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Ranolazine stabilizes cardiac Ryanodine receptors: a novel mechanism for the suppression of Early afterdepolarization and Torsade de Pointes in Long QT type 2

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Abstract

Background—Ranolazine (Ran) is known to inhibit multiple targets, including: the late Na⁺ current, I_{NaP} , the rapid delayed rectifying K⁺ current I_{Kr} , L-type Ca²⁺ current $I_{Ca,L}$, and fatty-acid metabolism. Functionally, Ran suppresses early afterdepolarization (EADs) and Torsade de Pointes (TdP) in drug-induced long QT type 2 (LQT2) presumably by decreasing intracellular [Na⁺]_i and Ca²⁺ overload. However, simulations of EADs in LQT2 failed to predict their suppression by Ran.

Objectives—To elucidate the mechanism(s) whereby Ran alters cardiac action potentials (APs) and cytosolic Ca²⁺ transients (Ca_iT) and suppresses EADs and (TdP) in LQT2.

Methods—The known effects of Ran were inserted in simulations (*Shannon* and *Mahajan* models) of rabbit ventricular APs and Ca_iT in control and LQT2 models and compared to experimental optical mapping data from Langendorff rabbit hearts treated with E4031 (0.5 M) to block I_{Kr} . Direct effects of Ran on cardiac ryanodine receptors (RyR2) were investigated in single channels and changes in Ca²⁺-dependent high-affinity ryanodine binding.

Results—Ran (10 μM) alone prolonged APDs (206±4.6 to 240±7.8 ms; p<0.05); E4031 prolonged APDs (204±6 to 546±35 ms) and elicited EADs and TdP that were suppressed by Ran (10 μM, n=7/7 hearts). Simulations (*Shannon* but not *Mahajan* model) closely reproduced experimental data except for EAD-suppression by Ran. Ran reduced P_o of RyR2 (IC₅₀=10±3 μM; n=7) in bilayers and shifted EC₅₀ for Ca²⁺-dependent ryanodine-binding from 0.42±0.02 to 0.64±0.02 M with 30 M Ran.

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Conclusions—Ran reduces P_o of RyR2, desensitizes Ca^{2+} -dependent RyR2 activation and inhibits Ca_i oscillations which represents a novel mechanism for its suppression of EADs and TdP.

Introduction

Ran (2–6 μ M) is approved for the treatment of *Angina Pectoris* and ischemic heart disease but its exact therapeutic mode of action remains controversial. Early studies suggested that Ran altered myocardial energy metabolism by reducing fatty acid oxidation and glucose oxidation.¹ The inhibition of fatty oxidation by Ran appeared at relatively high concentrations (12% inhibition at 100 μ M) which brought into question the validity of this mode of action.^{1–3} Alternatively, Ran at therapeutic doses (<10 μ M) was shown to inhibit the late sodium current, I_{Na}^4 . Besides its efficacy in the treatment of angina pectoris, Ran suppressed early afterdepolarizations (EADs) and Torsade de Pointes (TdP) in animal models of acquired long QT type 2 (LQT2)⁵ despite its tendency to prolong the QT interval by inhibiting the rapid, delayed rectifying K^+ current, I_{Kr} .⁶

The inhibition of the I_{Na} window results in a decrease of intracellular Na^+ and improved extrusion of Ca^{2+} via the Na-Ca exchange current, I_{NCX} .^{7–9} Inhibition of I_{Na} could account for the therapeutic effects of Ran because a reduced Ca_i load would reduce the bioenergetics stress, protect the heart from ischemic injury and suppress the incidence of EADs.

Two not mutually-exclusive mechanisms have been proposed to explain the generation of EADs. EADs could be elicited by the spontaneous re-activation of the L-type Ca^{2+} channels, $I_{Ca,L}$ which is triggered by a “ Ca^{2+} window current”, $I_{Ca,w}$ during the AP plateau.^{10–14} At normal heart rates, APDs are too short to permit $I_{Ca,w}$ but when the APD is prolonged as in LQT, there may be sufficient time to activate $I_{Ca,w}$.¹² Agents that prolong APDs such as BAY-K8644 that increases the amplitude and duration of $I_{Ca,L}$ ¹³ or I_{Kr} blockers¹⁴ elicited a slow “conditioning phase” perhaps due to $I_{Ca,w}$ followed by the faster EADs upstroke caused by a regenerative $I_{Ca,L}$.¹⁵ More compelling evidence has shifted the general consensus to Ca^{2+} overload as the primary trigger of EADs. In this case, long APDs result in a greater Ca^{2+} influx during the AP plateau phase, sarcoplasmic reticulum (SR) Ca^{2+} overload and spontaneous SR Ca^{2+} release where the elevation of cytosolic Ca^{2+} activates the depolarizing, forward mode of I_{NCX} that re-activates $I_{Ca,L}$ causing an EAD.^{16–18}

Computational models for profiling proarrhythmic risk have made significant advances. Highly sophisticated *in silico* models have been developed to predict the shape and time-course of APs and Ca_iT in ventricular myocytes¹⁹, with the *Shannon*²⁰ and the *Mahajan*²¹ models being specifically designed to incorporate experimentally determined properties of rabbit ventricular myocytes. The *Shannon* model contains a robust representation of excitation-contraction coupling where the properties of SR Ca^{2+} release include inactivation/adaptation and a non-linear dependence on SR Ca^{2+} -load. The *Mahajan* model includes a minimal seven-state Markovian model of $I_{Ca,L}$, which incorporates voltage-dependent inactivation (VDI) and Ca-dependent inactivation (CDI). Both models include advanced calcium cycling kinetics, critical for the development of EADs.

Here, we show that modeling the actions of Ran based on its IC_{50} values at its known targets failed to predict Ran’s suppression of EADs in LQT2. We hypothesize that antiarrhythmic effect of Ran in the setting of LQT2 cannot be understood without including additional sites of action that alter intracellular Ca^{2+} handling and which to date have not been identified. The current study investigates the effect of Ran on the incidence of EADs and TdP in a rabbit model of acquired LQT2 using simultaneous optical mapping of APs and Ca_iT . Experimental findings are compared to mathematical simulations of APs and Ca_i in rabbit

ventricular myocytes and tests the effects of Ran on RyR2 reconstituted in bilayers and Ca^{2+} -dependent RyR2 activation by measuring changes in high-affinity [^3H]ryanodine binding.

Materials and Methods

Heart Preparations and Optical Mapping

New Zealand White rabbits, (adult females >60 days old, ~2kg) were injected with pentobarbital (35 mg kg^{-1} , I.V.) and heparin (200 U kg^{-1}) via an ear vein; the heart was excised and retrogradely perfused through the aorta with Tyrode's solution (in mM): 130 NaCl, 24 NaHCO_3 , 1.0 MgCl_2 , 4.0 KCl, 1.2 NaH_2PO_4 , 50 dextrose, 1.25 CaCl_2 , at pH 7.4, gassed with 95% O_2 and 5% CO_2 . Temperature was maintained at $37.0 \pm 2^\circ\text{C}$ and perfusion pressure was adjusted to ~70 mmHg with a peristaltic pump.²² The atrio-ventricular node was ablated by cauterization to control rate (500–2000 ms). The heart was stained with a bolus of a voltage-sensitive dye (RH 237 or PGHI; 50 μl of 1 mgml^{-1} in dimethyl sulfoxide, DMSO) and a Ca^{2+} indicator (Rhod-2/AM, 300 μl of 1 mgml^{-1} in DMSO) delivered through the bubble trap, above the aortic cannula. The hearts were oriented to view the anterior surface, record control APs and Ca_iT then add E4031 (0.5 μM) and/or Ran to the perfusate. E4031 was purchased from Sigma-Aldrich (St Louis, MO) and Ran was the kind gift of Dr. Luiz Belardinelli (Gilead Sciences, Palo Alto, CA). The optical apparatus based on 2 (16×16 pixels) photodiode arrays has been previously described.^{22, 23} Each pixel viewed a $0.9 \times 0.9 \text{ mm}^2$ area of myocardium and images were acquired at 1,000 frames/s.

Single channel recordings of Cardiac Ryanodine Receptors (RyR2)

Cardiac SR vesicles (5–10 g/ml) isolated from sheep ventricles²⁴ were added to the *cis*-chamber of a planar bilayer setup containing 400 mM $\text{Cs}^+\text{CH}_3\text{O}_3\text{S}^-$, 25mM Hepes, pH 7.4, while the *trans*-side contained 40mM $\text{Cs}^+\text{CH}_3\text{O}_3\text{S}^-$, 25mM Hepes, pH 7.4. Bilayers were made of 5:3:2 PE:PS:PC (Avanti Polar Lipids – Coagulation reagent 1) painted across a 150 μm hole separating two compartments. Following fusion of an SR vesicle to the bilayer, 4M $\text{Cs}^+\text{CH}_3\text{O}_3\text{S}^-$, 25mM Hepes, pH 7.4 was added to the *trans*-side to equalize the salt concentration at 400 mM. Channel output was filtered at 0.8–1.0 kHz and traces were recorded at a holding potential of -40 mV , for not less than three minutes following an addition of Ran to the *cis*-chamber. Single channel analysis was carried out using the ClampFit program (Axon Instruments: pClamp software). The P_o (mean $\pm$ SE) normalized to 1 (control without Ran) was plotted as a function of [Ran] ($n=7$).²⁵

Ca^{2+} dependent ryanodine binding to RyR2

Equilibrium ryanodine-binding was measured as a function of free Ca^{2+} (Ca_f) in buffer containing 250mM KCl, 15mM NaCl, 2nM [^3H]ryanodine, 13nM unlabeled ryanodine, 20mM PIPES, pH 7.1 $\pm$ 30 M Ran at an SR concentration of 0.5 mg/ml, following 3 hours incubation at 37°C .²⁶ Nonspecific binding, measured in the presence of 200nM unlabeled ryanodine and 50 M Ca^{2+} +4 mM EGTA, was subtracted from all measurements. Ca^{2+} was buffered with EGTA and Ca_f was calculated with established binding constants and measured with a Ca^{2+} -selective electrode.

Simulations

APs and Ca_iT predicted by the Shannon²⁰ and Mahajan²¹ models were compared to optical recordings at various CLs (500, 1000, 2000 ms). The models were tested for their ability to generate EADs in a rabbit model of LQT2 (see supplement). Simulations of LQT2 included: a) a 50 % decrease of IKr , to mimic the effect of E4031, b) a 32% increase of $\text{I}_{\text{Ca,L}}$ to mimic

the increase in $I_{Ca,L}$ expression measured in females hearts and shown to be a key factor for the higher risk of TdP in female hearts,²⁷ c) an increase in cycle length to 1 or 2 s since bradycardia is a critical co-factor to promote EADs.

The multifaceted action of Ran was modeled by modifying the channel conductance based on the Hill equation using the following values: I_{Kr} ($IC_{50}=12\mu M$), I_{Na} ($IC_{50}=5.9\mu M$), $I_{Ca,L}$ ($IC_{50}=50\mu M$), and the sodium-calcium exchange current I_{NCX} ($IC_{50}=91\mu M$),⁶ (See supplement). The code for *Shannon* model was provided by Dr. Jose Luis Puglisi (Davis, CA) and was compiled in Matlab, while the code for the *Mahajan* model was provided by Dr. Alain Karma (Boston, MA) and was compiled in C++.

Analysis

APDs, Ca_iT , rise-times, durations, amplitudes and V_m to Ca_i delays were measured at regular intervals. Measurements of duration are expressed as mean SEM; Student's t-Test (paired) was applied to determine statistical significance based on $p < 0.05$.

Results

Effects of Ran on APs and Ca_iT before and after I_{Kr} block

Figure 1 illustrates representative traces of optical APs and Ca_iT from the base of the left ventricle. Control recordings showed that Ca_iT followed the AP upstroke by 10 ms and recovered after the local repolarization (panel A). E4031 ($0.5\mu M$) added to the perfusate prolonged APD, induced Ca_iT oscillations and elicited EADs (panel B, $n=7/7$). The latter increased in frequency degenerating into salvos of EADs (< 10 min). However, perfusion with Ran ($10\mu M$) plus E4031 suppressed EADs within 5 min (panel C) and abolished the progressive worsening of the electrical instabilities to TdP. In panels A' to D', the order of addition of the two drugs was reversed; Ran ($10\mu M$) added alone prolonged APDs (204.6.1 to 240.7.8 ms; $p < 0.05$), Ca_iT (249.23.5 to 275.43.1 ms) and Ca_i rise-time (26.1.2 to 42.3.0 ms; $p < 0.05$) (compare A' to B'). The subsequent addition of E4031 ($0.5\mu M$) failed to prolong APDs and to elicit EADs (panel C'). Ran was then washed out while keeping E4031 resulting in a marked APD prolongation, giving rise to EADs and TdP (panel D'). The washout of Ran exposed the proarrhythmic effect of E4031 and the protective effects of Ran. Table 1 summarizes the statistically significant effects of Ran and E4031 on APDs and Ca_iT .

Simulations

Selection of the model—Figure 2A compares the APs and Ca_iT obtained with the *Shannon* (a) or *Mahajan* (b) model and the experimental data (c) at three different CLs (500, 1,000 and 2,000 ms). In Figure 2B, APD_{90} , Ca_iTD_{75} , Ca_i rise-time and Peak- Ca_iT amplitude were calculated and compared at the three CLs for the experimental data (blue bars), the *Shannon* (red bars) and the *Mahajan* (green bars) models. Based on quantitative comparisons, APD_{90} for the two models predicted closely the optical recordings at 500ms CL; but at 1,000ms CL, the *Shannon* was close but the *Mahajan* model deviated significantly predicting longer APD_{90} than optical APD_{90} and at 2,000 ms CL, the *Shannon* model underestimated and the *Mahajan* model overestimated the experimental APD_{90} (Fig. 2Ba). The duration of Ca_iT measured at 75% recovery to baseline, Ca_iTD_{75} were similar for the *Shannon* and experimental values but the *Mahajan* model deviated significantly, at all three CLs. Similarly, the *Shannon* and experimental values were considerably closer to each other than the values predicted by the *Mahajan* model for the rise time and peak of Ca_iT . For peak- Ca_iT the signals were normalized at 500 ms CL and changes in peak- Ca_iT were compared for the longer CLs. The *Mahajan* model predicted markedly slower rise-times and

smaller peak-amplitudes of Ca_iT and rather abnormal shape and time courses of Ca_iT , particularly at longer CLs.

The *Shannon* model generated EADs by inserting LQT2 simulations (see methods). However, the *Mahajan* model failed to generate EADs (Figure 2C). Based on the closer fit to experimental data and ability to generate EADs, we chose the *Shannon* model to simulate the actions of Ran.

Simulation of the Actions of Ranolazine—The effect of Ran was simulated based on its IC_{50} values on the major ionic currents.²⁸ However, the model failed to predict the suppression of EADs by Ran (Figure 3), suggesting that our understanding of the actions of Ran was incomplete.

Effects of Ranolazine on RyR2—Cardiac SR vesicles isolated from sheep ventricles were fused to planar bilayers and single channel open-probability (P_o) was recorded in the presence of $50\mu\text{M}$ Ca^{2+} on the *cis*-side at pH 7.4. Figure 4A illustrates single channel recordings as a function of [Ran] from the same bilayer. Figure 4B plots the normalized P_o (derived from 2 min of continuous recordings) as a function of [Ran]. The data were fit to a four parameter logistic curve (Sigma-Plot) which yielded an IC_{50} of RyR2 inhibition equal to $10\pm 3\mu\text{M}$ (mean $\pm$ SE, $n=7$). In figure 4C, equilibrium high-affinity ryanodine binding is plotted vs. $[\text{Ca}^{2+}]$ in the absence and presence of 30 M Ran. This data were fit to a Hill Plot. Ran shifts the EC_{50} for Ca^{2+} dependent activation of ryanodine binding from 0.42 ± 0.02 to $0.64\pm 0.02\mu\text{M}$, but has negligible effect on the degree of cooperativity (Hill number=2.5) or the maximum level of ryanodine binding. At 10 and 30 μM Ran, the Ca^{2+} dependence of $[\text{^3H}]\text{ryanodine}$ binding shifted to the right, respectively by 80 (not shown) and 220 (Figure 4C) nM Ca^{2+} .

Simulations II

To fully simulate the effects of Ran, P_o of RyR2 was modified vs. [Ran] according to single channel bilayer experiments, in addition to its other targets. The model correctly predicted experimental findings of Ran under normal and LQT2 condition.

In controls (Figure 5), Ran at $5\mu\text{M}$ prolonged APD by 8ms and decreased peak- Ca_iT by 17%. While at $10\mu\text{M}$, Ran prolonged APD by 15ms, increased Ca_iT rise-time by 1.9 fold and decreased peak- Ca_iT by 35% as previously reported.²⁸ The simulation also predicts that Ran reduces the AP ‘notch’ most likely due to its effect of the late Na^+ current but does not alter the voltage during the AP plateau phase. The effect of Ran on the notch is not detected by optical AP measurements because optical recordings smooth out the notch since they represent the sum of thousands of APs from myocytes under the field-of-view. In LQT2 (Figure 6), Ran ($5\mu\text{M}$) decreased Ca_i overload but was not effective at suppressing EADs. At $10\mu\text{M}$, Ran suppressed EADs and reduced Ca_i overload, highlighting a concentration-dependent suppression of EADs.

Figure 7 investigates the mechanisms of action of Ran in LQT2 by testing its known effects, except for the inhibition of RyR2, and then including RyR2 inhibition. The stimulation demonstrates that RyR2 inhibition by Ran is required to suppress EADs.

Discussion

The effects of Ran on the normal AP and its suppression of EADs in LQT2 hearts were readily reproduced using optical mapping. The main finding was that the *Shannon* model reproduced the experimentally measured AP and Ca_iT at various CLs and the initiation of EADs. However, the model failed to predict the suppression of EADs and TdP after

inserting the currently known effects of Ran which contradicted experimental observations. Our data further shows that Ran reduced P_o of RyR2 reconstituted in planar bilayers and desensitized RyR2 to Ca^{2+} -dependent activation. Mathematical simulations that included changes in the P_o of RyR2 caused by Ran, predicted the suppression of EADs indicating that the antiarrhythmic effects of Ran are dependent on its effect on RyR2. More precisely, the model required a reduction of P_o of 50% (as would be expected by $\sim 10\mu M$ Ran) along with the inhibition of I_{Na} to protect the heart from SR Ca^{2+} overload and to suppress EADs. At $10\mu M$, Ran acts at multiple targets;²⁸ and by inhibiting I_{Kr} , Ran prolongs APDs and QT intervals yet paradoxically does not induce but suppresses TdP in experimental LQT1-3 models⁹. Although Ran alone prolonged APDs, when added after E4031, Ran reduced APDs (Figure 1C). Moreover, when hearts were treated with Ran followed by E4031, Ran was considerably more effective at reducing E4031-induced APD prolongation (Figure 1C'). Although the final concentration of the two drugs is the same, the order of their addition produced different results. Electrophysiological studies of HERG channels expressed in HEK-293 cells indicated that Ran and E4031 shared the same binding domain to inhibit I_{Kr} but that Ran could not competitively displace E4031.²⁹ Hence, there are two reasons why the two conditions differ. a) When E4031 is added first, Ran cannot displace E4031 but E4031 can displace Ran from HERG. b) E4031 causes Ca_i overload, oscillation, high plateau Ca_i and spontaneous SR Ca^{2+} release which activates I_{NCX} that prolongs APDs, Ran can reduce spontaneous SR Ca^{2+} release by stabilizing RyR2 but does not lower plateau Ca_i (Figure 1C) but when added first Ran inhibits the subsequent E4031-induced Ca_i overload (Figure 1C') thereby reducing APD more effectively. In clinical studies, Ran lowered the incidence of arrhythmias in survivors of acute coronary syndrome³⁰ and studies in patients with atrial fibrillation are promising.³¹ Ran has been found to alter Ca^{2+} handling by increasing the latency of Ca^{2+} waves and reducing the severity of SR Ca^{2+} overload in LQT3 induced by ATX-II³²; an effect that would result in reduced likelihood of Ca^{2+} overload-induced triggered activity.

Mathematical simulation can provide a powerful tool to investigate the mechanisms of drug action but first it was necessary to select and validate the model based on experimental observation. The *Shannon* model produced APs and Ca_iT , which were close but not identical to the optical data; an important difference being the lack of APD prolongation with longer cycle lengths (Fig. 2A) which persisted with predictions of APD prolongation caused by Ran. The *Mahajan* model produced unrealistic Ca_iT at slower rates exhibiting a long rise-time to a first peak followed by a gradual rise of Ca_i before recovering to baseline (Fig. 2Ab). Also, the peak- Ca_iT collapsed compared at faster rates (Fig. 2Bd). Insertion of the effects of Ran in the *Shannon* model of LQT2 failed to predict Ran's antiarrhythmic effect (Fig. 3).

There are precedents of drug with established 'modes-of-action' being re-discovered for new properties and/or sites-of-action. For instance, flecainide, a Class Ic antiarrhythmic known for its inhibition of the late I_{Na} was found to decrease the open-time of RyR2 channels and prevent spontaneous SR Ca^{2+} -release in catecholaminergic polymorphic ventricular tachycardia.³³ Similarly, Ran reduced P_o of RyR2 with an IC_{50} of $10\mu M$ (Figure 4). The Shannon model predicts that RyR2 inhibition by Ran does not alter peak $I_{Ca,L}$ but blunted $I_{Ca,L}$ reactivation through a 27% reduction of I_{NCX} (-1.827 to -1.329 pA/pF) (See Figure 1 in Supplement). Hence, Ran inhibition of RyR2 primarily reduced I_{NCX} thereby suppressing EADs (as in Figure 7). Moreover, lower [Ran] ($5\mu M$) did not subdue EADs (Fig. 6) in agreement with previous findings.⁵ [Ran] subdues arrhythmias at $10\mu M$ but not when [Ran] falls below the concentration required for RyR2 inhibition. In support of this hypothesis, the MERLIN-TIMI 36 trial documented Ran's antiarrhythmic efficacy at $10\mu M$ but not lower concentrations.³⁰

Limitations: The *Shannon* model is based on ionic currents measured from rabbit and guinea pig (I_{Ks}) myocytes³⁴ and the modulation of ionic currents by Ran was measured using guinea pig myocytes.^{6, 28} However, species-differences appear to be negligible because Ran has been shown to have similar antiarrhythmic properties in guinea pigs²⁸, dogs⁵ and rabbits³⁵.

In summary, our study applied computational techniques to discern discrepancies in the pharmaceutical actions of Ran which encouraged us to pursue alternative explanations. It allowed us to experimentally identify a new target and to confirm its validity. The findings show that in LQT2, Ran prevents excessive Ca^{2+} load by stabilizing RyR2 and desensitizing RyR2's activation by Ca_i resulting in the suppression of EADs and TdP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Key Words & Abbreviations

Ran	Ranolazine
APDs	Action potential durations
CL	cycle length
EADs	Early afterdepolarizations
TdP	Torsade de Pointes
LQT2	Long QT type 2
Ca_i	intracellular free calcium
SR	sarcoplasmic reticulum
RyR2	Cardiac Ryanodine Receptor
P_o	open probability

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Figure 1. Ran Suppresses EADs and TdP in LQT2

Left Panel: V_m (blue) and Ca_i (red) measured from the same site at the base of the heart.

A: Control AP and Ca_iT with the heart was paced at 2 s CL

B: 10 min after E4031 ($0.5\mu\text{M}$)

C: 10 min after E4031 plus Ran ($10\mu\text{M}$)

Right Panel: The two drugs were added in the reverse order.

A': Control, 15 min after Ran B': 10 min after Ran plus E4031

C': 5 min after washout of Ran but with E4031

D': Washout of Ran prolonged APD and unmasked EADs due to the presence of E4031

Figure 3. Simulation I - Antiarrhythmic effect of Ranolazine

Steady state APs and Ca_i Ts for the last four beats in a train of 75 pulses at 2 s CL under LQT2 condition (black); under LQT2 plus 10 μ M Ran (blue) as described in 'Supplement'. Ran failed to suppress EADs.

Figure 4. Effects of Ranolazine on P_o of RyR2 and Ca^{2+} -dependent [3H]ryanodine binding

A: Characteristic single channel fluctuations following fusion of cardiac SR vesicles to a planar bilayer as a function of [Ran]. P_o was measured in the presence of 50 μ M Ca^{2+} to maintain a highly active channel (i.e. $P_o \sim 0.5$) and was averaged over 2 min of continuous recordings. c = closed, o = open state.

B: Normalized $P_o \pm SE$ vs. [Ran], $n=7$.

C: Ryanodine-binding vs. free [Ca^{2+}] (Ca^{2+} -selective electrode). Ryanodine binding was measured ± 30 M Ran with SR vesicles (0.5 mg/ml), data are average $\pm SE$ ($n=4$).

Figure 5. Simulated effect of Ranolazine on APs and Ca_iTs

Top traces: Steady state APs and Ca_iTs at 500 ms CL from the *Shannon* model at control (blue), 5 μM (black) and 10 μM (red) Ran. Bottom traces: APs and Ca_iTs shown at faster sweep speed.

Figure 6. Simulation II- Various [Ran] in LQT2 model

APs and Ca_i Ts from the *Shannon* model showing the last four beats from a train of 75 pulses at 2 s CL (black), with LQT2 (red), LQT2 plus 5 μM Ran (blue) and LQT2 with 10 μM Ran (green). EADs persisted with 5 μM but not 10 μM Ran.

Figure 7. Antiarrhythmic effect of Ranolazine when RyR2 is inhibited

APs and Ca_i Ts (Shannon model) showing the last four beats after 75 pulses at 2-s CL with LQT2 (black); with LQT2 and 10 μ M Ran but without (blue) and with RyR2 inhibition (red).

Table 1

Summary of Parameters

	APD (ms)	Ca _i T Duration (ms)	V _m -Ca _i delay (ms)	Ca _i T Rise- Time (ms)
Control (n=8)	204 6.1	249 23.5	6.3 0.7	26 1.2
E4031 only (n=7)	546 34.9 *	582 21.9 *	5.8 0.7	43 2.7 *
Ranolazine only (n=6)	240 7.8 *	275 43.1	5.8 0.8	42 3.0 *
E4031+Ranolazine (n=7)	306 27.1 *!	343 51.0 *!	6.0 1.3	43 4.6 *

* versus Control p <0.05; versus E4031 only p <0.05