Ranolazine for Congenital and Acquired Late I\textsubscript{Na} -Linked Arrhythmias
In Silico Pharmacological Screening

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**Rationale:** The antiarrhythmic ranolazine blocks the human ether-a-go-go–related gene–based current I\textsubscript{Kr} at therapeutic concentrations and causes QT interval prolongation. Thus, ranolazine is contraindicated for patients with preexisting long-QT and those with repolarization abnormalities. However, with its preferential targeting of late I\textsubscript{Na} (I\textsubscript{nat}), patients with disease resulting from increased I\textsubscript{nat} from inherited defects (eg, long-QT syndrome type 3 or disease-induced electric remodeling (eg, ischemic heart failure) might be exactly the ones to benefit most from the presumed antiarrhythmic properties of ranolazine.

**Objective:** We developed a computational model to predict if therapeutic effects of pharmacological targeting of I\textsubscript{nat} by ranolazine prevailed over the off-target block of I\textsubscript{Kr} in the setting of inherited long-QT syndrome type 3 and heart failure.

**Methods and Results:** We developed computational models describing the kinetics and the interaction of ranolazine with cardiac Na\textsuperscript{+} channels in the setting of normal physiology, long-QT syndrome type 3–linked ΔKPQ mutation, and heart failure. We then simulated clinically relevant concentrations of ranolazine and predicted the combined effects of Na\textsuperscript{+} channel and I\textsubscript{Kr} blockade by both the parent compound ranolazine and its active metabolites, which have shown potent blocking effects in the therapeutically relevant range. Our simulations suggest that ranolazine is effective at normalizing arrhythmia triggers in bradycardia-dependent arrhythmias in long-QT syndrome type 3 as well tachyarrhythmogenic triggers arising from heart failure–induced remodeling.

**Conclusions:** Our model predictions suggest that acute targeting of I\textsubscript{nat} with ranolazine may be an effective therapeutic strategy in diverse arrhythmia-provoking situations that arise from a common pathway of increased pathological I\textsubscript{nat}. *(Circ Res. 2013;113:e50–e61.)*

**Key Words:** computational model ■ heart failure ■ late I\textsubscript{Na} ■ long-QT syndrome type 3 ■ ranolazine

The Cardiac Arrhythmia Suppression Trial (CAST) and Survival With Oral d-sotalol (SWORD) trial showed that common antiarrhythmics increased mortality and risk of sudden cardiac death in postinfarction patients.\textsuperscript{1,2} Almost 30 years after CAST began, there is still no way to differentiate potentially useful from potentially harmful drugs.\textsuperscript{3}

Classification of antiarrhythmic drugs is based on the primary effect of the drug, known as the Singh–Vaughn Williams classification.\textsuperscript{5} Although this method is straightforward, it fails to describe the complex kinetics of the drug–channel interaction, contributions from charged and uncharged species, and the effects of nonspecific drugs on multiple ion channels.

For example, although lidocaine is specific for Na\textsuperscript{+} channels,\textsuperscript{5} flecainide blocks K\textsuperscript{+} channels in some species,\textsuperscript{6,7} the ryanodine receptor,\textsuperscript{8} and the L-type calcium current\textsuperscript{9} in others. Drug–channel interactions also are modified by cellular action potential (AP) properties, including morphology, duration, and frequency. Strong bidirectional feedback exists because drugs alter the AP waveform, which in turn affects the potency of drugs. Electrotonic coupling in tissue leads to even more complex responses to drug application that may not be apparent in cellular-level studies.

Off-target effects are of special interest for the novel antianginal agent ranolazine. Although ranolazine preferentially...
blocks the late component of the Na+ current, late \( I_{NaL} \) (a depolarizing current), ranolazine also interacts with and reduces the repolarizing human ether-a-go-go–related gene (hERG) current \( I_{Na} \) with therapeutic concentrations.\(^{10}\) The result is a mild concentration-dependent QTc prolongation.\(^{11}\) Ranolazine is thus contraindicated for patients using other QT-prolonging drugs, those with preexisting QT prolongation,\(^{12}\) or ostensibly those with any form of decreased repolarization reserve. However, patients with QT prolongation from increased \( I_{NaL} \) from either inherited defects or disease-induced electric remodeling might be exactly the ones to benefit most from selective targeting of \( I_{NaL} \).

The wealth of genetic information in recent years has led to an increased understanding of how genotype underlies clinical phenotype. For example, the long-QT syndrome type 3 (LQT3), a subset of the congenital long-QT syndrome, is a clinical phenotype. For example, the long-QT syndrome type 3 (LQT3), a subset of the congenital long-QT syndrome, is a group of inherited Na+ channel mutations that are characterized by a delay in cardiac cellular repolarization, manifesting as a prolongation of the QT interval on the ECG, resultant cardiac arrhythmias, and sudden death.\(^{13}\) LQT3 mutations manifest clinically similarly (a prolongation on the ECG) but are heterogeneous in mechanisms. Thus, it is not surprising that they also exhibit varied responses to antiarrhythmic drugs.\(^{14–17}\)

Acquired arrhythmia syndromes such as ischemic heart failure present their own specific challenge, namely the vast heterogeneity of disease phenotypes and the continuum of severity existing throughout the natural course of disease. As opposed to genetically linked ion channel mutations, which have a fairly defined mechanistic basis, the emergent effects of heart failure–associated arrhythmias are the result of multiple intersecting, deranged, and physiological compensatory processes.\(^{18}\)

Another confounding factor in accurate interpretation of antiarrhythmic drug effects results from drug metabolism. For example, ranolazine is extensively biotransformed into active metabolites that exhibit strikingly different affinities to cardiac ion channels than the parent compound.\(^{19,20}\) Thus, ex vivo channel expression and cell studies performed outside the physiological milieu where drug metabolism is absent must be interpreted with caution.

In a recent study,\(^{21}\) we developed a computational modeling approach, informed and validated by experimental data, that simulated the interaction kinetics of the antiarrhythmic drugs flecainide and lidocaine with cardiac Na+ channels. We then used the model to predict the drug effects on human ventricular cellular and tissue electric activity and, in the setting of 1 common arrhythmia trigger, spontaneous ventricular ectopy. The model predicted when clinically relevant concentrations of the antiarrhythmic drugs, flecainide and lidocaine, would exacerbate, rather than ameliorate, arrhythmia. Here, we expand this computational framework to predict the effects of promising genotype-specific therapeutic candidates for inherited LQT3–linked arrhythmias as well as acquired arrhythmia syndromes (eg, heart failure) on emergent electric activity in virtual cells and tissue. Computational analyses of disease-specific alterations and pharmacology present an opportunity to screen drugs for improved phenotype for a given disease process and when a drug should be excluded if it exacerbates arrhythmogenic potential.

## Methods

Detailed methods are available in the Online Data Supplement. Source code is available on request.

## Results

To compare the potential usefulness of ranolazine as an antiarrhythmic in the setting of LQT3, we expanded an existing theoretical model of Na+ channel gating to include drug interactions (Online Data Supplement), which takes into account channel conformation dependence of drug accessibility and binding affinity and channel kinetics after drug binding.\(^{22,23}\)

From experimentally obtained data, we first developed a model of ranolazine interaction with the wild-type cardiac Na+ channel as described in the Online Data Supplement.\(^{21}\) A depiction of the model is shown in Figure 1A. The model contains 8 discrete background states to represent the drug-free channel conformations (black) and 8 additional states (green) that represent drug-bound channel states. We also have included 4 additional states (not shown for clarity) to represent channel bursting—a small population of channels that transiently fail to inactivate, producing a persistent Na+ current that represents 0.1% of the peak Na+ current as described for wild-type Na+ channels.\(^{25}\) The drug channel representation is based on assumptions derived from the modulated receptor hypothesis, which suggests that any discrete conformational state of the channel can exist in a drug-free or drug-bound form.\(^{31,22}\)

Ranolazine binds to closed cardiac Na+ channels (inhibitory concentration \( IC_{50}=165 \) \( \mu \)mol/L), indicated by resting tonic block at hyperpolarized membrane potentials that favor the closed channel conformation as in Figure 1B—dose-dependent tonic block of peak current in solid line. Ranolazine also tonically blocks late Na+ current with higher affinity (\( IC_{50}=5 \) to 21 \( \mu \)mol/L).\(^{10,26}\) Simulated tonic block of late current is shown in Figure 1B (dashed line). The clinically relevant concentration range of drug is shown in yellow. Unlike some Na+ channel–blocking drugs,\(^{15,27}\) ranolazine does not bind to inactivated Na+ channels, because no shift in steady-state inactivation is observed on drug application (Figure 1C). In response to repetitive depolarization, ranolazine exhibits potent, open-state, use-dependent block (UDB; \( IC_{50}=100.5 \) \( \mu \)mol/L), resulting from cumulative build-up of drug-bound channels and incomplete recovery during the interstimulus interval.
(Figure 1D). UDB by ranolazine is frequency-dependent, with marked increases in UDB observed at faster pacing frequencies (Figure 1E). Ranolazine also dramatically slows Na\(^+\) channel recovery from UDB after a rapid series of depolarizing pulses (Figure 1F).

We then optimized a model of the LQT3-linked Na\(^+\) channel mutation ΔKPQ to fit experimental data obtained from drug-free ΔKPQ mutant channels (Online Figure I) using the methods as described for wild-type and as described previously. Notably, aside from a bursting-induced persistent Na\(^+\) current of ≈0.5% of the peak Na\(^+\) current, the ΔKPQ channel recovers slightly faster from inactivation but has similar mean open time, peak current density, steady-state availability, and activation, making ΔKPQ a particularly well-suited mutation to compare with wild-type for efficacy of mutation-specific persistent Na\(^+\) current blockade.

Next, we modeled ranolazine effects on ΔKPQ mutant channels. Figure 2 shows the model fits (lines) to data
Ranolazine Binds to the Promiscuous Drug Target hERG

Ranolazine is a potentially promising therapeutic for LQTS patients because of its targeting of \( I_{\text{Na}} \), but is contraindicated for LQTS patients because of off-target interactions with the promiscuous drug target hERG, which underlies the key human repolarizing current \( I_{\text{Na}} \). The rapid kinetic interaction of ranolazine with hERG yields frequency-independent block, thus allowing for a much simplified model representation compared with that required for the Na\(^+\) channel.\(^{34}\) Thus, to account for the off-target interactions of ranolazine with hERG, we incorporated a concentration-dependent block of \( I_{\text{Na}} \) peak using a concentration–response relationship with a Hill coefficient of 1 (\( n=1 \)). Multiple studies concur that ranolazine blocks hERG with an \( IC_{50} \) of \( 12 \mu M \).\(^{10,34} \) Clinical studies also suggest hERG blockade; administration of 2 to 6 \( \mu M \) ranolazine yields a proportional increase in QTc of 2 to 6 ms.\(^{26,35} \) We performed simulations in a 1-dimensional (1D) transmural tissue informed by human data (Online Data Supplement) comprising 165 cardiac cells and simulated the effect of 6 \( \mu M \) ranolazine on the computed electrogram generated by the model. In the model, we observed a marked QTc prolongation of 40 ms, a prediction that was not consistent with the clinical data.

A survey of the literature revealed a plausible and testable explanation for the discrepant model predictions and clinical findings. Pharmacokinetic studies of ranolazine suggest extensive metabolism via cytochrome P450, family 3, sub-type A–mediated pathways of biotransformation, with <5% of the parent compound unmetabolized.\(^{36} \) Four predominant metabolites were identified in healthy volunteers at plasma concentrations 30% to 40% of the parent compound, all of which produce a substantially weaker inhibition of \( I_{\text{Na}} \) (40%–50% inhibition at 50 \( \mu M /L \)). \( IC_{50} \) values for an additional

7 metabolites tested were all >50 \( \mu M /L \).\(^{26} \) Importantly, in contrast, all 11 metabolites potently inhibited \( I_{\text{Na}} \) by 12% to 57% at 10 \( \mu M /L \).\(^{26} \)

In light of this pharmacokinetic data, we used the model to make a prediction about the role of weaker ranolazine metabolite inhibition of \( I_{\text{Na}} \) to explain the clinically observed changes in QTc on ranolazine administration. Shown in Figure 3A (Online Figure II) are computed electrograms from transmural tissues spanning the range of measured affinities (50–12 \( \mu M /L \) for the parent compound ranolazine and its metabolites on \( I_{\text{Na}} \). Notably, an intermediate value that best reflects the physiological situation encompassing a weighted average of \( I_{\text{Na}} \) inhibition from high-affinity block by ranolazine and low-affinity block by metabolites produced 8 ms prolongation of computed QTc at 6 \( \mu M /L \) ranolazine, fully consistent with clinical data.\(^{26} \) In Figure 3B, the predicted concentration-dependent increase in QTc with increasing doses of ranolazine is shown. Low-dose ranolazine (2 \( \mu M /L \)) increased QTc by 2.5 ms, whereas high-dose ranolazine (10 \( \mu M /L \)) increased the QTc by 14 ms. The simulated QTc-prolonging effects are approximately linear, with a slope of \( \approx 3 \) ms per 1000 ng/mL, consistent with the clinically observed change of 2.4 ms per 1000 ng/mL.\(^{26} \)
Potential for Ranolazine to Normalize ΔKPQ Arrhythmia Triggers

To explore the potential for ranolazine to improve abnormal cellular electric phenotypes arising from the ΔKPQ mutation, we incorporated the channel model with and without drug in the O’Hara et al36 (Figure 4, left) and Grandi et al37 (Figure 4, right) human ventricular myocyte models. The ten Tusscher et al38,39 model is shown in Online Figure III. We conducted simulations in the full complement of existing human ventricular AP models to ensure model independence of our predictions. Consistent with experimental data30,32,40 and previous computationally based studies, the ΔKPQ mutation led to dramatic AP duration (APD) prolongation that worsened with slowing of pacing frequency. As shown in Figure 4 for each model, after 500 stimuli at bradycardic pacing intervals, the ΔKPQ mutation resulted in persistent late Na+ current (Figure 4B) and continued arrhythmogenic early afterdepolarizations (EADs) that arose from an extended phase 2 plateau (Figure 4A), which allowed for reactivation of the L-type Ca2+ channel (Figure 4C). For Figure 4B and 4C, peak currents of both Na+ and L-type Ca2+ currents are off-scale. Within the therapeutically relevant range, both high-dose (10 μmol/L, teal lines) and low-dose (5 μmol/L, red lines) ranolazine normalized the ΔKPQ AP morphology, an effect that was model-independent.

Figure 4D shows a summary of the effects of clinically relevant concentrations of ranolazine on ΔKPQ APD and cellular excitability (upstroke velocity of the AP) for simulated epicardial cells at nominal pacing (basic cycle length, 1000). Over the clinically relevant dosing regimen (1–10 μmol/L), ranolazine effectively normalizes APD without compromising cellular excitability, a potentially confounding occurrence and cellular-level marker that was previously shown to be strongly proarrhythmic in coupled tissue.21 Because there was minimal upstroke velocity depression, we further tested supratherapeutic ranolazine (15 and 20 μmol/L) and found similar results.

Efficacy of Ranolazine to Normalize Pause-Induced EADs

It has been widely documented that LQT3-linked arrhythmias are typically preceded by sinus pauses and short-long-short sequences.41–45 The presumed mechanisms have been shown experimentally and predicted computationally and result from the emergence of EADs on APs triggered after a pause. Thus, ideal drug therapy for LQT3 patients must normalize arrhythmia triggers occurring subsequent to long diastolic intervals.
We used computational 1D transmural tissue models to test the potential for ranolazine to normalize APs after long rest intervals in coupled tissue.

Shown in Figure 5A is a space–time–membrane voltage plot showing the last 3 S1 beats (stars) at basic cycle length 750 (after steady-state pacing, 500 beats), followed by an S2 (arrow) stimulus applied after a 1.05-second pause. Underneath each voltage in the time plot is a computed electrogram from the tissue. The electrogram in Figure 5A shows an early downward deflection attributable to EAD generation that occurs first in endocardial cells. Flattening of the electrogram then occurs and finally a positive t-wave deflection as epicardial cells repolarize before endocardial cells. Figure 5B and 5C shows the effect of pretreatment with moderate (5 μmol/L) and high (10 μmol/L) clinical doses of ranolazine. The model predicts that 5 μmol/L ranolazine improves the cellular phenotype after the pause but is unable to fully normalize the arrhythmogenic trigger after the pause (Figure 5B). High-dose ranolazine application (Figure 5C), however, completely prevents EAD formation after a long pause.

We next quantified the effect of high and low concentrations of ranolazine to prevent pause-induced arrhythmia triggers over a physiologically relevant pause interval range. Shown in Figure 5D is the increase in pause length threshold for EAD normalization after pretreatment with drug at 5 and 10 μmol/L ranolazine after pacing to steady state at basic cycle length of 750. The simulations suggest that high-dose ranolazine can normalize EADs when the incident pause is <1150 ms. Supratherapeutic levels of ranolazine, which in Figure 4 is shown to maintain upstroke velocity in single cells, substantially increases the pause duration safety window before arrhythmogenic EADs are noticed (2150 ms at 20 μmol/L).

Thus far, our model simulations have suggested that within the clinically relevant dosages, ranolazine resolves arrhythmia triggers that result from persistent LQT3-linked Na+ current. We next wanted to test whether ranolazine had the potential to normalize arrhythmia triggers stemming from acquired dysfunction such as human heart failure, which has been linked to a pathological increase in INa,L and suggested as a potential therapeutic target.46,47

**Formulation of a Human Heart Failure Model**

The range of heart failure phenotypes is complex, and there currently exists no adequate computational model that incorporates the myriad ionic and hormonal dysregulation found in end-stage ischemic heart disease. We thus turned to the literature48–50 to find the most up-to-date and reproducible human heart failure data and incorporated the deranged ionic fluxes into the Grandi et al51 human ventricular model. We chose the model of Grandi et al because it incorporates intricacies of Ca2+ handling that are known to play a key role in Ca2+-induced arrhythmia triggers. We combined the Grandi et al model of the AP with the Soltis and Saucerman51 formulation of the β-adrenergic pathway. This includes Ca2+/calmodulin-dependent protein kinase II and protein kinase A signaling, important regulatory pathways shown to be upregulated in human heart failure.52 Complete details of our human heart failure model formulation can be found in Online Table VI.

In Figure 6, we show cellular simulations generated by the human heart failure model. Figure 6A shows a prolonged APD under the influence of 1 μmol/L isoproterenol, consistent with experiments.49,53 Other important ionic fluxes include an outward shift in Na+/Ca2+ exchange (NCX) current (Figure 6C), reduced intracellular Ca2+ transient (Figure 6D), a delayed recovery of sarcoplasmic reticulum Ca2+ load (Figure 6E), an increased INa,L optimized to yield a 1% late current (Figure 6F),54 and an increased intracellular Na+ concentration (Figure 6G)55 also attributable to increased INa,L and Na+ leak current (INa,leak), and decreased Na+/K+-ATPase (NKA). Summary data comparing experiment to simulation are shown in Figure 6H.
Efficacy of Ranolazine to Ameliorate Heart Failure–Induced Arrhythmia Triggers

A hallmark arrhythmia trigger in human heart failure is the occurrence of Ca\(^{2+}\)-induced delayed afterdepolarizations (DADs). Although the complete pathway is not fully elucidated, Ca\(^{2+}\) modulation of the Na\(^{+}\) channel has been demonstrated via Ca\(^{2+}\)/calmodulin-dependent protein kinase II, and multiple upstream pathways converge on pathological INaL (e.g., increased mitochondrial oxidative phosphorylation, increased ROS, increased Na+/H+ exchange, decreased NKA). Increased I\(_{\text{NaL}}\) and increased intracellular Na\(^{+}\) then lead to increased [Ca\(^{2+}\)] via NCX and ultimately mechanical and electric instabilities (e.g., DADs, beat-to-beat variability in APD), leading to further ischemia and ventricular arrhythmias.

In Figure 7 (and expanded analysis in Online Figures V–VII), we tested the effects of ranolazine to inhibit DAD generation under conditions of heart failure in the presence of β-adrenergic stimulation. After conditions of tachycardic pacing (basic cycle length, 500), Figure 7B (left) depicts a nonstimulated DAD beat (red arrowhead) not present in control conditions (Figure 7A; Online Figure V). Moderate dose of ranolazine (5 μmol/L) is sufficient to inhibit its occurrence (Figure 7B, right; Online Figure VI). Online Figure VII depicts high-dose ranolazine (10 μmol/L). Figure 7C depicts a more severe derangement (20% decrease in NKA; 6-fold increase in I\(_{\text{Na,leak}}\), eliciting 4 spontaneous beats (red arrowheads, Figure 7C, left), which is again ameliorated by 5 μmol/L ranolazine (Figure 7C, right). An expanded analysis of ionic current changes for this severe condition (Figure 7C) is in Online Figure XI. Of note, no data exist for the affinity of ranolazine to I\(_{\text{Na,leak}}\); for these simulations, we assumed that I\(_{\text{Na,leak}}\) had similar affinity to wild-type I\(_{\text{NaL}}\) (6 μmol/L). For completeness, we also tested differing ranolazine affinities to I\(_{\text{Na,leak}}\) in Online Figures IX and X. Figure 7D summarizes the results of simulations spanning physiologically reasonable range of combinations of increased Na\(^{+}\) leak and decreased NKA. When Na\(^{+}\) leak is increased 10-fold, full repolarization failure occurs over all conditions tested (blue circles in top row of Figure 7D, left). Figure 7D, right, shows ranolazine is
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...effective at restoring repolarization and inhibiting DAD generation in the majority of physiologically plausible conditions tested (compare blue circles and red arrowheads, left, to filled circles, right).

Finally, in Figure 8 and Online Figure XIII, we probed the model components to reveal the ionic mechanism for ranolazine efficacy for one of the conditions tested in Figure 7, namely an 8-fold increase in Na⁺ leak and 10% decrease in NKA (condition in Figure 7B). As compared with control (Figure 8, column 1), the heart failure condition exhibits an ≈20% increase in [Na⁺]i (Figure 8B, column 2 vs column 1), which slows inward NCX (Ca²⁺ extrusion) and enhances outward NCX (Ca²⁺ entry, at the beginning of the AP). This coupled with AP prolongation due to extensive ionic remodeling in HF allows increased Ca²⁺ entry and maintains adequate sarcoplasmic reticulum Ca²⁺ load and Ca²⁺ transient despite reduced sarco/endoplasmic reticulum Ca²⁺-ATPase function. However, [Na⁺]-induced Ca²⁺ enhancement in combination with hypersensitive ryanodine receptors causes diastolic sarcoplasmic reticulum Ca²⁺ release and the occurrence of a spontaneous Ca²⁺ transient (Figure 8C, column 2 vs column 1, red arrowhead). This Ca²⁺ is extruded by NCX, which generates an inward current (Figure 8D, column 2, red arrowhead) that depolarizes the membrane potential leading to I_{Ca,L} activation and triggers AP (Figure 8A, column 2 vs column 1, red arrowhead). Note that the more depolarized resting membrane potential in HF (due to decreased I_{K1} and increased I_{Na,leak}) is likely to favor AP triggering. Application of 5 μmol/L ranolazine partly normalizes [Na⁺]i (Figure 8B, column 3 vs column 1) and inward NCX (Figure 8D, column 3) and abolishes the spontaneous Ca²⁺ transient (Figure 8C, column 3) and triggered AP (Figure 8A, column 3). Notably, ranolazine also hyperpolarizes the resting membrane potential, thus elevating the threshold for triggered diastolic events. These simulations are fully consistent with recent experimental data for a hypertrophic cardiomyopathy experimental model with ranolazine⁶⁴ and suggest that even moderate dose of ranolazine may be an appropriate antiarrhythmic therapeutic for the prevention of arrhythmia triggers driven by spontaneous sarcoplasmic reticulum Ca²⁺ release.

Discussion

Recently, there has been interest in the antiarrhythmic potential of the novel antianginal agent, ranolazine, the first Food and Drug Administration–approved drug that specifically blocks the late component of the Na⁺ current. Like most antiarrhythmics that target cardiac ion channels (eg, flecainide and amiodarone), ranolazine blocks multiple channels, including the repolarizing hERG current I_{K1}, with therapeutic concentrations. The result is a mild concentration-dependent QTc prolongation seen in patients with chronic stable angina.¹¹ Because of this, ranolazine is contraindicated for patients using other QT-prolonging drugs, those with preexisting QT prolongation,¹² and those with repolarization abnormalities.

In this study, we sought to use a computationally based approach to determine whether ranolazine’s unintended
pathological block of promiscuous K⁺ channels would prevail over therapeutic drug effects in 2 specific patient populations: LQT3–ΔKPQ carriers and those with acquired arrhythmias arising from heart failure.

With regard to congenital LQT3, although many in vitro studies have suggested ranolazine as an ideal therapeutic, to date, only 1 clinical study has been performed on a small number (5 carriers) of ΔKPQ mutation–afflicted patients. Moss et al showed an unequivocal decrease in QTc with ranolazine treatment in these patients (4% decrease in QTc at ≈5 μmol/L), but because of its small size and limited end points, it is unclear if ranolazine would be effective at preventing bradyarrhythmias rather than just impacting surrogate markers of arrhythmia (eg, the corrected QT interval).

When we tested ranolazine in cellular simulations, we found another potential mechanism of safety that, unlike other Na⁺ channel blockers (eg, flecainide and lidocaine), ranolazine does not cause a decrease in cellular excitability and tissue conduction velocity. Another drug that had similar promise was the lidocaine oral analog mexiletine. Although mexiletine was proven useful in a small clinical trial of LQT3 patients, it has proarrhythmic side effects like many other antiarrhythmic drugs. Sustained ventricular tachycardia has been reported, as well as exacerbation of arrhythmia in 10% to 15% of patients. More importantly for the LQT3 patient population, in which the characteristic phenotype is a bradyarrhythmia, mexiletine is associated with sinus node depression, resulting in sinus bradycardia and prolonged sinus node recovery time, potentially exacerbating the arrhythmia phenotype.

Clinical studies have shown that administration of ranolazine in the clinic (2–6 μmol/L) causes proportional increases in QTc of 2 to 6 ms, presumably arising from hERG block. Therefore, we would have expected to see ≈6 ms increase in the QTc on our computed electrograms when model tissues were pretreated with 6 μmol/L ranolazine with incorporation of the rapid blockade of hERG. Instead, we observed a dramatic QTc prolongation (40 ms), a prediction that was not consistent with the clinical data (Figure 3A).

However, when we additionally considered the effects of active metabolites of ranolazine, the simulation confirmed clinical findings. Like most drugs, ranolazine undergoes extensive metabolism, primarily via the cytochrome P450, family 3, subtype A system, with <5% of the parent compound excreted in the urine unchanged. All 11 active metabolites potently inhibit I\textsubscript{Na}, by 12% to 57% at 10 μmol/L, similar to the parent compound.

In contrast, the 4 predominant metabolites comprising 30% to 40% of the parent compound produce substantially weaker inhibition of I\textsubscript{Kr} (40%–50% inhibition at 50 μmol/L). IC\textsubscript{50} values for an additional 7 metabolites tested were all >50 μmol/L. Our model simulations suggested that a weighted average of the affinities of parent compound and active metabolites led to an apparent affinity for ranolazine...
and metabolites for $I_{\text{Na}}$ in the range of 35 μmol/L, causing the clinically observed moderate changes in QTc. Thus, there exists a large margin of safety for drug administration of ranolazine that reflects the difference in ranolazine affinity, and targeting, for $I_{\text{NaL}}$ and $I_{\text{NaK}}$.

We validated our model predictions against surrogate markers of arrhythmia risk (eg, normalization of the QT interval) and then sought to determine if ranolazine could prevent pause-induced EADs, a clinically significant precedent event to torsades de pointes. We found that a pacing interval of 750 ms, a minimal pause (≈100 ms) that extended the diastolic interval beyond normal (S2=850 ms), induced AP prolongation and EAD triggers in a transmural tissue model. Pretreatment of the cardiac fiber with high therapeutic ranolazine (10 μmol/L) delayed the onset of EAD generation by 35% (S2=1150 ms compared with 850 ms in drug-free conditions).

In a comprehensive review of the incidence of pause-dependent torsadogenic arrhythmias in congenital LQTS, Viskin et al. found an average precedent pause was ≈1000±300 ms, similar to the threshold range we predicted with ranolazine treatment. We then tested supratherapeutic ranolazine (15–20 μmol/L) because of the promising results of robust upstroke velocity in single cells, and we found a dramatic increase in the safety window of a pause necessary to elicit an EAD (2150 ms). This suggests that high-dose ranolazine may reduce the need for cardiac pacing, which itself perpetuates the short-long sequence of torsades de pointes, but clinical studies of ranolazine with Holter ECG recording will be needed to confirm this clinically relevant prediction.

By targeting pathological late Na+ current, ranolazine shows therapeutic promise for treatment of $I_{\text{Na}}$-induced arrhythmias, both congenital and acquired. Mechanistically, ranolazine does this by limiting [Na+]i, and restoring normal NCX forward mode that limits Ca2+ entry via NCX and speeds up Ca2+ extrusion, by shortening APD, thus further limiting Ca2+ entry, and by hyperpolarizing the membrane potential, which elevates the threshold of triggered activity (presumably by ranolazine’s effect on $I_{\text{NaL}}$, a hypothesis borne out by recent experiments and suggested by the model). We simulated a physiologically realistic transmural ventricular cardiac tissue based on data obtained from transmural wedge preparations from both normal and failing human myocardium. The simulations recapitulated a modest decrease in QTc with therapeutic ranolazine (5 μmol/L) and showed that higher dose of ranolazine (10 μmol/L) can decrease the QTc interval at static pacing (≈12% decrease in QTc).

For LQT–AKPQ carriers, ranolazine can also ameliorate the effects of pause-induced EADs, a hallmark clinical precedent to torsades de pointes. In the heart failure setting, we found that even moderate dose of ranolazine (5 μmol/L) was predicted to nearly normalize many of the derangements in intracellular ionic concentrations and aberrant currents, which led to an effective abolishment of arrhythmia triggers. Of note, this therapeutic effect may be specific to LQT arising from elevated $I_{\text{NaL}}$. In the absence of substantial $I_{\text{NaK}}$, the model predicts that ranolazine will prolong APD and consequently QT interval (Online Figure XII).

Because there exists heterogeneity of heart failure phenotype severity, we attempted to survey a wide parameter space, including the effects of varying $I_{\text{NaL}}$ and $I_{\text{NaK}}$. We found that phenotypes arising from a large component of $I_{\text{NaL}}$ are more susceptible to ranolazine blockade than those from decreased NKA expression. We also found that even a moderate dose of ranolazine (5 μmol/L) shows efficacy in suppressing spontaneous depolarizations (Figure 7, right), and high-dose ranolazine (10 μmol/L) suppresses all but the 2 most severe phenotypes (Online Figure VII).

In summary, we have built genotype-specific computational models of the LQT3–AKPQ mutation and a human heart failure model to specifically test a therapeutic intervention that targets the aberrant molecular mechanism (persistent late Na+ current) in 2 different pathological settings. Our multidimensional framework largely relies on experimental functional data but is refined and validated by clinical electrophysiological data from numerous clinical trials. The results of our study suggest that the therapeutic potential of ranolazine derives largely from metabolism of the parent compound into active metabolites that show significant selectivity between repolarizing current blockade ($I_{\text{Na}}$) and pathological current blockade ($I_{\text{NaL}}$). Computational modeling of the effects of metabolism is, thus, vitally important for accurate and physiologically realistic electrophysiological models of drug blockade. Our studies extend the results of the clinical literature to show that ranolazine further ameliorates the effects of specific torsadogenic activation sequences common to LQT carriers as well as selectively targeting upstream pathways, which lead to mechanical and electric instability within the ischemic heart failure setting. Both results suggest potential avenues for further clinical testing.

This study represents one step toward construction of an in silico, high-throughput drug testing system based on specific genetic defects as well as a continuum of acquired syndromes. Computational models of the kind we present in this study can be used to test vast parameter spaces that include variation in severity of disease. This may allow for rapid preclinical identification of potentially proarrhythmic or antiarrhythmic agents with high fidelity, unencumbered by problems inherent to large-scale clinical trials, which are heterogeneous in patient population and disease severity. Our approach constitutes a tractable methodology to determine which agents merit further testing with experiments and tailored clinical trials.

Disclosures

The research was supported by the American Heart Association (GIAs #10GRNT3880050, 13GRNT143700019, Western States Affiliate), the National Institutes of Health NHLBI R01-HL-085592, NHLBI R01-HL-085592-S1 (to C.E.C.), MSTP Grant: 5 T32 GM 07739 (J.D.M.) and by AHA 10PRE3650037 (J.D.B.), NHLBI R01-HL-56810 (to R.S.K.), R01-HL105242 and P01-HL80101 (D.M.B.). C.E. Clancy and D.M. Bers received research grants from Gilead Sciences (beginning May 2013). Gilead Sciences was not involved in the design, funding, execution, or interpretation of this study. The other authors report no conflicts.

References

2. Waldo AL, Cannam AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt B, Pratt CM, Schwartz PJ, Veltri EP. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote
We developed a computational framework to predict the effects of ranolazine in 2 distinct disease states marked by late Na current, inherited long-QT syndrome type 3, and heart failure.

What Is Known?

- Drug therapy for long-term management of cardiac arrhythmia has had limited success, in part, because it is difficult to predict how drug therapy will alter the emergent electric behavior of the heart.
- Ranolazine is contraindicated for patients using drugs that prolong the QT interval or those with preexisting QT prolongation because its off-target effects could worsen these conditions.
- Patients with QT prolongation attributable to inherited defects or disease might benefit from drugs like ranolazine that target the pathological late Na current.

What New Information Does This Article Contribute?

- We developed a computational framework to predict the effects of ranolazine in 2 distinct disease states marked by late Na current, inherited long-QT syndrome type 3, and heart failure.

What Is Known?

- Ranolazine shortens repolarization in patients with sustained inward sodium current due to type-3 long-QT syndrome.
- Patients with mutations of the SCN5A and HERG genes have different responses to Na+ channel blockade and to increases in heart rate. Implications for gene-specific therapy.

What Is New?

- Computationally Based Cardiac Drug Screening

Novelty and Significance

- The computational modeling framework is used to improve understanding of arrhythmogenic drug actions across multiple spatial scales of the cardiac system, from molecule to channel, to cell, and to tissue.
- Our simulated data suggest that ranolazine may be effective in preventing multiple types of arrhythmia triggers that arise from late INa.

In this study, we adopted an interdisciplinary approach combining laboratory experiments, computational biology, high-performance computing, and clinical observation. Collectively, this led to the development of a computational approach to predict the effects of a drug in specific disease states that promote cardiac arrhythmia. We used this approach to predict if ranolazine would be useful in 2 distinct clinical syndromes associated with an increase in pathological Na. This framework forms a base that can be readily expanded for virtual drug screening of other agents.
Ranolazine for Congenital and Acquired Late I_{Na}-Linked Arrhythmias: In Silico Pharmacological Screening

Jonathan D. Moreno, Pei-Chi Yang, John R. Bankston, Eleonora Grandi, Donald M. Bers, Robert S. Kass and Colleen E. Clancy

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Supplemental Material

Ranolazine for congenital and acquired late $I_{\text{Na}}$ linked arrhythmias: in silico pharmacologic screening

Jonathan D. Moreno, MD, PhD, Pei-Chi Yang, PhD, John R. Bankston, PhD, Eleonora Grandi, PhD, Donald M. Bers PhD, Robert S. Kass, PhD, and Colleen E. Clancy, PhD

Materials and Methods Summary

A computational Markov model of the WT ranolazine, ΔKPQ drug-free, and ΔKPQ ranolazine, drug channel interaction was formulated via numerical optimization from experimentally derived rate constants as previously described. Channel models recapitulated many features of $Na^+$ channel blockade including time and voltage dependent recovery, frequency and use-dependent block, as well as tonic block. The drug channel model was incorporated into a computational model of the human ventricular myocyte as described previously. All source code used for simulations in this paper is available upon request. Full methods are below.

Full Materials and Methods

Simulations were encoded in C/C++ and run on a Sun Fire X4440 x64 Server and multiple Apple Intel based Mac Pros 3.0 GHz 8-Core using OpenMP with the Intel ICC compiler version 11.1. Numerical results were visualized using MATLAB R2012a by The Math Works, Inc. All parameter optimization source code used in this paper is available and can be obtained by emailing ceclancy@ucdavis.edu.

Inclusion of Bursting States in the Wild-Type Model

The wild-type drug-free model was used as previously described, but now includes a bursting regime. To model bursting states, a “burst” mode of gating from C3, C2, C1, and O, that includes 3 closed states and an open state is added to the model, and denoted with the prefix B (BC3, BC2, BC1, BO). The rates governing the transition between background and burst modes ($\mu_1 = \text{entry into bursting mode}, \mu_2 = \text{egress from bursting mode}$) are time independent and represent the probability of transitioning between the two modes of gating. Initial estimates were taken from 2. The bursting rate constants ($\mu_1$ and $\mu_2$) were optimized (all other rate constants held constant) to yield a sustained inward current of ~0.1% of the peak $Na^+$ current at tonic pacing (BCL = 3000) for WT, and a sustained inward current of 1% for the heart failure (HF) model. Further details about modeling sustained inward Na current can be found in Clancy et al. 2.

Online Table I

Transition rates Drug free WT $Na^+$ channel (ms$^{-1}$)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC3 $\rightarrow$ IC2, C3 $\rightarrow$ C2</td>
<td>$\alpha_11 = 8.5539/(7.4392e^{-2}\exp(-V/17.0)+2.0373e^{-1}\exp(-V/150))$</td>
</tr>
<tr>
<td>IC2 $\rightarrow$ IF, C2 $\rightarrow$ C1</td>
<td>$\alpha_{12} = 8.5539/(7.4392e^{-2}\exp(-V/15.0)+2.0373e^{-1}\exp(-V/150))$</td>
</tr>
<tr>
<td>C1 $\rightarrow$ O</td>
<td>$\alpha_{13} = 8.5539/(7.4392e^{-2}\exp(-V/12.0)+2.0373e^{-1}\exp(-V/150))$</td>
</tr>
<tr>
<td>IC2 $\rightarrow$ IC3, C2 $\rightarrow$ C3</td>
<td>$\beta_{11} = 7.5215e^{-2}\exp(-V/20.3)$</td>
</tr>
<tr>
<td>IF $\rightarrow$ IC2, C1 $\rightarrow$ C2</td>
<td>$\beta_{12} = 2.7574\exp(-V/5)/20.3$</td>
</tr>
<tr>
<td>O $\rightarrow$ C1</td>
<td>$\beta_{13} = 4.7755e^{-1}\exp(-V/10)/20.3$</td>
</tr>
<tr>
<td>IC3 $\rightarrow$ IC3, C2 $\rightarrow$ C2, IF $\rightarrow$ C1</td>
<td>$\alpha_3 = 5.1458e^{-6}\exp(-V/8.2471)$</td>
</tr>
<tr>
<td>C3 $\rightarrow$ IC3, C2 $\rightarrow$ IC2, C1 $\rightarrow$ IF</td>
<td>$\beta_3 = 6.1205\exp(V/12.542)$</td>
</tr>
<tr>
<td>O $\rightarrow$ IF</td>
<td>$\beta_2 = 13.370\exp(V/43.749)$</td>
</tr>
<tr>
<td>IF $\rightarrow$ O</td>
<td>$\beta_{2x} = (\alpha_13 \cdot \alpha_2 \cdot \alpha_3) / (\beta_13 \cdot \beta_3)$</td>
</tr>
<tr>
<td>O $\rightarrow$ IS</td>
<td>$\alpha_x = 3.4229e^{-2}a_2$</td>
</tr>
<tr>
<td>IS $\rightarrow$ O</td>
<td>$\beta_x = 1.7698e^{-2}a_3$</td>
</tr>
<tr>
<td>C3, C2, C1, O $\rightarrow$ BC3, BC2, BC1, BO</td>
<td>$\mu_1 = 2.0462e^{-7}$ (WT); 2.7252e^{-7} (HF)</td>
</tr>
<tr>
<td>BC3, BC2, BC1, BO $\rightarrow$ C3, C2, C1, O</td>
<td>$\mu_2 = 8.9731e^{-4}$ (WT); 1.9701e^{-4} (HF)</td>
</tr>
</tbody>
</table>
Optimization procedure for ΔKPQ mutant sodium channel

Five pacing protocols were optimized: steady state availability \(^3\) (shown to be similar to WT \(^4\)), steady state activation \(^5\) (shown to be similar to WT \(^4\)), recovery from inactivation at -90mV \(^6\), recovery from use-dependent block at -100mV, and time constant of inactivation from the open state \(^6\). The model was further constrained by channel mean open time \(^4\).

A cost function for each protocol was defined as the sum of squared differences between experiment and simulation. The total cost function (sum of the individual protocol errors) was then minimized and converged when a tolerance of 0.01 for the change in parameters was achieved. The initial conditions were set as the optimized WT Na\(^+\) channel recently published \(^1\). For the aforementioned protocols, entry and egress from the bursting state (\(\mu_1 \) and \(\mu_2\), respectively) were set at 0 (no bursting during optimization).

After initial optimization, bursting states were added to the model \(^7\), and denoted with the prefix \(B\) (\(BC3, BC2, BC1, BO\)), as described for wild-type. The rate constants of the bursting regime (\(\mu_1, \mu_2\)) were then optimized (all other rate constants held constant) to yield a sustained inward current of either 0.5\% (O’Hara-Rudy model \(^8\)) or 1\% (ten Tusscher \(^9\) or Grandi-Bers model \(^10\)) of the peak Na\(^+\) current \(^4,11\) at tonic pacing (BCL = 3000).

**Online Table II**

<table>
<thead>
<tr>
<th>Transition rates</th>
<th>ΔKPQ Mutant Na(^+) channel (ms(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC3 (\rightarrow) IC2, C3 (\rightarrow) C2, BC3 (\rightarrow) BC2</td>
<td>(a_{11} = 1.6662 \times 10^4 / (6.7574 \times 10^{-2} \exp(-V/17.0) + 8.0935 \times 10^{-2} \exp(-V/150)))</td>
</tr>
<tr>
<td>IC2 (\rightarrow) IF, C2 (\rightarrow) C1, BC2 (\rightarrow) BC1</td>
<td>(a_{12} = 1.6662 \times 10^4 / (6.7574 \times 10^{-2} \exp(-V/17.0) + 8.0935 \times 10^{-2} \exp(-V/150)))</td>
</tr>
<tr>
<td>C1 (\rightarrow) O, BC1 (\rightarrow) BO</td>
<td>(a_{13} = 1.6662 \times 10^4 / (6.7574 \times 10^{-2} \exp(-V/17.0) + 8.0935 \times 10^{-2} \exp(-V/150)))</td>
</tr>
<tr>
<td>IC2 (\rightarrow) IC3, C2 (\rightarrow) C3, BC2 (\rightarrow) BC3</td>
<td>(b_{11} = 1.4984 \times 10^4 \exp(-V/20.3))</td>
</tr>
<tr>
<td>IF (\rightarrow) IC2, C1 (\rightarrow) C2, BC1 (\rightarrow) BC2</td>
<td>(b_{12} = 1.0868 \times 10^4 \exp(-V/20.3))</td>
</tr>
<tr>
<td>O (\rightarrow) C1, BO (\rightarrow) BC1</td>
<td>(b_{13} = 1.9349 \times 10^4 \exp(-V/20.3))</td>
</tr>
<tr>
<td>IC3 (\rightarrow) C3, IC2 (\rightarrow) C2, IF (\rightarrow) C1</td>
<td>(a_{3} = 2.6699 \times 10^{-6} \exp(-V/7.5168))</td>
</tr>
<tr>
<td>C3 (\rightarrow) IC3, C2 (\rightarrow) IC2, C1 (\rightarrow) IF</td>
<td>(b_{3} = 1.7538 \times 10^4 \exp(V/11.010))</td>
</tr>
<tr>
<td>O (\rightarrow) IF</td>
<td>(a_{2} = 7.6104 \exp(V/214.37))</td>
</tr>
<tr>
<td>IF (\rightarrow) O</td>
<td>(b_{2} = (\alpha_{13} \alpha_{2} \alpha_{3}) / (\beta_{13} \beta_{3}))</td>
</tr>
<tr>
<td>O (\rightarrow) IS</td>
<td>(a_{x} = 8.6589 \times 10^{-2} \alpha_{2})</td>
</tr>
<tr>
<td>IS (\rightarrow) O</td>
<td>(b_{x} = 1.4265 \times 10^{-2} \alpha_{3})</td>
</tr>
<tr>
<td>C3 (\rightarrow) BC3, C2 (\rightarrow) BC2, C1 (\rightarrow) BC1</td>
<td>(\mu_1 = 1.4397 \times 10^{-2} (0.5% \text{ late}); 2.6589 \times 10^{-2} (1% \text{ late}))</td>
</tr>
<tr>
<td>C1 (\rightarrow) BC1, O (\rightarrow) BO</td>
<td>(\mu_2 = 5.6593 \times 10^{-2} (0.5% \text{ late}); 4.6274 \times 10^{-2} (1% \text{ late}))</td>
</tr>
</tbody>
</table>

Optimization procedure for the drug channel interaction

**Online Table III: Situation-Dependent Affinities of Ranolazine to the WT and ΔKPQ Na\(^+\) Channel (Experimental Data)**

<table>
<thead>
<tr>
<th>Situation-Dependent Affinities</th>
<th>WT</th>
<th>ΔKPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tonic Block of Late I(_{Na})</strong></td>
<td>6 µM (^{12})</td>
<td>12.66 µM, (n = 0.7301)*</td>
</tr>
<tr>
<td>(I_{Kr})</td>
<td>12 µM (^{13})</td>
<td>12 µM (^{13})</td>
</tr>
<tr>
<td><strong>Tonic Block of Peak I(_{Na})</strong></td>
<td>165.2 µM, (n = 1.623)*</td>
<td>120.8 µM, (n = 1.115)*</td>
</tr>
<tr>
<td><strong>Use-Dependent Block of I(_{Na})</strong></td>
<td>100.5 µM, (n = 1.015)*</td>
<td>83.11 µM, (n = 0.9082)*</td>
</tr>
</tbody>
</table>

\(n = \text{Hill coefficient}\)

* Kass laboratory
The Na⁺ drug-channel model parameters for the on and off rates of ranolazine are taken from experiments where available. These include diffusion rates that indicate drug on rates \( k_{on} = \text{[drug]}^* D \) (diffusion rate) and affinities (Kd) to discrete conformations that determine drug off rates \( k_{off} = \text{Kd}^*D \) (diffusion rate). The diffusion rate for ranolazine was assumed similar to other local anesthetics \(^{14,15}\), and set at 5500 M⁻¹ms⁻¹ in the computational model. Rates were also constrained by experimental data (described in detail below) and microscopic reversibility as in Colquhoun \(^{16}\).

**Optimization of Wild-Type and Ranolazine Model**

Five experimental protocols were used to constrain the model: steady state availability (SSA), tonic block (TB) of peak and late current, use-dependent block (UDB), recovery from UDB (RUDB), and frequency-dependent UDB (FDUDB). Charged drug rate constants \((ax1, bx1, a_{13c}, a_{22}, b_{33}, a_{33}, \text{Kd}_{0,Bursting})\), and neutral drug rate constants \((ax2, a_{13n}, a_{-22}, b_{-33})\) were optimized.

Open state affinity for the charged form of ranolazine was derived from the Kd value from use-dependent block (UDB), and assumed to measure affinity to the open state. 100.5 µM was set as \( \text{Kd}_0 \) – the Kd at 0 mV. Closed state affinity of charged drug was then calculated using Eyring rate theory for the voltage dependence of rate constants (\( \text{Kd} = \text{Kd}_0^*e^{-(d*V*F/(R*T))} \)) \(^{17}\). For example, the computed Kd value at -100 mV for charged ranolazine is computed to be 1578 µM.

Bursting state affinity for charged ranolazine was initially set at the value found by assuming the affinity of tonic block of late \( I_{\text{Na,L}} \) was equal to Kd at -100 mV. Using \( \text{Kd} = \text{Kd}_0^*e^{-(d*V*F/(R*T))} \), \( \text{Kd}_0 \) was then calculated and used as an initial value in the optimization. For example, the affinity of TB \( I_{\text{Na,L}} \) for WT is 6 µM \(^{12}\), if that value is assumed to equal \( \text{Kd}_{-100\text{mV}}, \text{Kd}_0^* \) was initially set at 0.3822 µM.

Affinities of the neutral fraction of ranolazine to drug-bound states \((\text{Kd}_{\text{neutral}}, \text{Kd}_{\text{inactive\_neutral}}, \text{Kd}_{\text{closed\_neutral}})\) were initially held constant and assumed similar to flecainide \(^1,18\); because the model gave acceptable fits to the data, those rates were kept. The optimized rate constants are shown in the table below.

**Optimization of ΔKPQ and Ranolazine Model**

Four experimental protocols were used to constrain the model: tonic block (TB) of peak and late current, use-dependent block (UDB), recovery from UDB (RUDB), and frequency-dependent UDB (FDUDB). Charged drug rate constants \((ax1, bx1, a_{13c}, a_{22}, b_{33}, a_{33}, \text{Kd}_{0,Bursting})\), and neutral drug rate constants \((ax2, a_{13n}, a_{-22}, b_{-33}, \text{Kd}_{\text{neutral}}, \text{Kd}_{\text{inactive\_neutral}}, \text{Kd}_{\text{closed\_neutral}})\) were optimized.

Open state affinity of the charged form of ranolazine was derived from the Kd value from use-dependent block (UDB), as described above for WT, and set at 83.11 µM. Closed state affinity of charged drug was then calculated using Eyring rate theory for the voltage dependence of rate constants (\( \text{Kd} = \text{Kd}_0^*e^{-(d*V*F/(R*T))} \)) \(^{17}\). Bursting state affinity for charged ranolazine was initially set at the value found by assuming the affinity of tonic block of late \( I_{\text{Na,L}} \) was equal to Kd at -100 mV, as described above for WT, and \( \text{Kd}_{0,Burst} \) was initially set at 0.8064 µM.

Affinities of the neutral fraction of ranolazine to drug-bound states \((\text{Kd}_{\text{neutral}}, \text{Kd}_{\text{inactive\_neutral}}, \text{Kd}_{\text{closed\_neutral}})\) were initially set to flecainide \(^1,18\), but were allowed to change throughout the optimization. The optimized rate constants are shown in the table below.
Online Table IV

WT Ranolazine

Transition rates (ms⁻¹)

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{on}$ = $k_{closed, on}$</td>
<td></td>
</tr>
<tr>
<td>$k_{off}$ = $k_{closed, off}$</td>
<td></td>
</tr>
<tr>
<td>$k_{bursting, on}$ = $k_{closed, bursting, on}$</td>
<td></td>
</tr>
<tr>
<td>$k_{bursting, off}$ = $k_{closed, bursting, off}$</td>
<td></td>
</tr>
<tr>
<td>$k_{neutral, on}$</td>
<td></td>
</tr>
<tr>
<td>$k_{neutral, off}$</td>
<td></td>
</tr>
<tr>
<td>$k_{neutral, inactivated, on}$</td>
<td></td>
</tr>
<tr>
<td>$k_{neutral, closed, on}$</td>
<td></td>
</tr>
<tr>
<td>$k_{neutral, closed, off}$</td>
<td></td>
</tr>
</tbody>
</table>

$D^1C3 \rightarrow D^1C2, D^1C3 \rightarrow D^1C2,$
$D^1C2 \rightarrow D^1C1, D^1C2 \rightarrow D^2C1,$
$D^2C2 \rightarrow D^2C1, D^2C2 \rightarrow D^2C3,$
$D^2C3 \rightarrow D^2C2, D^2C3 \rightarrow D^2C2,$
$D^2IF \rightarrow D^1C2, D^2C1 \rightarrow D^1C2,$
$D^2IF \rightarrow D^2C1, D^2C1 \rightarrow D^2C2,$
$D^2DIF \rightarrow D^2C2, D^2C1 \rightarrow D^2C2,$
$D^2DIF \rightarrow D^2C2, D^2C1 \rightarrow D^2C2,$

$D^1O \rightarrow D^1IS$  $a_{11}$
$D^1IS \rightarrow D^1O$  $a_{12}$
$DO \rightarrow DI^S$  $a_{13} = 3.6811 \times a_{13}$
$D^1C1 \rightarrow D^1O$  $a_{13} = 2.3570 \times 10^{-2} a_{13}$
$D^1O \rightarrow D^1C1$  $b_{13} = (b_{13} k_{con} k_{off} a_{13})/(k_{on} k_{off} a_{13})$
$DO \rightarrow DC^1$  $b_{13} = (b_{13} k_{on} a_{13} n_{k_{off}})/(k_{off} a_{13} k_{on})$
$DI^S \rightarrow DO$  $\beta_{x2} = (\beta_{x} k_{on} a_{x}^2 k_{off})/(a_{x} k_{on}^2 k_{off})$
$D^1O \rightarrow D^1IF$  $\beta_{x2} = 6.8705 \times 10^4 a_{2}$
$DO \rightarrow D^1IF$  $\beta_{x2} = 2.1182 \times 10^2 a_{2}$
$D^1IF \rightarrow D^1O$  $\beta_{x2} = (a_{13} c_{a_{22}} a_{33})/(a_{13} c_{a_{22}} a_{33})$
$DI^F \rightarrow DO$  $\beta_{x2} = (\beta_{33} a_{33} a_{22} a_{33})/(\beta_{33} a_{33} a_{22})$
$D^2C3 \rightarrow D^2C1, D^2C2 \rightarrow D^2C1, D^2IF \rightarrow D^2C1$  $\beta_{33} = 1.7561 \times 10^5 a_{3}$
$DC^3 \rightarrow D^2C3, DC^2 \rightarrow D^2C2, DC^1 \rightarrow D^2C1$  $\beta_{33} = 1.2197 \times 10^3 a_{3}$
$D^1C3 \rightarrow D^1C2, D^1C3 \rightarrow D^1C2, D^1C1 \rightarrow D^1C1$  $\beta_{33} = 4.0832 \times 10^2 a_{3}$
$D^2C3 \rightarrow D^2C2, D^2C2 \rightarrow D^2C1$  $\beta_{33} = 4.0832 \times 10^2 a_{3}$

Diffusion 5500 M⁻¹ms⁻¹
### Online Table V

**ΔKPQ Ranolazine**

<table>
<thead>
<tr>
<th>Transition rates (ms⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{on} = k_{closed, on}$</td>
</tr>
<tr>
<td>$k_{off} = k_{closed, off}$</td>
</tr>
</tbody>
</table>

| $k_{bursting, on} = k_{closed, bursting, on}$ | $[\text{D+}]^*\text{Diffusion}$ |
| $k_{bursting, off} = k_{closed, bursting, off}$ | $k_{d, Bursting,Open}^*\text{Diffusion}$ ; $(k_{d, Bursting,Open} = 3.8367\times10^{-6}\exp(-0.7\times V/F/R\times T))$ |

| $k_{neutral, on}$ | $\alpha_{11}$ |
| $k_{neutral, off}$ | $2.6479\times10^{-6}\text{Diffusion}$ |
| $k_{neutral, inactivated, on}$ | $19.034\times10^{-6}\text{Diffusion}$ |
| $k_{neutral, inactivated, off}$ | $61.842\times10^{-6}\text{Diffusion}$ |

| $D^{'IC3} \rightarrow D^{'IC2}, D^{'C3} \rightarrow D^{'C2}$, | $\alpha_{12}$ |
| $D^{'IC2} \rightarrow D^{'IF}, D^{'C2} \rightarrow D^{'C1}$, | $\beta_{11}$ |
| $D^{'IC2} \rightarrow D^{'IC3}, D^{'C2} \rightarrow D^{'C3}$, | $\beta_{12}$ |
| $D^{'IF} \rightarrow D^{'IC2}, D^{'C1} \rightarrow D^{'C2}$, | $\alpha_{13c} = 4.7577\times10^{-1}\alpha_{13a}$ |
| $D^{'IF} \rightarrow D^{'C1}$ | $\beta_{13c} = 3.4549\times10^{-4}\beta_{13}$ |

| $D^{'O} \rightarrow D^{'IS}$ | $\beta_{x1} = 2.1775\times10^{-2}\beta_{x}$ |
| $D^{'IS} \rightarrow D^{'O}$ | $\alpha_{x2} = 3.8814\times10^{-2}\alpha_{x}$ |
| $D^{'C1} \rightarrow D^{'O}$ | $\alpha_{13c} = 4.7577\times10^{-1}\alpha_{13a}$ |
| $D^{'C1} \rightarrow D^{'C2}$, | $\alpha_{13n} = 3.3694\times10^{1}\alpha_{13n}$ |
| $D^{'O} \rightarrow D^{'C1}$ | $b_{13c} = (\beta_{13c} \times k_{on} \times k_{off} \times a_{13c}) / (k_{on} \times k_{off} \times a_{13c})$ |
| $D^{'C1} \rightarrow D^{'O}$ | $b_{13n} = (\beta_{13n} \times k_{on} \times k_{off} \times a_{13n}) / (k_{on} \times k_{off} \times a_{13n})$ |
| $D^{'IS} \rightarrow D^{'O}$ | $\beta_{x2} = (\beta_{x} \times k_{on}^* \times a_{x2} \times k_{off}^*) / (\beta_{x} \times k_{on}^* \times k_{off})$ |
| $D^{'O} \rightarrow D^{'IF}$ | $\alpha_{22} = 8.8205\times10^{1}\alpha_{22}$ |
| $D^{'O} \rightarrow D^{'IF}$ | $\beta_{22} = (\alpha_{13c} \times a_{22}^* \times a_{33}) / (\beta_{13c} \times \beta_{33})$ |
| $D^{'IF} \rightarrow D^{'O}$ | $\beta_{22} = (\alpha_{33} \times a_{13n} \times a_{22}) / (\beta_{33} \times \beta_{13n})$ |
| $D^{'IF} \rightarrow D^{'IF}$ | $\beta_{33} = 3.4549\times10^{4}\beta_{33}$ |
| $D^{'C3} \rightarrow D^{'IC3}, D^{'C2} \rightarrow D^{'IC2}, D^{'C1} \rightarrow D^{'IF}$ | $\beta_{33} = 4.2894\times10^{3}$ |

| Diffusion | $5500 \text{ M}^{-1}\text{ms}^{-1}$ |
Simulation of $I_{K_r}$ Blockade

To simulate the effects of ranolazine on $I_{K_r}$ current, we decreased the peak conductance, $G_{IK_r}$ in a concentration dependent fashion using a concentration response relationship with a Hill coefficient of $1 \ (n = 1)$ as follows:

$$G_{IK_r} = G_{IK_r,\text{max}} \left( \frac{1}{1 + (\text{Drug} / IC_{50})^n} \right)$$

where $G_{IK_r,\text{max}}$ is the nominal conductance value from the given human ventricular myocyte model used (O’Hara-Rudy $^8$, ten Tusscher $^9$, or Grandi-Bers $^{10}$) and $IC_{50}$ is the concentration of drug that produces a 50% inhibition of $I_{K_r}$ current.

Cellular simulations

The model formulation for virtual (O’Hara-Rudy $^8$, ten Tusscher $^9$, or Grandi-Bers $^{10}$ with Soltis-Saucerman model $^{19}$) epicardial cells was used with the published Na$^+$ channel replaced with the model described here. State probabilities in the Markov model of the Na$^+$ channel were computed by an implicit Trapezoidal numerical method. The numerical method for updating the voltage was forward Euler.

The Soltis-Saucerman model of CaMKII and PKA signaling pathways $^{19}$ was merged with the Grandi-Bers human model $^{10}$. We used the Soltis-Saucerman model as a template to replace each ionic current with the Grandi-Bers model except for the L-type calcium channels (LTCC, the seven-state Markov model). We then adjusted the Ca$^{2+}$ current amplitude in the Grandi-Bers model to match current experimental human data $^{20}$ and $G_{Kr}$ was increased by 3 fold. We also replaced the nominal Na$^+$ channel model with our Na$^+$ drug-channel models as described above. Isoproterenol was set to 0 in mutant simulations (Figure 4).

For the O’Hara-Rudy ΔKPQ model, we made a heterozygote mutant (50% mutant Na$^+$ channels, 50% WT Na$^+$ channels), and included 0.5% late Na$^+$ current, as this model easily produced EADs and exhibited repolarization failure with 1% late Na$^+$ current. For the ten Tusscher ΔKPQ model, we made a heterozygote with 1% late Na$^+$ current. The Grand-Bers ΔKPQ model was a heterozygote with 1% late Na$^+$ current.

One-dimensional simulations

One-dimensional simulations were modified with experimental transmural data from $^{21}$ which show a linear decrease in APD from endocardium to epicardium. The maximal conductance $G_{Ks}$ was monotonically increased from 0.15 at the endocardium (cell 1) to 0.30 at the epicardium (cell 165). Cells $1 – 40$ were endocardial, and cells $41 – 165$ were epicardial, and utilized the nominal values of $G_{Ks}$ of ten Tusscher $^9$. These ionic conductances gave an endocardial APD of 379 ms, an epicardial APD of 335 ms, and a QTc of 391.75 ms (at 1Hz pacing), consistent with experimental optical imaging data $^{21,22}$. For O’Hara-Rudy $^8$ and Grandi-Bers $^{10}$ model without β-adrenergic stimulation, the maximal conductance $G_{Kr}$ was monotonically increased from 0.046 and 0.03 at the endocardium (cell 1) to 0.0598 and 0.035 at the epicardium (cell 165), respectively. Cells $1 – 40$ were endocardial, and cells $41 – 165$ were epicardial. These ionic conductances gave an endocardial APD of 268 ms in O’Hara and 355 ms in Grandi model, an epicardial APD of 212 ms in O’Hara and 330 ms in Grandi model, and a QTc of 300 ms in O’Hara and 360 ms in Grandi model (at 1Hz pacing).
A 165-cell cable was unstimulated for 10 minutes without drug. Drug was then “applied” and cells paced (-250 pA/pF for 1 ms) for 500 beats at a given pacing cycle length and drug concentration. The numerical method was forward Euler.

**Pseudo ECG computation**

Extracellular unipolar potentials \( \Phi_E \) generated by the fiber in an extensive medium of conductivity \( \sigma_e \), were computed from the transmembrane potential \( V_m \) using the integral expression as in Plonsey and Barr \(^{23}\) and Gima and Rudy \(^{24}\):

\[
\Phi_E(x',y',z') = \frac{a^2 \sigma_i}{4 \sigma_e} \int \left( -\nabla V_m \right) \cdot \frac{1}{r} \, dx
\]

where \( \nabla V_m \) is the spatial gradient of \( V_m \), \( a \) is the radius of the fiber, \( \sigma_i \) is the intracellular conductivity, and \( r \) is the distance from a source point \((x, y, z)\) to a field point \((x', y', z')\). \( \Phi_E \) was computed at an electrode site 2.0 cm away from the distal end along the fiber axis \(^{25}\).

**Cell Expression and Electrophysiology of the ΔKPQ mutation**

Site-directed mutagenesis was done on \( \text{Na}_V \)1.5 in pcDNA3.1 using the Quik Change site-directed mutagenesis kit (Stratagene). Whole cell recordings were made on Human Embryonic Kidney (HEK) 293 cells expressing WT and mutant \( \text{Na}_V \)1.5 channels along with h\( \beta \)1 subunits (Lipofectamine, Invitrogen).

Patch clamp procedures were used with the following internal solution (in mM): 50 aspartic acid, 60 CsCl, 5 Na2ATP, 11 EGTA, 10 HEPES, 4.27 CaCl\(_2\) (resulting in a final [Ca\(^{2+}\)] of 100 nM), and 1 MgCl\(_2\), pH 7.4 adjusted with CsOH. The external solutions for measurement of all Na\(^{+}\) channel activity contained (in mM): 130 NaCl, 2 CaCl\(_2\), 5 CsCl, 1.2 MgCl\(_2\), 10 HEPES, and 5 glucose, pH 7.4 adjusted with NaOH.

TTX was purchased from Ascent Scientific (UK). Ranolazine was purchased from Sigma Aldrich (St. Louis, MO). Drugs were applied locally to the outside of the cell being patched via homemade perfusion system using microfluidic valves (Lee Co, Essex, CT). Currents were measured at room temperature (~23 °C). Pipettes were borosilicate from VWR (West Chester, PA). Typical pipette resistance was between 1.5 and 3 M\( \Omega \). After whole cell configuration is achieved only cells with access resistance less than 7 M\( \Omega \) are recorded. Membrane currents were measured with Axopatch 200B amplifiers (Axon Instruments, Foster City, CA). Capacitance and series resistance compensation were carried out using analog techniques according to the amplifier manufacturer (Axon Instruments, Foster City, CA). Only cells with access resistance and peak current that, after compensation, have voltage errors less than 5 mV are used for analysis. PClamp8 (Axon Instruments) was used for data acquisition and initial analysis. Analysis was carried out in Excel (Microsoft), Origin 7.0 (Microcal Software, Northampton, MA), and programs written in Matlab (The Mathworks, Natick, MA). Analyzed data are shown as mean +/- S.E.M. Statistical significance was tested using Student's t test; \( p < 0.05 \) was considered statistically significant.

**Simulation of human heart failure**

**Cell model**

The Grandi-Bers model with Soltis-Saucerman \( \beta \)-adrenergic signaling pathway (see above) was used for heart failure simulations. The epicardial cell was paced to 1060 seconds with 2 Hz pacing (BCL 500), and spontaneous activity was observed after stimulus removal.
CaMKII and PKA regulation
For the CaMKII and PKA phosphorylation, we added the regulation on $I_{Ks}$, LTCC and RyR as in Soltis-Saucerman 19, highlighting the following regulatory pathways: (1) $I_{Ks}$ is regulated by PKA phosphorylation; (2) $I_{CaL}$ and RyR opening are modulated by CaMKII and PKA; (3) RyR leak is CaMKII dependent; (4) CaMKII and PKA phosphorylate PLB that alternate SERCA fluxes; (5) the cystic fibrosis transmembrane conductance regulator Cl$^-$ current regulated by PKA, and (6) Troponin I (TnI) is regulated by PKA. Here we also included (7) the effect of PKA to phosphorylate PLM and increase NKA activity in the model 26.

Current density changes in heart failure
To simulate human heart failure (HF), we modified the current density changes in HF shown in Online Table V, below. In addition, CaMKII expression is increased in failing human myocardium 27. We simulated CaMKII overexpression (CaMKII-OE) as in Soltis-Saucerman 19: (1) Increased $I_{to,slow}$ amplitude and $I_{to}$ recovery from inactivation by CaMKII-OE (See 19 for detailed equations); and (2) CAMKII-OE effects has been shown to shift $I_{Na}$ to the hyperpolarizing direction, delay recovery from inactivation. To simulated CaMKII-OE effects alterations to $I_{Na}$: rate constant $\beta_3$ was increased by 2.4-fold.

Online Table VI: Current density changes induced in the failing heart

<table>
<thead>
<tr>
<th>Ionic current</th>
<th>Percentage Change</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{NaL}$</td>
<td>10x increase (0.1% → 1%)</td>
<td>Human</td>
<td>28</td>
</tr>
<tr>
<td>$I_{to,fast}$</td>
<td>36% decrease</td>
<td>Human</td>
<td>29</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>25% decrease</td>
<td>Human</td>
<td>30, 31</td>
</tr>
<tr>
<td>SERCA</td>
<td>36% decrease</td>
<td>Human</td>
<td>32</td>
</tr>
<tr>
<td>$k_{leak}$ (SR leak)</td>
<td>3.5-fold increase</td>
<td>Rabbit</td>
<td>33</td>
</tr>
<tr>
<td>$I_{Na, leak}$ *</td>
<td>16-fold increase</td>
<td>Rabbit</td>
<td>34</td>
</tr>
<tr>
<td>$I_{NaK}$ (Na$^+$/K$^+$-ATPase)*</td>
<td>10 - 42% decrease</td>
<td>Human</td>
<td>35-37</td>
</tr>
</tbody>
</table>

*Note: Models of modified $I_{NaK}$ and $I_{Na, background}$ currents are set within the range of experimental data and parameter space is analyzed in Figures 7 and 8.

SR – sacroplasmic reticulum

Simulation of the drug interaction with Na$^+$ leak current ($I_{Na,Leak}$)
To simulate the effects of drugs on Na$^+$ leak current during heart failure, we decreased the peak conductance of $I_{Na,Leak}$ in a concentration-dependent manner using a concentration response relationship with a Hill coefficient (n) as follows:

$$G_{Na,Leak} = G_{Na,Leak,\text{max}} \left( \frac{1}{1 + (\text{Drug}/IC_{50})^n} \right)$$

where $G_{Na,Leak,\text{max}}$ is the nominal conductance from the Grandi model, and $IC_{50}$ corresponds to the sensitivity of the channels to ranolazine assuming peak current affinity (165.2 µM, $n = 1.6$), or late current affinity (6 µM, $n = 1$) 12. In Online Figure VII and VIII, we assume peak current affinity, and in Figure 7, 8, Online Figure V and VI, we assume late current affinity.
Online Figure I: ΔKPQ mutant Na⁺ channel kinetics.
(A) Schematic of the bursting states. In B – D, the points are experiment, lines are simulation.
(B) Recovery from UDB induced by trains of 100 pulses (-10 mV for 25 ms at 25 Hz) from -100 mV in drug free conditions. Test pulses (-10 mV) were after variable recovery intervals at -100 mV. Currents were normalized to tonic block. (C) Recovery from inactivation induced by a depolarizing pulse (-10 mV for 100 ms) from -90 mV. Test pulses (-10 mV) were after variable recovery intervals at -90 mV. (D) Time constant of inactivation, induced from a holding potential of -100 mV to indicated voltages. (E) Mean open time at -30 mV. (F) Optimization of entry and egress from the bursting mode (µ₁ and µ₂ respectively) to induce a 0.5% (black) or 1% (red) persistent inward Na⁺ current. (G) Current – voltage relationship indicating ΔKPQ has nearly double current density as compared to WT.
Online Figure II: Consideration of ranolazine metabolites for $I_{kr}$ inhibition predicts clinical QTc prolongation.

Shown in (A) are computed ECGs of a 165-cell cardiac fiber with 6 µM ranolazine (therapeutic concentration), and varying values for IC$_{50}$ of $I_{kr}$ inhibition (see Supplementary Information). $I_{kr}$ inhibition at IC$_{50} =$ 50 µM (green) produces a 2.5 ms QTc prolongation; $I_{kr}$ inhibition at IC$_{50} =$ 12 µM (blue) prolongs QTc by 20.3 ms; $I_{kr}$ inhibition at IC$_{50} =$ 35 µM (red) prolongs QTc by 5.45 ms. (B) Concentration-dependent ΔQTc is approximately linear over the therapeutic range of ranolazine. See text for details. (C) and (D) are for the Grandi-Bers human model: QT prolongation with IC$_{50} =$ 12 µM, 35 µM, and 50 µM are 59 ms, 8 ms, and 1 ms, respectively.
Online Figure III: Effects of a pause on incident EADs generated in the subsequent beat for the ten Tusscher human ventricular myocyte model.

The effects of the ΔKPQ mutation are shown for the ten Tusscher human ventricular myocyte model, and yields qualitatively similar results to the O’Hara Rudy and the Grandi-Bers models shown in Figure 4. Panel (A) depicts cellular APs, (B) depicts Na⁺ currents (peak off scale), and (C) depicts the L-type Ca²⁺ currents (peak off scale). In all three models, low (5 µM), and high (10 µM) ranolazine progressively shortens the APD but fails to fully normalize to WT (blue line). Panel (D) depicts concentration dependent effects of ranolazine on action potential duration (APD) and upstroke velocity (UV) at BCL 1000. A comparison to Figure 4 reveals that the O’Hara Rudy and Grandi-Bers models are much more effective at normalizing the APD in the...
highest concentrations tested. For all three models, UV remains robust with high therapeutic concentrations of drug. Panels (E-G) are the tissue level simulations. Panel (E) shows a space-time plot of 3 S1 beats at BCL1000 (after steady state pacing – 500 beats) in the absence of drug. A 2000 ms pause, followed by an S2 stimulus elicits an EAD throughout the 165-cell cardiac fiber. With pretreatment of 10 µM ranolazine (F), the S2 stimulus fails to elicit an EAD throughout the fiber and monotonic repolarization is restored. For (E) and (F), x-axis is time, y-axis is cell number, z-axis is voltage. A computed ECG is underneath the space-time plot. (G) The pause necessary to elicit an EAD throughout a 165-cell cardiac fiber with assessed in 5 ms increments for two pacing cycles (60 BPM – solid line, 100 BPM – dotted line) with 10 µM ranolazine after steady state pacing at the given cycle length (500 beats).
Online Figure IV: Comparison of Ca\textsuperscript{2+} transient in control and HF conditions
Shown in (A) are cellular APs in the control (black) and heart failure (HF condition same as in Figure 6 - red) at BCL 1000 ms without β-adrenergic stimulation. (B) Intracellular Na\textsuperscript{+} concentration with 1 µM (black) and 0 µM isoproterenol applications (red) in control case. (C) In the heart failure model, intracellular Na\textsuperscript{+} concentration with 1 µM (black) and 0 µM isoproterenol applications (red) in control case.
Online Figure V: Summary data of DAD formation in the drug-free conditions of Figure 7. Action potentials shown for each condition in Figure 7, drug free. Row (A) indicates 10-fold Na⁺ leak, (B) indicates 8-fold Na⁺ leak, (C) indicates 6-fold Na⁺ leak, and (D) indicates 4-fold Na⁺ leak. Column 1 corresponds to 10% decreased NKA, column 2 is 20% decrease, and column 3 is 30% decrease. As in Figure 7, filled circles indicate absence of DADs, upside-down red triangles indicate presence of DADs, and stop signs (☐) indicate repolarization failure.
Online Figure VI: DAD abolishment with ranolazine 5 µM when Na⁺ leak current is sensitive at an affinity equivalent to late Na⁺ current blockade.

Summary data and expanded AP waveforms assuming that ranolazine affinity is equivalent to late current affinity (IC₅₀ = 6 µM – Figure 7). Row (A) indicates 10-fold Na⁺ leak, (B) indicates 8-fold Na⁺ leak, (C) indicates 6-fold Na⁺ leak, and (D) indicates 4-fold Na⁺ leak. Column 1 corresponds to 10% decreased NKA, column 2 is 20% decrease, and column 3 is 30% decrease. Filled black circles (●) indicate absence of DADs, upside down red triangles indicate presence of DADs. See Supplementary Information for details on calculation of Na⁺ leak current blockade.
Online Figure VII: DAD abolishment with ranolazine 10 µM when Na⁺ leak current is sensitive at an affinity equivalent to late Na⁺ current blockade.

Summary data and expanded AP waveforms assuming that ranolazine affinity is equivalent to late current affinity (IC₅₀ = 6 µM – Figure 7). Row (A) indicates 10-fold Na⁺ leak, (B) indicates 8-fold Na⁺ leak, (C) indicates 6-fold Na⁺ leak, and (D) indicates 4-fold Na⁺ leak. Column 1 corresponds to 10% decreased NKA, column 2 is 20% decrease, and column 3 is 30% decrease. As expected, with ranolazine 10 µM, more DADs are abolished, compared to low-dose drug (Figure 7 and Online figure 5). Filled black circles (•) indicate absence of DADs, upside down red triangles indicate presence of DADs. See Supplementary Information for details on calculation of Na⁺ leak current blockade.
Online Figure VIII: Ionic mechanisms of suppression of triggered activity with application of ranolazine in a HF condition

The HF model (condition from Figure 7B shown here: 10% decreased NKA, 8-fold increased $I_{\text{Na,Leak}}$) shown in the red traces displays many characteristic features of heart failure phenotypes found in the literature including (A) increased APD, presence of triggered beats (in this case, DAD – red arrowhead) and diastolic depolarization of the resting membrane potential, (B) an increased Na$^+$ load, (C) a slight decreased Ca$^{2+}$ transient and blunted decay, and (D) large forward mode NCX generated as a result of increased Ca$^{2+}$ loading. This Ca$^{2+}$ extrusion process depolarizes the membrane potential and leads to $I_{\text{Na}}$ activation and a triggered beat. Application of low (5 µM, blue, left column) and high (10 µM, blue, right column) dose ranolazine partly normalizes [Na$^+$]i (row B) and forward mode NCX (row D), abolishes the spontaneous Ca$^{2+}$ transient (row C), triggered AP (row A), and partially restores the resting membrane potential, thus elevating the threshold for triggered diastolic events. Many of these results were recently confirmed in a hypertrophic cardiomyopathy experimental model 38; application of ranolazine shortened the AP, reduced the occurrence of triggered activity, reduced the Ca$^{2+}$ transient and accelerated its decay, and hyperpolarized the resting membrane potential.
Online Figure IX: DAD abolishment with ranolazine 5 µM when Na⁺ leak current is sensitive at an affinity equivalent to peak Na⁺ current blockade.

Because no data exists for the affinity of ranolazine to the Na⁺ leak current, we show both summary data assuming that ranolazine affinity is equivalent to late current affinity (IC₅₀ = 6 µM – Figure 7, Online Figures VI – VIII), and here, where ranolazine affinity is assumed equivalent to peak current affinity (IC₅₀ = 165.2 µM). As expected, with Na⁺ leak current affinity equivalent to peak current affinity (165.2 µM), fewer DADs are abolished, given the same concentration of ranolazine. Row (A) indicates 10-fold Na⁺ leak, (B) indicates 8-fold Na⁺ leak, (C) indicates 6-fold Na⁺ leak, and (D) indicates 4-fold Na⁺ leak. Column 1 corresponds to 10% decreased NKA, column 2 is 20% decrease, and column 3 is 30% decrease. Filled black circles (✿) indicate absence of DADs, upside down red triangles indicate presence of DADs. See Supplementary Information for details on calculation of Na⁺ leak current blockade.
Online Figure X: DAD abolishment with ranolazine 10 µM when Na⁺ leak current is sensitive at an affinity equivalent to peak Na⁺ current blockade.

Here, we show the same analysis as in Online Figure VII where ranolazine affinity is assumed equivalent to peak current affinity (IC₅₀ = 165.2 µM), but with high dose (10 µM) ranolazine. Row (A) indicates 10-fold Na⁺ leak, (B) indicates 8-fold Na⁺ leak, (C) indicates 6-fold Na⁺ leak, and (D) indicates 4-fold Na⁺ leak. Column 1 corresponds to 10% decreased NKA, column 2 is 20% decrease, and column 3 is 30% decrease. Filled black circles (●) indicate absence of DADs, upside down red triangles indicate presence of DADs, and green asterisk indicates subthreshold DAD. See Supplementary Information for details on calculation of Na⁺ leak current blockade.
Online Figure XI: Intracellular ion concentrations and expanded analysis for case in Figure 7C (20% decrease NKA, 6-fold increase in Na⁺ leak).

Shown in row (A) are cellular APs, (B) intracellular Na⁺ concentration, (C) intracellular Ca²⁺ concentration, and (D) Na⁺/Ca²⁺ exchange current. Column 1 WT drug-free, column 2 depicts 20% decrease NKA, 6-fold increased Na⁺ leak (case C from Figure 7), and columns 3 is the same condition with 5 µM ranolazine. The arrowheads indicate non-paced (DAD) beats. As in Figure 7C, only low dose (5 µM) ranolazine is required to abolish the non-paced DAD beat shown in column 2.
Online Figure XII: Ranolazine application in the absence of late Na⁺ current
Action potential (AP) with 100% block of late sodium current (black) and the same condition with ranolazine 10 μM (red) at 1Hz pacing frequencies.
Online Figure XIII: Sensitivity analysis of ionic currents contributing to triggered APs

The current densities changed in the HF were varied by increased or decreased 10%. The baseline model was HF condition of 6-fold increased in Na⁺ leak & 20% decreased in NKA, which induced four trigger action potentials. The y-axis indicates number of triggered action potentials.
Online Figure XIV: Analysis of composite effects of ranolazine

Here, we show the composite characteristics of ranolazine with $I_{Na}$ and $I_{Kr}$ effects (green) as seen experimentally. We then deconstructed the contributions to each current and show the effects of a hypothetical “pure $I_{Na}$ block ranolazine” (red), or “pure $I_{Kr}$ block ranolazine” (blue). Panels (A) and (B) show the effects of 5 µM and 10 µM, respectively. Consistent with experiments and our hypothesis, a pure $I_{Na}$ block would serve to decrease the APD as compared to control (black), and a pure $I_{Kr}$ block would tend to increase the APD as compared to control. True ranolazine (simulated in green) shows composite characteristics and lengthens the APD modestly.
Online Figure XV: Analysis of ranolazine on a simulated guinea-pig ventricular myocyte

To compare APD prolongation with experimental data, we simulated the effects of 50 µM ranolazne on a simulated guinea-pig ventricular myocyte model at 1.5 Hz. Consistent with experiments, ranolazine lengthens the APD by 14% (as compared to 22% experimentally).
REFERENCES


