EADs in M cell and Purkinje fiber preparations pretreated with other I_{Kr} blockers such as d-sotalol. D-Sotalol (100 μM) produced a remarkable prolongation of repolarization and induced EADs in both M cell and Purkinje fiber preparations. Ranolazine caused a concentration-dependent abbreviation of the action potential and abolished the EADs. A similar effect of ranolazine (5–20 μM) to suppress EAD activity and abbreviate APD was observed in 4/4 M cell and 3/3 Purkinje fiber preparations. Moreover, ranolazine was found to be effective in reducing TD-APD, the interval between the peak and end of the T wave (Tpeak-Tend) and TDR under long QT conditions (D-sotalol, moxifloxacin and ATX-II)(data not shown).

Discussion

The effects of ranolazine on cardiac ion currents at concentrations within the therapeutic range (i.e., 2–6 μM) include inhibition of I_{Kr}, late I_{Na} and late $I_{Ca,L}$. Ranolazine inhibition of I_{Kr} prolongs APD and its effect to inhibit late I_{Na} and late $I_{Ca,L}$, abbreviates APD. The net effect and clinical consequence of inhibition of these ion channel currents is a modest increase in the mean QTc interval over the therapeutic range. The drug differs significantly from other agents that block I_{Kr} and induce TdP. Ranolazine-induced prolongation of the APD is rate independent

(i.e., does not display reverse rate dependent prolongation of APD), and is not associated with EADs, triggered activity, an increase in spatial dispersion of repolarization or polymorphic VT. Indeed, rather than displaying arrhythmogenic activity, ranolazine, via its actions to suppress EADs and reduce TDR, possesses significant antiarrhythmic activity, acting to suppress the arrhythmogenic effects induced by a variety of other QT-prolonging drugs.

Drugs with QT prolonging properties have attracted considerable attention in recent years due to their proclivity to induce life-threatening cardiac arrhythmias, such as Torsade de Pointes[9–11]. More than 50 commercially available or investigational non-cardiovascular and 20 cardiovascular non-antiarrhythmic drugs have been shown or are suspected to have proarrhythmic effects.

The mechanisms underlying TdP have long been a matter of debate. Recent studies have identified dispersion of repolarization secondary to accentuation of electrical heterogeneities intrinsic to ventricular myocardium as the substrate and EADs as the trigger for the development of TdP. [8, 10, 12–15]

Ventricular myocardium is comprised of at least three electrophysiologically distinct cell types: epicardial, M and endocardial. M cells are distinguished by having action potentials that prolong disproportionately relative to the action potentials of other ventricular myocardial cell types in response to a slowing of rate and/or in response to many QT-prolonging drugs.[16–18] The ionic basis for these features include the presence of a smaller slowly activating delayed rectifier current (I_{Ks}) [19], a larger late I_{Na}[20], and $I_{Na\text{-}Ca}$ [21]. I_{Kr} and I_{K1} are similar in the three transmural cell types. M cells, like Purkinje fibers, develop EADs in response to agents and pathophysiologic conditions that reduce the repolarization reserve of the ventricular myocardium. Epicardial and endocardial cells generally do not.

Most drugs that prolong the QT interval accentuate the normal transmural heterogeneity of final ventricular repolarization by causing a preferential prolongation of the action potential of M cells. I_{Kr} blockers, including d-sotalol, almokalant, E-4031, moxifloxacin and erythromycin augment transmural dispersion of repolarization as a consequence. These agents cause relatively little prolongation of the APD of epicardial and endocardial cells, because these cell types possess a much more prominent I_{Ks} as compared to the M cell. A similar preferential prolongation of the M cell APD is seen with agents that increase calcium current (I_{Ca}) such as Bay K 8644 as well as with agents that increase late I_{Na} such as ATX-II, anthopleurin-A and DPI 201-106. An exception to this rule applies to agents that block I_{Ks}, which cause a similar percentage of APD prolongation in the three transmural cell types.

A more complex electrophysiological effect is observed with drugs affecting two or more ion channels, such as amiodarone, sodium pentobarbital, quinidine, cisapride and azimilide. Amiodarone is a potent antiarrhythmic agent used in the management of both atrial and ventricular arrhythmias. In addition to its β-blocking properties, amiodarone is known to block late I_{Na}, I_{Ca}, I_{Kr} and I_{Ks}. The efficacy of the amiodarone and its low incidence of proarrhythmia relative to other agents with Class III actions are attributable to this complex multi-channel inhibition.[22] When administered chronically, amiodarone increases QT without augmenting spatial dispersion of repolarization, unlike other I_{Kr} blockers. [23–25] In some cases transmural dispersion of repolarization is reduced.[23] Chronic amiodarone therapy can also suppress the effect of other I_{Kr} blocker, like d-sotalol, to increase TDR or induce EADs. [23] Thus, chronic amiodarone alters cellular electrophysiology of ventricular myocardium so as to reduce TDR and suppress EADs, especially under conditions in which they are accentuated. The drugs potent inhibition of late I_{Na} is thought to play a key role.

The multi-channel inhibition, particularly the ability to potently block late I_{Na}, has been suggested to underlie the effect of I_{Kr} blockers to prolong QT without creating the substrate or trigger for the development of TdP. Indeed, this feature could contribute to the suppression of EADs and reduction of spatial dispersion of repolarization, the substrate and trigger for TdP. This is the case for amiodarone and sodium pentobarbital, as well as for high concentrations of quinidine and cisapride. [24, 26–28] Our data suggest that ranolazine fits this pharmacologic profile as well. Like amiodarone and sodium pentobarbital, ranolazine produces a preferential prolongation of epicardial APD_{90}, leading to a reduction in transmural dispersion of repolarization. The opposite effects of ranolazine on M and Purkinje fiber to that of epicardial APD is likely due to the more prominent late I_{Na} in the M cell and Purkinje fiber than in epicardial cells [20]. Ranolazine is among the most potent late I_{Na} blockers reported. It causes a decrease in net inward current in the M cells and Purkinje fiber, but a decrease in net outward current in epicardium. The effect of ranolazine to block late I_{Na} and late I_{Ca} likely underlie its effect to suppress EAD activity. Thus, unlike other I_{Kr} blockers, ranolazine does not lead to the development of TdP, either spontaneous or stimulation-induced. Of note, ranolazine has recently been evaluated in an anesthetized dog model with acute complete atrioventricular block, a model susceptible to drug-induced polymorphic VT. At doses that prolonged the QT interval by approximately 5% to 11% above control, ranolazine did not cause spontaneous TdP or TdP facilitated by iv bolus of phenylephrine (which increases susceptibility to TdP) in five dogs, whereas sotalol induced TdP in all five dogs under these conditions.[29] Recent studies involving isolated guinea pig and rabbit hearts have also reported failure of ranolazine to induce TdP, but it effectiveness to suppress TdP induced by selective I_{Kr} blockers (E-4031) and agents that augment late I_{Na} (ATX-II). [29] In summary, the available data suggest that ranolazine, in addition to its anti-anginal actions, may possess important antiarrhythmic activity.

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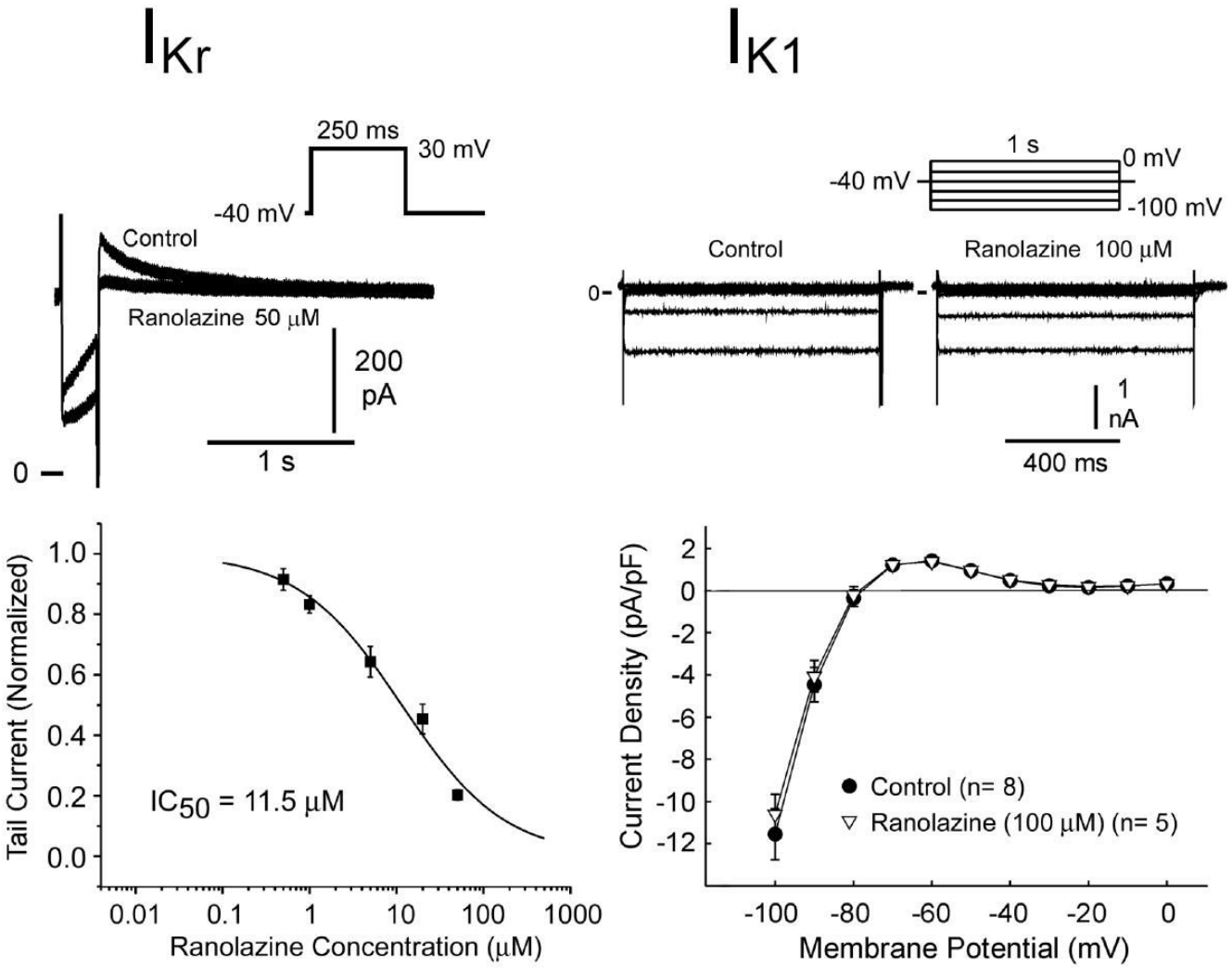

Figure 1.

Effect of ranolazine on the rapidly activating component of the delayed rectifier current (I_{Kr}, left) and the inward rectifier current (I_{K1}, right) in canine left ventricular myocytes. Top panels illustrate the voltage protocol and representative current traces. Bottom panels are the concentration-response relationships. Data are presented as mean ± S.E.M. (n = 5–8).

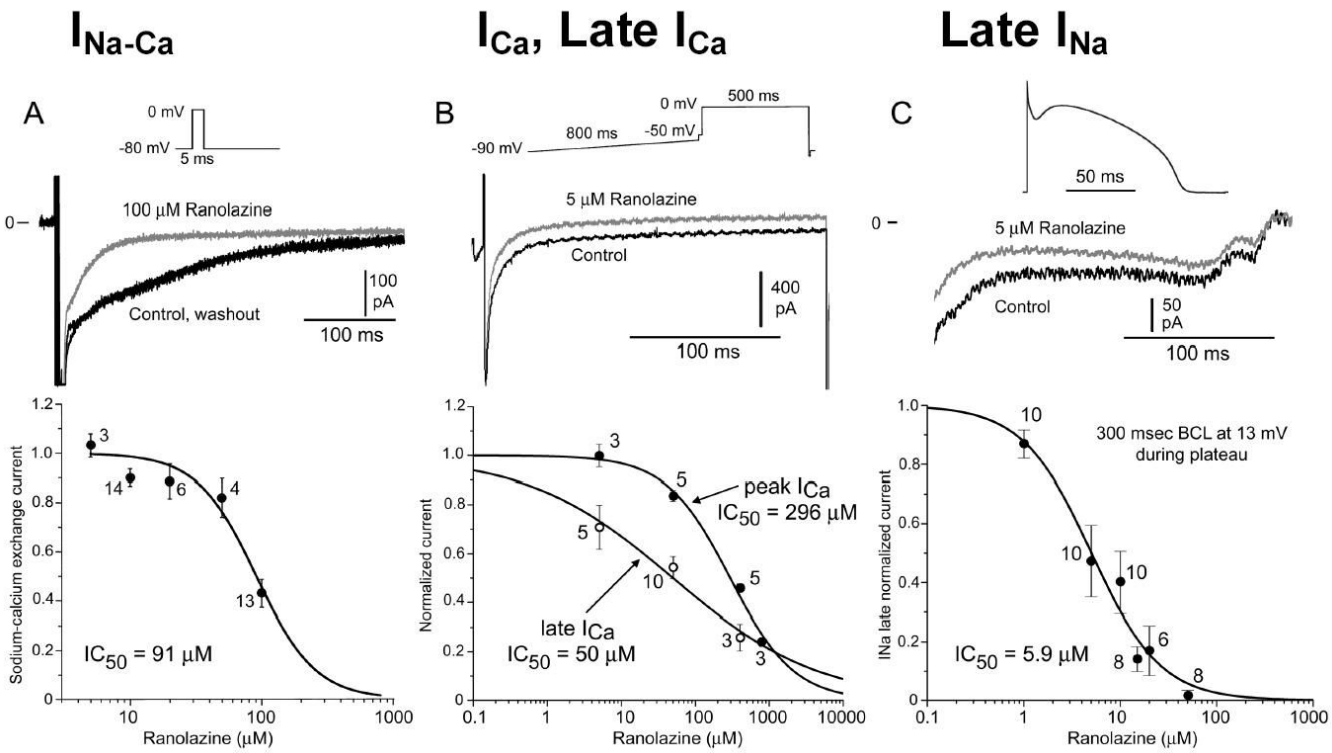

Figure 2.

Effect of ranolazine on the sodium calcium exchange current (I_{Na-Ca}, left), peak and late calcium channel current (I_{Ca} and late I_{Ca}, middle) and late sodium current (late I_{Na}, right). Top panels show the voltage protocols used and representative current traces. Bottom panels are the concentration-response relationships. Data are presented as mean ± S.E.M. (n = 3 – 14 as indicated over data points)

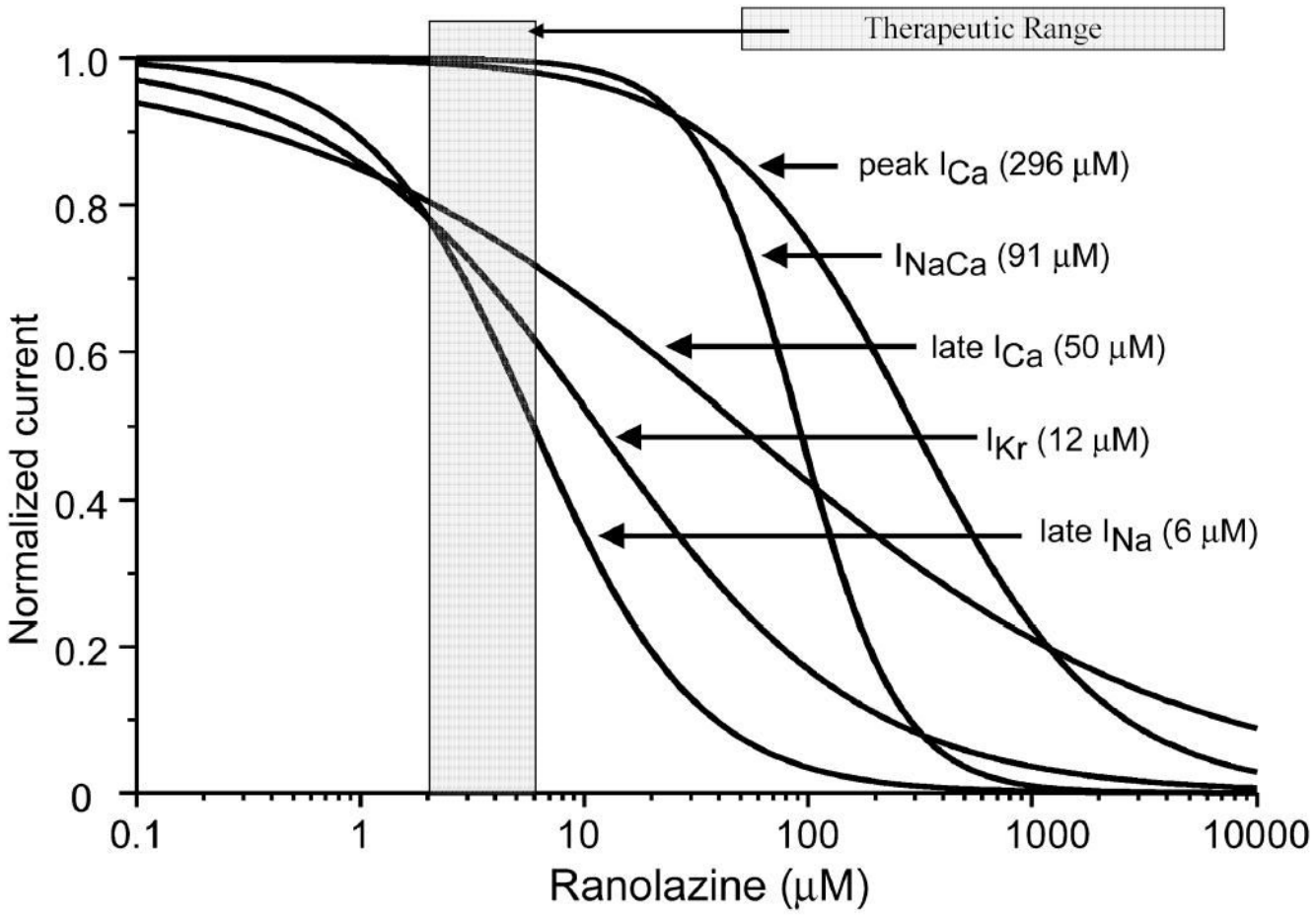

Figure 3.

Summary of the concentration–response relationships for the effect of ranolazine to inhibit inward and outward ion channel currents in canine ventricular myocytes. Numbers inside the parentheses are IC_{50} values for the effect of ranolazine to inhibit the rapidly activating delayed rectifier potassium current (I_{Kr}), late sodium current (late I_{Na}), peak calcium current (I_{Ca}), late I_{Ca}, and the sodium-calcium exchange current (I_{Na-Ca}).

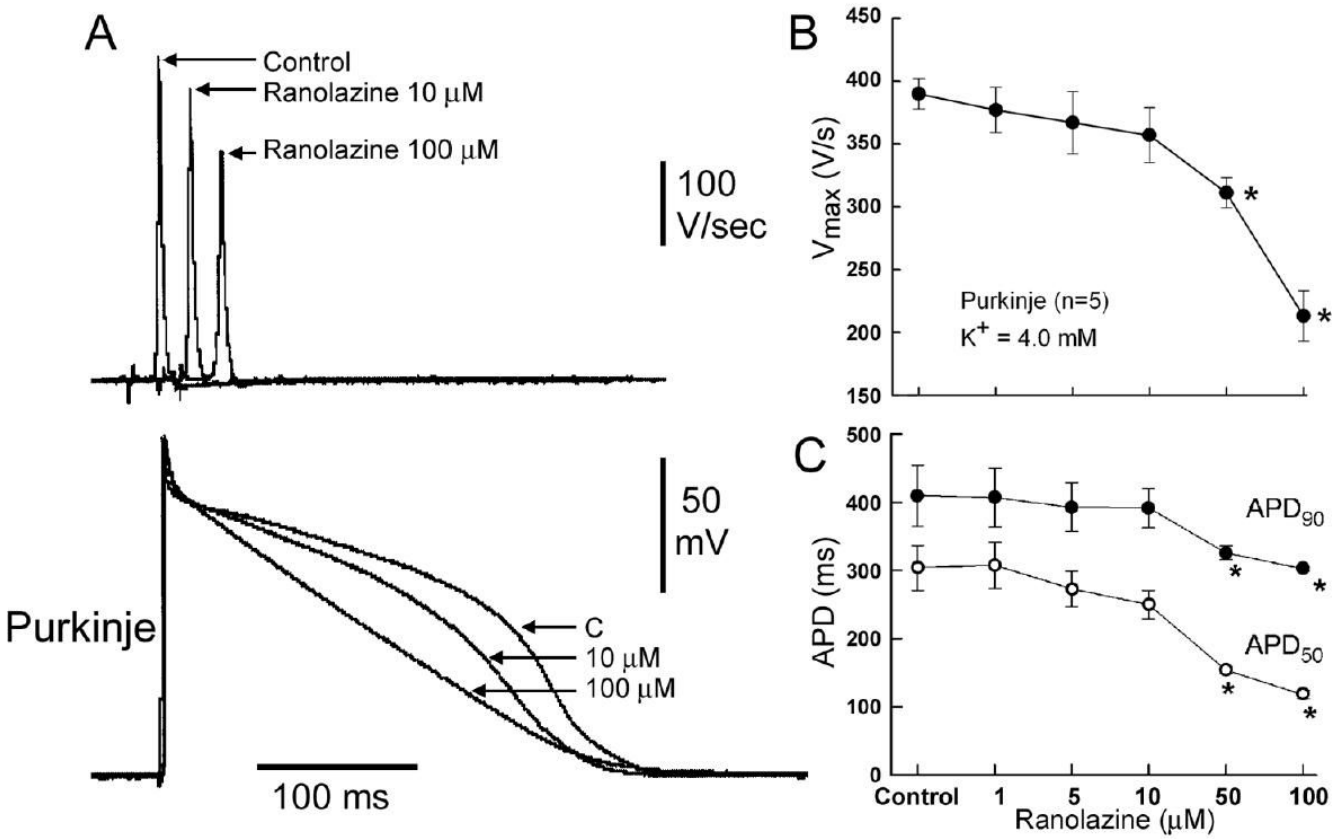

Figure 4.

Concentration -dependent effects of ranolazine on the rate of rise of the upstroke of a Purkinje fiber action potential (V_{max}). **A:** Shown are superimposed action potentials and corresponding differentiated upstrokes (dV/dt) recorded in the absence and presence of ranolazine (1–100 μM) (BCL=500 ms). **B:** Concentration-response relationship of ranolazine's effect to reduce V_{max}. Data are presented as mean ± S.E.M. (n=5).

C: Concentration-dependent effect of ranolazine on action potential duration at 50 and 90% repolarization ($APD_{50}$and APD_{90}). BCL=2000 ms. Values are mean ± SEM. * − 0.05 vs. control

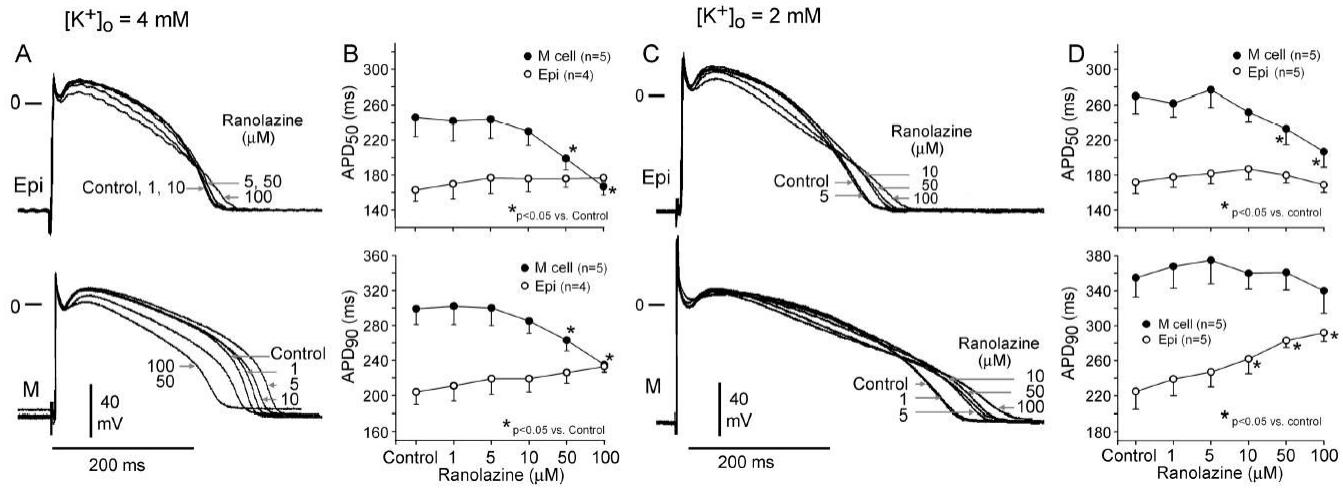

Figure 5.

Left Panel: 4 mM $[K^+]_o$. Effects of ranolazine on epicardial and M cell action potentials. **A:** Superimposed transmembrane action potentials recorded under control conditions and following the addition of progressively higher concentrations of ranolazine (1–100 μM). **B:** Concentration-response curves for the effect of ranolazine on action potential duration (APD_{50} and APD_{90}). **Right Panels:** 2 mM $[K^+]_o$. Effects of ranolazine on epicardial and M cell action potentials recorded at a pacing cycle length of 2000 ms and $[K^+]_0$ = 2 mM. **C:** Shown are superimposed transmembrane action potentials recorded in the absence and presence of ranolazine (1–100 μM). **D:** Concentration-dependent effect of ranolazine on action potential duration (APD_{50} and APD_{90}). BCL=2000 ms. Data presented as mean ± SEM.

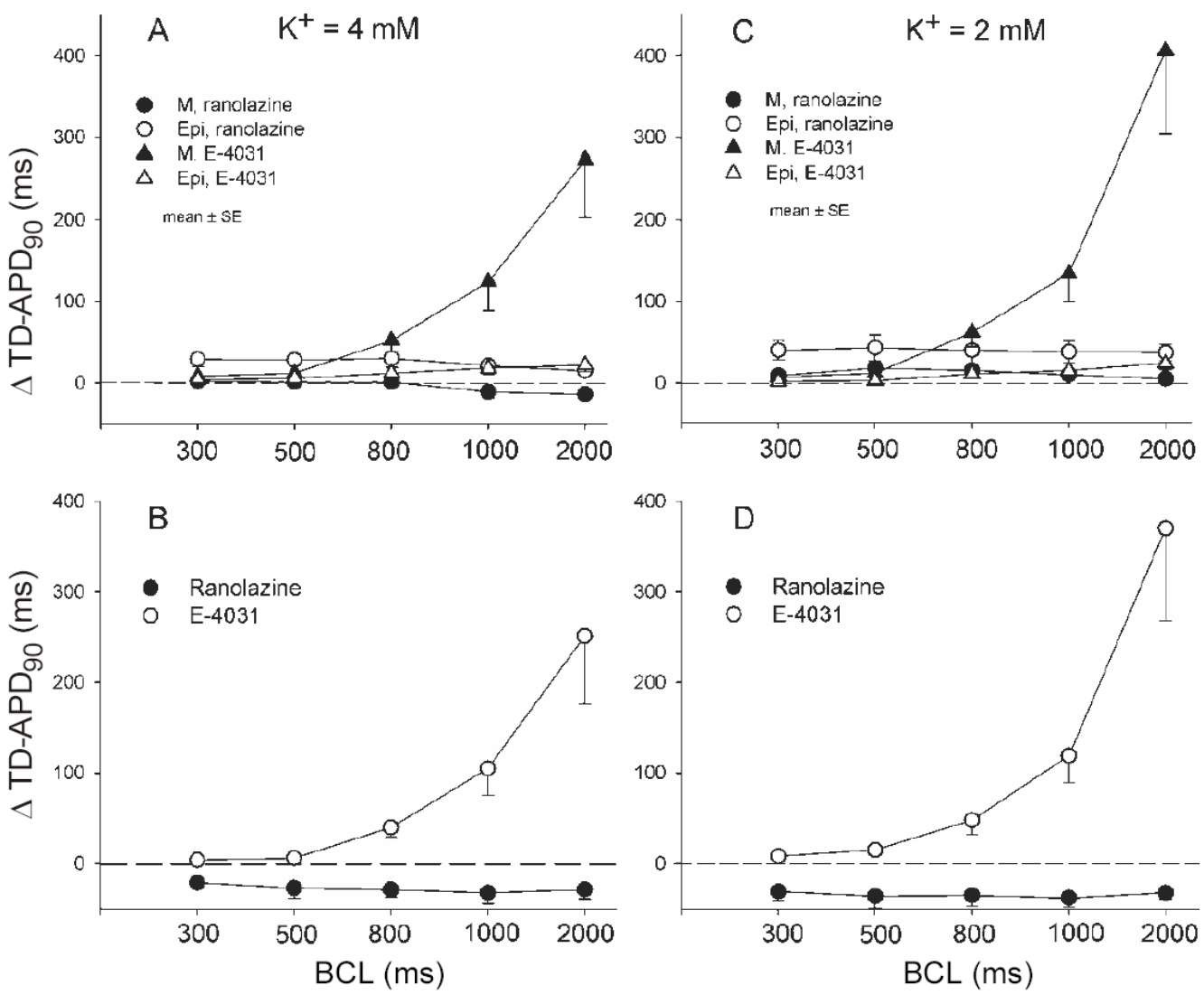

Figure 6.

Rate-dependent effect of ranolazine (10 μM) and E-4031 (1 μM) on action potential duration (APD) in canine ventricular M cell and Epicardial tissue slices in the presence of 4 mM (A and B) and 2 mM (C and D) $[K^+]_o$. **A and C:** Rate-dependence of E-4031 and ranolazine-induced change in APD_{90} of M and epicardial cells, respectively. **B and D:** Rate-dependence of drug-induced change in transmural dispersion of action potential duration ($TD\text{-}APD_{90}$). Values are mean ± SEM.

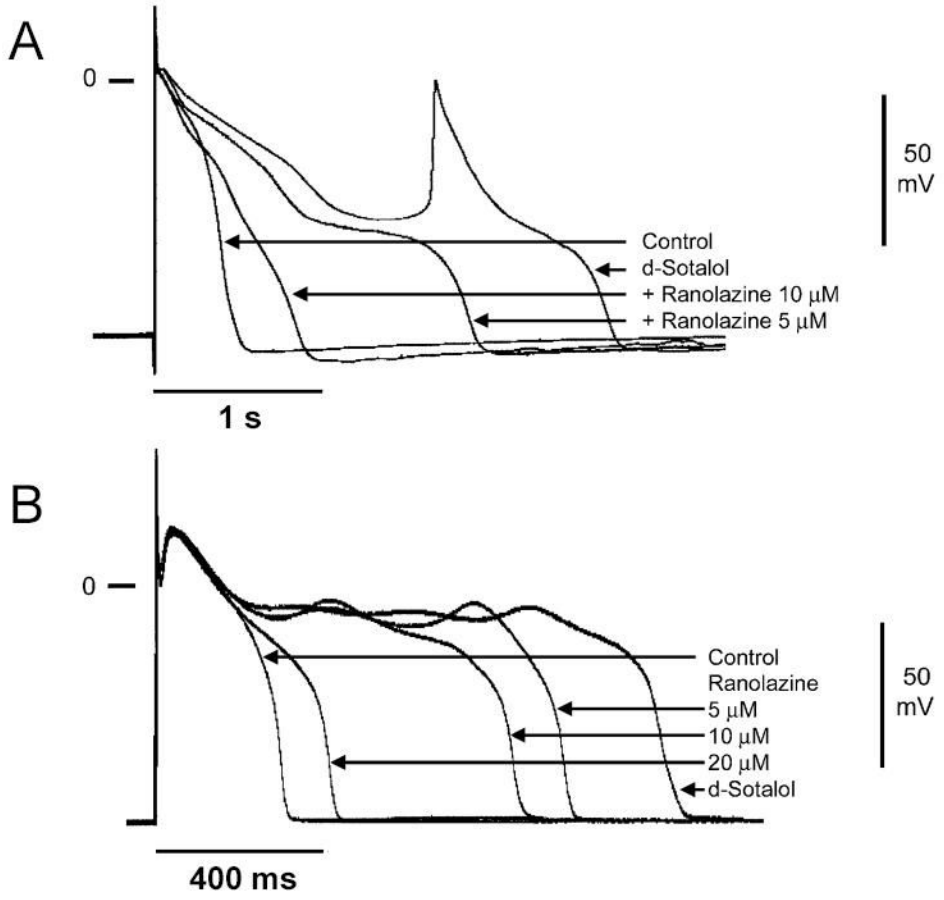

Figure 7.

Effect of ranolazine to suppress d-sotalol-induced early afterdepolarizations (EAD) in M cell and Purkinje fiber preparations. **A:** Shown are superimposed transmembrane action potentials recorded from a Purkinje fiber preparation in the presence of I_{Kr} block (100 μM d-sotalol), and following addition of increasing concentrations of ranolazine (5 and 10 μM) in the continued presence of d-sotalol. $[K^+]_o$=3 mM. BCL=8000 ms and **B:** Superimposed transmembrane action potentials recorded from an M cell preparation under control conditions, in the presence of I_{Kr} block (100 μM d-sotalol), and following increasing concentrations of ranolazine (5, 10, and 20 μM) in the continued presence of d-sotalol. BCL=2000 ms.

TABLE 1

Effects of ranolazine on phase 0 amplitude, resting membrane potential (RMP), and overshoot of action potential in M cell and epicardial preparations at a BCL of 500 ms.

M Cell	Ranolazine Concentration (μM)					
	Control	**1**	**5**	**10**	**50**	**100**
Amplitude	107±6	109±4	114±4	113±4	104±3	91±8*
RMP	−86±2	−86±1	−86±1	−88±1	−84±2	−82±3
Overshoot	21±6	23±5	27±3	25±3	19±1	9±6

Epicardial Cell						
	Control	**1**	**5**	**10**	**50**	**100**
Amplitude	95±2	93±2	101±1	94±2	86±6	93±2
RMP	−84±2	−84±2	−89±1	−88±1	−86±1	−85±2
Overshoot	11±1	10±2	12±1	8±2	0±6	8±4

Data are all in mV and expressed as mean±SEM, n=5 for all,

* - $p<0.05$ vs. control

Table 2

Summary of the effects of ranolazine on the action potential duration (APD), QT interval and transmural dispersion of repolarization (TDR) in the canine arterially-perfused left ventricular wedge preparation.

4 mM $[K^+]_o$

Concentration	Epicardium APD_{50}	APD_{90}	M region APD_{50}	APD_{90}	QT	TDR
control	180.4 ± 8.0	219.7 ± 9.6	218.0 ± 7.5	270.3 ± 9.6	276.7 ± 8.7	33.4 ± 3.2
1 μM	185.6 ± 7.5	228.0 ± 9.5	216.6 ± 5.0	271.4 ± 8.6	283.8 ± 8.6*	28.7 ± 2.5
5 μM	189.5 ± 9.4	233.0 ± 9.5	220.6 ± 6.1	281.1 ± 9.1	295.1 ± 9.5*	31.0 ± 3.8
10 μM	188.9 ± 10.1	238.3 ± 10.2	219.6 ± 5.8	284.9 ± 9.8	301.3 ± 9.0*	30.7 ± 4.5
100 μM	164.3 ± 7.6	233.0 ± 7.5	188.6 ± 6.0*	278.0 ± 8.1	306.9±11.7‡*	28.2 ± 5.3

3 mM $[K^+]_o$

Concentration	Epicardium APD_{50}	APD_{90}	M region APD_{50}	APD_{90}	QT	TDR
Control	186.4 ± 8.6	234.9 ± 10.3	228.6 ± 12.9	285.9 ± 14.3	293.0 ± 12.6	29.5 ± 7.7
1 μM	192.2 ± 9.2	240.0 ± 11.0	229.8 ± 13.9	289.7 ± 14.6	300.3 ± 12.8	28.7 ± 7.2
5 μM	193.6 ± 9.1	250.6 ± 10.8	226.5 ± 11.7	298.4 ± 13.7	314.4 ± 11.5*	26.0 ± 5.4
10 μM	198.9 ± 7.7	262.1 ± 8.2	236.4 ± 8.9	307.9 ± 7.0	318.8 ± 8.8	25.0 ± 5.6
100 μM	181.6 ± 14.7	285.3 ± 10.1*	198.4 ± 12.0	312.2 ± 9.6	347.7 ± 16.2‡*	12.9 ± 3.7

* p<0.05 vs. control. n=7 (unless otherwise noted);

‡ n=5 All values are mean ± SEM in ms; BCL=2000 ms

* p<0.05 vs. control. n=5 (unless otherwise noted);

‡ n=3 All values are mean ± SEM in ms; BCL=2000 ms