Ranolazine stabilizes cardiac ryanodine receptors: A novel mechanism for the suppression of early afterdepolarization and torsades de pointes in long QT type 2

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

OBJECTIVE To elucidate the mechanism(s) whereby Ran alters cardiac action potentials (APs) and cytosolic Ca^{2+}transients and suppresses EADs and TdP in LQT2.

METHODS The known effects of Ran were included in simulations (Shannon and Mahajan models) of rabbit ventricular APs and Ca^{2+}transients in control and LQT2 models and compared with experimental optical mapping data from Langendorff rabbit hearts treated with E4031 (0.5 μM) to block the rapid delayed rectifying K^+current. Direct effects of Ran on cardiac ryanodine receptors (RyR2) were investigated in single channels and changes in Ca^{2+}-dependent high-affinity ryanodine binding.

RESULTS Ran (10 μM) alone prolonged action potential durations (206 $\pm$ 4.6 to 240 $\pm$ 7.8 ms; $P < 0.05$); E4031 prolonged action potential durations (204 $\pm$ 6 to 546 $\pm$ 35 ms; $P < 0.05$) and elicited EADs and TdP that were suppressed by Ran (10 μM; n = 7 of 7 hearts). Simulations (Shannon but not Mahajan model) closely reproduced experimental data except for EAD suppression by Ran. Ran reduced open probability (P_o) of RyR2 (half maximal inhibitory concentration = 10 $\pm$ 3 μM; n = 7) in bilayers and shifted half maximal effective concentration for Ca^{2+}-dependent ryanodine binding from 0.42 $\pm$ 0.02 to 0.64 $\pm$ 0.02 μM with 30 μM Ran.

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.

KEYWORDS Ranolazine; Action potential durations; Cycle length; Early afterdepolarizations; Torsades de pointes; Long QT type 2; Intracellular free calcium; Sarcoplasmic reticulum; Cardiac ryanodine receptor; Open probability; Shannon-Puglisi model; Mahajan UCLA model; Modeling action potential and calcium transients

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **Ca_i** = intracellular free calcium; **Ca_iT** = Ca^{2+}transients; **CL** = cycle length; **EAD** = early afterdepolarization; **IC_{50}** = half maximal inhibitory concentration; **$I_{Ca,L}$** = L-type Ca^{2+}current; **$I_{Ca,w}$** = Ca^{2+}window current; **I_{Kr}** = rapid component of the delayed rectifying K^+current; **$I_{Na,L}$** = late Na^+current; **I_{NCX}** = Na^+-Ca^{2+} exchange current; **LQT2** = long QT type 2; **P_o** = open probability; **Ran** = ranolazine; **RyR2** = cardiac ryanodine receptor; **SR** = sarcoplasmic reticulum; **TdP** = torsades de pointes

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Introduction

Ranolazine (Ran; 2–6 μM) 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 acid oxidation by Ran appeared at relatively high concentrations (12% inhibition at 100 μM), which brought into question the validity of this mode of action.[1–3] Alternatively, Ran at therapeutic doses (<10 μM) 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 torsades 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]

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The inhibition of the I_{Na} window results in a decrease of intracellular Na^+ and an improved extrusion of Ca^{2+} via the Na^+-Ca^{2+} exchange current (I_{NCX}).[7–9] Inhibition of I_{Na} could account for the therapeutic effects of Ran because a reduced intracellular free calcium (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 reactivation of the L- type Ca^{2+} current ($I_{Ca,L}$), which is triggered by the "Ca^{2+} window current" ($I_{Ca,w}$) during the action potential (AP) plateau.[10–14] At normal heart rates, action potential durations (APDs) are too short to permit $I_{Ca,w}$, but when the APD is prolonged as in LQT2, there may be sufficient time to activate $I_{Ca,w}$.[12] Agents that prolong APDs, such as BAY-K8644, which increases the amplitude and duration of $I_{Ca,L}$[13] or I_{Kr} blockers,[14] elicited a slow "conditioning phase" perhaps owing to $I_{Ca,w}$ followed by the faster EAD 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 reactivates $I_{Ca,L}$, 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^{2+} transients (Ca_iT) in ventricular myocytes,[19] 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 nonlinear dependence on the SR Ca^{2+} load. The Mahajan model includes a minimal 7-state Markovian model of $I_{Ca,L}$ that incorporates voltage-dependent inactivation and Ca-dependent inactivation.

Both models include advanced calcium cycling kinetics, critical for the development of EADs. Here, we show that modeling the actions of Ran on the basis of its half maximal inhibitory concentration (IC_{50}) values at its known targets failed to predict Ran's suppression of EADs in LQT2. We hypothesize that the 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 that 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 used to test the effects of Ran on cardiac ryanodine receptor (RyR2) reconstituted in bilayers and Ca^{2+}-dependent RyR2 activation by measuring changes in high-affinity [^{3}H]ryanodine binding.

Materials and methods

Heart preparations and optical mapping

New Zealand white rabbits (adult female rabbits >60 days old, ~2 kg) were injected with pentobarbital (35 mg/kg intravenous) and heparin (200 U/kg) through an ear vein; the heart was excised and retrogradely perfused through the aorta with Tyrode's solution: 130 mM NaCl, 24 mM $NaHCO_3$, 1.0 mM $MgCl_2$, 4.0 mM KCl, 1.2 mM NaH_2PO_4, 50 mM dextrose, 1.25 mM $CaCl_2$, at pH 7.4, gassed with 95% O_2 and 5% CO_2. Temperature was maintained at (37.0 ± 2)°C, and perfusion pressure was adjusted to ~70 mm Hg with a peristaltic pump.[22] The atrioventricular node was ablated by cauterization to control the heart rate (500–2000 ms). The heart was stained with a bolus of a voltage-sensitive dye (50 μL of RH 237 or PGH1 of 1 mg/mL in dimethyl sulfoxide) and a Ca^{2+} indicator (Rhod-2/AM, 300 μL of 1 mg/mL in dimethyl sulfoxide) delivered above the aortic cannula through the bubble trap. The hearts were oriented to view the anterior surface, record control APs and Ca_iT, and 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 using 2 (16 × 16 pixels) photodiode arrays has been previously described.[22,23] Each pixel viewed a 0.9 × 0.9 mm^2 area of the myocardium, and images were acquired at 1000 frames/s.

Single-channel recordings of RyR2

Cardiac SR vesicles (5–10 g/mL) isolated from sheep ventricles[24] were added to the cis chamber of a planar bilayer setup containing 400 mM $Cs^+CH_3O_3S^-$, 25 mM Hepes, pH 7.4, while the trans side contained 40 mM $Cs^+CH_3O_3S^-$, 25 mM Hepes, pH 7.4. Bilayers were made of 5:3:2 1,2-diphytanoyl-sn-glycero-3-phophoehtanolamine (PE)/1,2-diphytanoyl-sn-glycero-3-phosphoserine (PS)/1,2-diphytanoyl-sn-glycero-3-phosphocholine (PC) (Coagulation Reagent 1, Avanti Polar Lipids, Inc, Alabaster, AL) painted across a 150 μm hole separating 2 compartments. Following fusion of an SR vesicle to the bilayer, 4 M $Cs^+CH_3O_3S^-$, 25 mM Hepes, pH 7.4, was added to the trans side to equalize the salt concentration at 400 mM. The 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 3 minutes following an addition of Ran to the cis chamber. Single-channel analysis was carried out by using the Clamp-Fit program (pCLAMP software, Axon Instruments, Grand Terrace, CA). The open probability (P_o; mean ± SE) normalized to 1 (control without Ran) was plotted as a function of [Ran] (n = 7).[25]

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