Ionic mechanisms of electrophysiological heterogeneity and conduction block in the infarct border zone

Keith F. Decker and Yoram Rudy
Cardiac Bioelectricity and Arrhythmia Center, Department of Biomedical Engineering, Washington University, St. Louis, Missouri

Submitted 8 April 2010; accepted in final form 9 August 2010

Decker KF, Rudy Y. Ionic mechanisms of electrophysiological heterogeneity and conduction block in the infarct border zone. Am J Physiol Heart Circ Physiol 299: H1588–H1597, 2010—The incidence of ventricular arrhythmia in the healing phase after infarction has been linked to remodeling in the epicardial border zone (EBZ). Ionic models of normal zone (NZ) and EBZ myocytes were incorporated into one-dimensional models of propagation to gain mechanistic insights into how ion channel remodeling affects action potential (AP) duration (APD) and refractoriness, vulnerability to conduction block, and conduction safety postinfarction. We found that EBZ tissue exhibited abnormal APD restitution. The remodeled Na⁺ current (INa) and L-type Ca²⁺ current (ICa,L) promoted increased effective refractory period and prolonged APD at a short diastolic interval. While postrepolarization refractoriness due to remodeled EBZ INa was the primary determinant of the vulnerable window for conduction block at the NZ-to-EBZ transition in response to premature S2 stimuli, altered EBZ restitution also promoted APD dispersion and increased the vulnerable window at fast S1 pacing rates. Abnormal EBZ APD restitution and refactoriness also led to abnormal periodic conduction block patterns for a range of fast S1 pacing rates. In addition, we found that INa remodeling decreased conduction safety in the EBZ but that inward rectifier K⁺ current remodeling partially offset this decrease. EBZ conduction was characterized by a weakened AP upstroke and short intercellular delays, which prevented ICa,L and transient outward K⁺ current remodeling from playing a role in EBZ conduction in uncoupled tissue. Simulations of a skeletal muscle Na⁺ channel SkM1-INa injection into the EBZ suggested that this recently proposed antiarrhythmic therapy has several desirable effects, including normalization of EBZ effective refractory period and APD restitution, elimination of vulnerability to conduction block, and normalization of conduction in tissue with reduced intercellular coupling.

 SURVIVORS OF MYOCARDIAL INFARCTION are at an increased risk of ventricular arrhythmia in the days and weeks that follow the initial ischemic event. Years of experiments have been devoted to increasing our understanding of the pathophysiological basis for this enhanced risk (20, 39). A large body of research has focused on a canine model in which a transmural infarct is created by ligation of the left anterior descending coronary artery (10, 13–15, 36). Arrhythmias in this model are reentrant in nature and have been mapped to a thin rim of surviving epicardial tissue that borders the infarct [the epicardial border zone (EBZ)] (14). The initiation and maintenance of arrhythmia are thought to depend on the presence of a structurally and electrophysiologically remodeled substrate with enhanced vulnerability to a triggering event (29). Numerous studies have characterized the remodeling of ion channels (1–3, 11, 12, 21, 26, 30), Ca²⁺ handling (25, 31), and gap junctions (6, 28, 40) in the EBZ 3–5 days postinfarction. The clinical importance of arrhythmia and the wealth of experimental data characterizing arrhythmias and remodeling make the EBZ an excellent paradigm for computational modeling studies aimed at elucidating the underlying ionic mechanisms.

METHODS

Cell modeling. A recently published model of the canine epicardial myocyte (9) served as the control model in this study (Fig. 1). Rate-dependent properties of the control model, including APD adaptation, restitution, and accommodation, have been thoroughly validated in both cell and tissue simulations. Modifications to the control model are described in the Supplemental Material.¹ The EBZ model was derived from the NZ model by incorporating myocyte remodeling data from 3- to 5-day old canine infarct preparations. Remodeled currents incorporated into the EBZ model are shown in shaded circles.

¹ Supplemental Material for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.
in Fig. 1. See the Supplemental Material for a complete list of equations describing remodeling in the EBZ model.

Cell and strand simulations. Cell and strand models were paced for 1,800 s at a given SF cycle length (CLS1) with a constant K+ stimulus magnitude: −80 μA/μF and duration: 0.5 ms) to achieve steady state. Previous studies (34, 35) from our laboratory have used strand models to gain insights into the ionic mechanisms of propagation and conduction block. Parameter values used in the present study and a detailed validation of the strand model of propagation can be found in Ref. 34. In this study, strands were composed of 96 serially aligned cells connected by gap junctions with a direct stimulation of cell 1 (magnitude: −200 μA/μF and duration: 1 ms). A finite-difference approximation of the cable equation was solved using the Forward-Euler method with a constant time step (0.005 ms) and a spatial step (100 μM) equal to the cell length. No flux boundary conditions were used at each end of the strand. Strand models used in the simulation were as follows: 1) homogenous NZ (NZHOMO; 96 NZ cells), 2) homogenous EBZ (EBZHOMO; 96 EBZ cells), and 3) heterogenous NZ-EBZ (NZ-EBZHET; 48 NZ cells followed by 48 EBZ cells). In simulations where SkM1-Ih was added to the EBZ region, SkM1-Ih density (1.65 mS/m2) was adjusted to reproduce the experimental observation of an 80% increase in the EBZ maximal upstroke velocity (vmax) after SkM1-Ih injection (24).

APD was calculated as the time from peak upstroke membrane voltage (Vmax) to 95% repolarization (APD95) except as shown in Fig. 3, where APD at 50% repolarization (APD50) and 98% repolarization (APD98) are shown for a comparison with experimental results. Restitution was studied with a single additional beat at the S2 coupling interval (Clump) after pacing to steady state at a given CLS1. The effective refractory period (ERP) was defined as the smallest Clump for which AP propagation was successful. Postrepolarization refractoriness (PRR) was defined as ERP − APD98.

VW for conduction block at the NZ-to-EBZ transition was used as a quantitative measure of vulnerability to formation of the initial line of conduction block, a prerequisite for the initiation of reentry in the infarcted myocardium. To determine the VW, NZ-EBZHET strands were paced from the NZ end, and conduction was characterized as a function of CLS1 or Clump. The following pacing-dependent behaviors were observed: 1) no conduction (stimulus failed to capture at the NZ end), 2) EBZ conduction block (conduction succeeded in the NZ but failure was observed at the NZ-to-EBZ transition), and 3) NZ and EBZ conduction (conduction was successful in the NZ and EBZ regions). For pacing at a constant CLS1, the VW was defined as the range of Clump for which EBZ conduction block was observed. For restitution protocols, VW was defined as the range of Clump for which EBZ conduction block was observed.

Safety factor (SF) indicates the margin of safety of AP propagation and is a useful quantitative measure of conduction safety (34). A detailed description and discussion of the method for calculating SF can be found in Ref. 34. Briefly, SF for a cell during propagation was defined as follows:

\[
SF = \frac{\text{Charge generated by cell excitation}}{\text{Charge received for excitation}} = \frac{\int_I dt}{\int_I dt} + \frac{\int_I dt}{\int_I dt} A \frac{Q_{in}}{Q_{in}} > 0
\]

where \(I_C\) is the capacitative current of the cell of interest, \(I_{out}\) is the axial current between the cell of interest and its downstream neighbor, \(I_{in}\) is the axial current received by the cell of interest from its upstream neighbor, and \(Q_{in}\) is the time integral of transmembrane current. The integration limit \(A (Q_{in} > 0)\) used to calculate SF denotes the time period during which the cell has completed its sink-source cycle. The fraction of SF > 1 indicates the relative safety of conduction, whereas conduction fails for SF < 1 (34).

RESULTS

EBZ cell model. Figure 2 shows a comparison between model-generated and experimentally recorded EBZ remodeling data. The reduction of INa density and leftward shift in INa steady-state inactivation resulted in a reduced maximum AP upstroke velocity (Fig. 2A, left) and increased time constant of recovery of the reduced AP upstroke velocity (Fig. 2A, right), which were quantitatively consistent with experimental data (26). The model reproduced the reduced IcaL (Fig. 2B, left) and faster IcaL-voltage-dependent inactivation (Fig. 2B, right) recorded in the EBZ (1). Figure 2, C and D, shows reductions in the magnitude of K+ (26) and Cl− (2) components of the transient outward K+ currents (Ito1 and Ito2) in the EBZ, respectively. Delayed and inward rectifier K+ channel data are shown in Fig. 2E. The reduced fast and slow components of the delayed rectifier K+ current (IKr and IKs, respectively) (21), reduced inward rectifier K+ current (IK1) (26), and more rapid IKr activation (21) were reproduced by the EBZ model.

APD heterogeneity and conduction block. Figure 3A shows a comparison of steady-state APs [CLS1; 1 s and extracellular K+ concentration ([K+]o); 4 mM] in NZ (black) and EBZ (shaded) single cell simulations. The EBZ model AP reproduced the absence of a phase 1 notch (due to loss of Ito1) and AP triangulation (due to the reduction in IK1) observed experimentally (26). Both model and experiment data showed similar APD50 in the NZ and EBZ (Fig. 3B, left). In addition, the model reproduced the significant prolongation of EBZ APD at full repolarization observed in cell experiments with [K+]o = 4 mM (Fig. 3B, right). The AP triangulation, loss of the notch,
and prolonged EBZ cell APD at [K⁺]₀ = 4 mM were consistent with previous single cell modeling results (4, 17). Figure 3 shows a comparison of simulated APs from the middle cell of NZHOMO and EBZHOMO strands. An elevation of EBZ [K⁺]₀ was required to reproduce the marked elevation of EBZ resting Vᵐ observed in tissue experiments (36) (model: NZ, [K⁺]₀: 4.5 mM and Vᵐ: −78.6 mV; and experiment: NZ, Vᵐ: −90 mV and EBZ, Vᵐ: −79 mV). The degree of elevation of [K⁺]₀ required to reproduce the elevation of resting membrane potential (Vᵣ) observed experimentally was a critical determinant of EBZ repolarization and refractoriness. An EBZHOMO strand paced at CLₛ₁ = 1,000 ms with [K⁺]₀ = 4.5 mM had APD₉₅ = 306 ms, PRR = 12 ms, and ERP = 318 ms, whereas at elevated [K⁺]₀ = 7.6 mM, EBZ APD₉₅ = 253 ms, PRR = 74 ms, and ERP = 327 ms. Increasing [K⁺]₀ substantially increases PRR due to the markedly slower recovery of EBZ dV/dt max at elevated resting potentials (Fig. 2A, right). The elevation of EBZ [K⁺]₀ thus leads to a net increase in ERP of 9 ms, with slowing of Iₙa recovery due to resting Vᵐ elevation causing an increase in PRR and offsetting the ERP abbreviation due to APD shortening.

To understand the ionic mechanisms underlying the heterogeneity in the response of NZ and EBZ tissue to premature stimuli, APD restitution was characterized in NZHOMO and EBZHOMO strands. Figure 4 shows an examination of the ionic

Fig. 2. Comparison of model and experimental data. A: normal zone (NZ) and EBZ maximum action potential (AP) upstroke velocity (dV/dt max) and time constant of recovery of AP dV/dt max (τ_recovery) in model and experimental data (26). dV/dt max properties were determined as described in Ref. 26. Membrane voltage (Vᵐ) was held at 0 mV for 500 ms, followed by varying time intervals at the holding potential (V_hold). APs were elicited by the application of a stimulus after the release of voltage clamp at V_hold. Fully recovered dV/dt max was determined for an AP elicited after 500 ms at V_hold = −110 mV, τ_recovery was determined by an exponential fit to the time course of recovery of AP dV/dt max at V_hold = −80 mV. B: IₐCaL-voltage [current-voltage (I-V)] relationship and time constant of voltage-dependent inactivation (τ_VDI) in model and experimental data (1). C and D: NZ and EBZ I-V relationship of Iₙa1 (C) (26) and Iₙa2 (D) (2) in model and experimental data. E: ratio (EBZ to NZ) of Iₙa, density (21), Iₙa1 density (26), Iₙa2 density (21), and Iₙa (21) time constant of activation (τ_vactivation) in model and experimental data.
mechanisms underlying the altered refractoriness and restitution in the EBZ. Restitution in the EBZ was characterized by a CLS2 range with abnormally prolonged APD at short diastolic interval (DI; Fig. 4A, shaded curve). Restitution of APD50 showed similar behavior to that of APD95 (abnormal EBZ APD prolongation at short DI). In the NZ, successful conduction occurred at a DI of 5 ms (Fig. 4B, left) due to a near-complete recovery of $I_{\text{Na}}$ availability (Fig. 4C, left). In the EBZ, successful conduction did not occur until DI = 75 ms (Fig. 4B, right) due to the slow recovery of $I_{\text{Na}}$ from inactivation (Fig. 4C, right). Note that for restitution in an NZHOMO strand, $I_{\text{Na}}$ recovered rapidly with distance from the stimulus site, resulting in near-complete $I_{\text{Na}}$ activation at the downstream cell for which restitution is shown (cell 4B). In the EBZHOMO strand, reduced $I_{\text{Na}}$ availability and slow recovery from inactivation led to $I_{\text{Na}}$ activation that remained severely reduced at the stimulus site and the downstream cell (cell 4B) (see Supplemental Material, Supplemental Fig. S2 for further details). The weak $I_{\text{Na}}$ and diminished upstroke $V_m$ amplitude at DI = 75 ms were associated with abnormally prolonged EBZ APD. Peak $V_m$ during the upstroke was reduced to approximately $-20$ mV, resulting in slow $I_{\text{Ca,L}}$ activation (Fig. 4D, right) and slow $I_{\text{Ca,L}}$-dependent depolarization (Fig. 4B, right). In the NZ, positive $V_m$ promoted robust $I_{\text{Ca,L}}$ activation at short DI (Fig. 4D, left), in contrast to the slow, weak activation of $I_{\text{Ca,L}}$ observed in the EBZ. In addition, positive $V_m$ led to rapid $I_{\text{Kr}}$ activation (Fig. 4E, left) and promoted short APD at short DI in the NZ. In contrast, the suppression of EBZ $I_{\text{Kr}}$ (Fig. 4E, right) due to negative $V_m$ promoted APD lengthening at short DI. The critical role of slow $I_{\text{Kr}}$ recovery from inactivation and weak $I_{\text{Ca,L}}$ activation in leading to abnormal EBZ restitution was confirmed via additional simulations. The restoration of normal $I_{\text{Na}}$ or normal $I_{\text{Ca,L}}$ dramatically reduced the region of prolonged APD at short DI in the EBZHOMO strand (Supplemental Material, Supplemental Fig. S1).

We hypothesized that altered APD restitution in the EBZ leads to rate-dependent dispersion of APD in the heterogeneous myocardium. Figure 5A shows the rate dependence of APD in the heterogeneous strand simulation. The NZ-EBZHET strand was composed of 48 NZ cells and 48 EBZ cells, with APD adaptation shown for central NZ (cell 24) and central EBZ (cell 72) cells. APD heterogeneity between NZ and EBZ increased at CLS1 = 300 ms. Cells at the NZ-to-EBZ transition showed APD restitution, APD adaptation, and an AP shape intermediate between that of the NZ and EBZ due to electrotonic effects. The VW in NZ-EBZHET strand simulations was characterized via S1-S2 stimulation protocols to test the hypothesis that heterogeneity in APD and refractoriness would lead to a large, rate-dependent VW. Figure 5B shows the VW in an NZ-EBZHET strand in response to S2 stimuli as a function of CLS1. The width of the VW was 70 ms at CLS1 = 300 ms, 58 ms at CLS2 = 500, and 57 ms at CLS1 = 1,000 ms. The width of the VW at all CLS1 was much greater than the APD dispersion, demonstrating that PRR is the primary determinant of the width of the VW. However, the increased APD dispersion at CLS1 = 300 ms did lead to an increase in the VW relative to that at slower rates. Figure 5C and D, show the ionic mechanisms of increased APD dispersion at CLS1 = 300 ms. Conduction at CLS1 = 300 ms was characterized by reduced $I_{\text{Na}}$ activation (Fig. 5C, middle), a weakened AP upstroke (Fig. 5C, middle), $I_{\text{Ca,L}}$ activation (Fig. 5C, bottom), and prolonged APD relative to the NZ (Fig. 5C, top). At CLS1 = 1,000 ms, $I_{\text{Na}}$ was more fully recovered (Fig. 5D, middle), $I_{\text{Ca,L}}$ activation was less delayed (Fig. 5D, bottom), and APD heterogeneity between NZ and EBZ was decreased (Fig. 5D, top).

The effects of heterogeneity in NZ and EBZ APD and refractoriness were also explored during sustained pacing at the rapid S1 pacing rates that characterize tachycardia and are often required to induce arrhythmia experimentally. Figure 6A

Fig. 4. A: APD restitution measured in the central cell of homogenous NZ (NZHOMO) and homogenous EBZ (EBZHOMO) strands (CLS1 = 1 s). DI, diastolic interval. B–E: $V_m$ (B), $I_{\text{Na}}$ (C), $I_{\text{Ca,L}}$ (D), and $I_{\text{Kr}}$ (E) as a function of DI in NZ (left) and EBZ (right) strands. APs were time shifted to align dV/dtmax for each DI. APD was calculated at 95% repolarization. PRR, postrepolarization refractoriness.
Pacing over a CLS1 range from 190 to 260 ms resulted in 2:1 B–D stimulation at the NZ end. APD is shown for central NZ (cell 72) and increasingly slow and delayed activation of I_{Ca,L} (Fig. 6B, middle) and increasingly slow and delayed activation of I_{Ca,L} (Fig. 6B, bottom). The abnormal increase in APD with decreasing DI during APD restitution was therefore responsible for the region of 3:2 block. The 5:4 block pattern observed at CL_{S1} = 280 ms followed a similar I_{Na}^- and I_{Ca,L}^- dependent pattern of increasing APD with decreasing DI until block occurred. At CL_{S1} = 250 ms, 2:1 block at the NZ-to-EBZ transition occurred (Fig. 6C) since the ERP in the EBZ exceeded the pacing CL. At CL_{S1} = 200 ms (Fig. 6D), the pacing rate exceeded the ERP of both the NZ and EBZ, and 2:1 block occurred across the entire strand.

**Ionic mechanisms of conduction in the NZ and EBZ.** We hypothesized that remodeling of key determinants of conduction velocity and safety, including gap junction conductance (g_j), I_{Na}, I_{Ca,L}, and I_{Io1}, would lead to abnormal conduction in the EBZ. Figure 7, A and B, shows conduction safety and velocity as a function of g_j in NZ_{HOMO} and EBZ_{HOMO} strands. Despite the range of remodeling processes that occur in the EBZ, conduction safety and velocity remained near normal except at the most severe degrees of gap junction uncoupling. The ionic mechanisms promoting near-normal conduction in the EBZ were unclear, however, considering the key role of I_{Na} in conduction (34) and the severe remodeling of this current in the EBZ. It was therefore hypothesized that additional remodeling processes in the EBZ counteracted the effect of reduced I_{Na} and helped maintain the near-normal conduction. Additional simulations revealed a key role for I_{K1} remodeling in maintaining the near-normal conduction in the EBZ. When EBZ I_{K1} was restored to the larger magnitude that occurs in the NZ, SF was more severely reduced (Fig. 7A) and conduction block occurred at a lesser degree of uncoupling (Fig. 7B). Figure 7, C–E, shows a comparison of propagating APs, I_{K1}, and I_{Na} in uncoupled EBZ strands (g_j = 0.069 μS) with and without I_{K1} restored to its normal density. Reduction of I_{K1} (Fig. 7D) in the EBZ increased cell excitability, leading to a faster AP upstroke (Fig. 7C), increased I_{Na} activation (Fig. 7E), and thus increased SF.

Experiments and simulations have emphasized the importance of I_{Io1} (16) and I_{Ca,L} (22, 33, 34) as determinants of conduction safety in the context of gap junction uncoupling. It was therefore hypothesized that remodeling of EBZ I_{Io1} and I_{Ca,L} would have significant effects on conduction in the EBZ. Simulations using the NZ_{HOMO} strand model were consistent with the expected role of I_{Ca,L} and I_{Io1} in conduction (Fig. 8A, left). In uncoupled tissue, block of I_{Ca,L} reduced NZ SF, whereas block of I_{Io1} facilitated NZ conduction and increased SF (Fig. 8A, left). In contrast, EBZ_{HOMO} strand simulations showed that block of I_{Ca,L} and restoration of normal I_{Io1} both had a negligible effect on conduction safety (Fig. 8A, right). Figure 8, B–D, shows the role of I_{Io1} and I_{Ca,L} in conduction in NZ_{HOMO} and EBZ_{HOMO} strands at the smallest g_j for which conduction occurs (NZ critical g_j = 0.020 μS and EBZ critical g_j = 0.038 μS). Simulations are shown with normal I_{Io1} restored in the EBZ to determine the potential importance of this current in propagation. In the NZ_{HOMO} strand, robust I_{Na} led to a strong AP upstroke (maximum upstroke V_m = +18 mV) and a long delay (8.3 ms) between the excitation of the neighboring upstream and downstream cells (Fig. 8B, left). During this delay, I_{Ca,L} and I_{Io1} activation were both large (Fig. 8C, left). Integration of I_{Na}, I_{Ca,L}, and I_{Io1} during the delay between the activation of adjacent cells was used to characterize their relative contribution of excitatory charge during propagation (Fig. 8D, left). In the NZ, I_{Ca,L} contributed an excitatory charge on the order of that contributed by I_{Na}. Since I_{Io1} is a repolarizing current, it makes a negative contribution to excitation. In the NZ, this negative contribution was comparable with the positive contributions made by I_{Na} and I_{Ca,L}. In the
EBZ, the reduction in \( I_{\text{Na}} \) due to decreased density and slowed recovery from inactivation led to a very weak AP upstroke (maximum upstroke \( V_m = -13 \text{ mV} \); Fig. 8B, right). Intracellular conduction delay (3.2 ms) at the larger critical EBZ \( g_1 \) was much shorter than in the NZ (Fig. 8C). The decreased AP upstroke \( V_m \) led to much weaker activation of \( I_{\text{Ca,L}} \) and \( I_{\text{to1}} \) (restored to NZ density) in the EBZ (Fig. 8C, right), and the shorter intercellular delay limited the time period within which these currents could contribute to the charge to propagation. The charge contributions of \( I_{\text{Ca,L}} \) and \( I_{\text{to1}} \) to EBZ conduction were consequently negligible relative to the contribution of \( I_{\text{Na}} \) (Fig. 8D, right). These results suggest that EBZ \( I_{\text{Na}} \) remodeling prevents conduction at the severe levels of uncoupling, where intercellular delays are long and \( I_{\text{Ca,L}} \) and \( I_{\text{to1}} \) participation are significant. Conduction was also studied with the same level of uncoupling (\( G_i = 0.038 \mu \text{S} \)) in NZ\( _{\text{HOMO}} \) and EBZ\( _{\text{HOMO}} \) strands. At \( G_i = 0.038 \mu \text{S} \), EBZ conduction delay (3.2 ms) was prolonged relative to NZ (2.6 ms). In the NZ, the charge contributions of \( I_{\text{Ca,L}} \) (12.9 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)) and \( I_{\text{to1}} \) (−5.3 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)) were significant but smaller than that of \( I_{\text{Na}} \) (47.5 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)). However, charge contributions of \( I_{\text{Ca,L}} \) (1.3 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)) and \( I_{\text{to1}} \) (−0.005 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)) in the EBZ were even smaller relative to \( I_{\text{Na}} \) (25.1 \( \mu \text{A}\text{-ms-}\mu \text{F}^{-1} \)). EBZ remodeling thus increased \( I_{\text{Ca,L}} \) and \( I_{\text{to1}} \) participation relative to those in the NZ at equivalent levels of uncoupling.

**SkM1-\( I_{\text{Na}} \).** A recent study (24) has shown that an injection of SkM1-\( I_{\text{Na}} \) into the EBZ decreases the inducibility of sustained tachycardia and fibrillation. Strand simulations were used to seek insights into the ionic mechanism underlying the antiarhythmic action of SkM1-\( I_{\text{Na}} \). SkM1-\( I_{\text{Na}} \) was modeled via a 6-mV rightward shift in steady-state availability relative to NZ \( I_{\text{Na}} \) (Fig. 9A), and the density of injected EBZ SkM1-\( I_{\text{Na}} \) was adjusted to reproduce the 80% increase in EBZ \( dV/dt_{\text{max}} \) recorded in experiments (24). In EBZ\( _{\text{HOMO}} \) strand simulations, the increase in APD at short DI observed during EBZ restitution was attenuated (Fig. 9B), and PRR was dramatically reduced (Fig. 9C). Figure 9D shows that the addition of SkM1-\( I_{\text{Na}} \) dramatically increased the SF for conduction in the EBZ, especially in regions of severe uncoupling. To explore the potential effects of SkM1-\( I_{\text{Na}} \) on arrhythmia inducibility, the VW in NZ-EBZ\( _{\text{HET}} \) strands with SkM1-\( I_{\text{Na}} \) added to the EBZ region was assessed. The dramatic reduction in ERP in the SkM1-\( I_{\text{Na}} \)-treated EBZ region completely eliminated the VW for premature S2 stimuli at all CLS1 (Fig. 9D, compare with Fig. 5B). During pacing at fast CLS1 (without S2 stimulation), the VW was also completely eliminated after the addition of SkM1-\( I_{\text{Na}} \) (Fig. 9E, compare with Fig. 6A). These results suggest that the elimination of abnormal EBZ refractoriness and restitution are critical determinants of the antiarrhythmic action of SkM1-\( I_{\text{Na}} \).

**DISCUSSION**

An extensively studied experimental canine model of the 3- to 5-day-old infarct has provided critical insights into the nature of the arrhythmias that originate in the EBZ (10, 13–15, 36). Additional studies (1–3, 21, 25, 26, 28, 31) have characterized the ionic remodeling processes that accompany these arrhythmias. However, the mechanistic link between EBZ remodeling and increased susceptibility to arrhythmia remains incompletely understood. The present work yielded several novel mechanistic insights into ionic mechanisms of arrhythmia after infarction. First, EBZ tissue exhibits abnormal APD restitution, with a region of increasing APD at short DI.
short DI, slow recovery of $I_{Na}$ severely weakens the AP upstroke, causing abnormally slow and delayed $I_{Ca,L}$ activation and suppression of $I_{Kr}$ activation, prolonging APD. Second, prolonged refractoriness is the primary cause of the large VW in response to premature S2 stimuli. Abnormal EBZ restitution also promotes APD dispersion between the NZ and EBZ at fast CLS1 and increases the VW in response to S2 stimuli. Third, prolonged refractoriness in the EBZ also promotes a large VW during pacing at sufficiently fast pacing rates (CLS1 /H11021 300 ms).

Abnormal APD restitution increases the width of the VW by causing abnormal periodic EBZ conduction block. Fourth, despite severely reduced $I_{Na}$, EBZ SF for conduction and conduction velocity are near normal except in severely uncoupled tissue. The detrimental effect of reduced $I_{Na}$ on conduc-

tion is partially offset by the reduction in EBZ $I_{K1}$, which facilitates conduction. Fifth, although $I_{Ca,L}$ and $I_{to1}$ play a role in conduction in the NZ, remodeling of these currents does not significantly affect SF in the EBZ. Reduced EBZ $I_{Na}$ decreases upstroke $V_{m}$ during propagation, suppressing $I_{Ca,L}$ and $I_{to1}$ activation and precluding any role for these currents in conduction safety. Finally, the antiarrhythmic effects of SkM1-$I_{Na}$ injection are likely attributable to a range of desirable actions, including 1) elimination of abnormal APD restitution, 2) shortening of EBZ ERP, and 3) an increased SF for conduction in

**Fig. 7.** Role of $I_{K1}$ in NZ and EBZ conduction. A and B: safety factor (SF; A) and conduction velocity (CV; B) as a function of gap junction conductance ($g_{j}$) in NZHOMO, EBZHOMO, and EBZHOMO strands with normal $I_{K1}$ restored. C–E: $V_{m}$ (C), $I_{K1}$ (D), and $I_{Na}$ (E) for propagating APs in EBZHOMO strands with remodeled and normal $I_{K1}$ (greatly reduced gap junction coupling, $g_{j} = 0.069$ $\mu$s). The SF, CV, and APs for each strand type are shown at the central cell (cell 48).

**Fig. 8.** Role of $I_{Ca,L}$ and $I_{to1}$ in NZ and EBZ conduction. A: SF for propagation as a function of gap junction coupling in NZHOMO (left) and EBZHOMO (right) strands. B: $V_{m}$ of upstream and downstream cells during propagation in NZHOMO (left) and EBZHOMO (right) strands at the minimum $g_{j}$ (critical $g_{j}$) for which propagation occurred. C: activation of $I_{Na}$, $I_{K1}$, and $I_{Ca,L}$ for a propagating AP at the critical $g_{j}$ in NZHOMO (left) and EBZHOMO (right) strands. Normal $I_{Na}$ was included in the EBZHOMO strand simulation to assess its potential role in propagation. D: charge contribution ($Q$) of $I_{Na}$, $I_{Ca,L}$, and $I_{to1}$ to propagation at the critical $g_{j}$ in NZHOMO (left) and EBZHOMO strands with normal $I_{to1}$ restored (right). SF, CV, and APs for each strand type are shown at the central cell (cell 48).
uncoupled tissue. Elimination of abnormal restitution and shortening of ERP in the EBZ by SkM1-I_{Na} addition eliminates the VW in heterogeneous strand simulations.

Previous computational studies have provided important insights into the ionic determinants of EBZ ERP and repolarization (4), the role of heterogeneous gap junction coupling in stabilizing reentrant circuits (5), and the role of elevated Ca^{2+}/calmodulin-dependent kinase II (CaMKII) in increased ERP and susceptibility to conduction block in the EBZ (8, 17). The present work compliments these previously published studies in several ways. APD restitution is thought to be a critical determinant of reentry initiation and maintenance but has not been explored in detail in previous EBZ modeling studies. Slow conduction and localized gap junction uncoupling are thought to be important in arrhythmogenesis postinfarction, but the contribution of the range of EBZ remodeling processes to conduction in this setting has not been explored.

Previous simulations studies have emphasized the importance of I_{Na} remodeling in ERP prolongation (4, 8), slowed conduction (4, 8), and formation of the VW (8). The present study demonstrates that remodeling of I_{Na} has several additional important consequences, including a reduction in SF, alteration of the roles of I_{to1} and I_{Ca,L} in conduction, and alteration of restitution kinetics.

The present study demonstrates that abnormal EBZ restitution promotes dispersion of refractoriness and increases the VW. The period of increasing APD at short DI in the EBZ is caused by slow I_{Na} recovery (due to I_{Na} remodeling and elevated V_{rest}) and decreased I_{Ca,L} density. EBZ APs where a weak upstroke (V_{m} ~ 15 mV) results in slow and delayed I_{Ca,L} activation and AP prolongation are analogous to those observed in the epicardium in response to I_{Na} block (23) or increased I_{to1} (7). The results of the present study suggest that a sufficiently negative peak AP upstroke can lead to delayed I_{Ca,L} activation and AP prolongation, even in the absence of large I_{to1}.

The ionic mechanisms of APD restitution and the response to rapid pacing have not been studied in detail in previous EBZ experimental and modeling studies. Heterogeneity in the response of NZ and EBZ tissue at fast CLS1 and short DI is no doubt critical to the initiation and maintenance of reentry and is an important area of further experimental and modeling studies. V_{rest} (36), I_{Na} remodeling (3), and I_{Ca,L} remodeling (3) vary regionally, and this spatial variation may be an additional determinant of APD dispersion and arrhythmogenesis through effects on EBZ APD and ERP at fast CLS1.

The healing infarct is characterized by compromised I_{Na} (30), localized gap junction uncoupling (6, 40), and depolarized V_{rest} (36). The inducibility of sustained reentry depends on the safety and velocity of conduction in the context of this remodeling. It is well established that reduced I_{Na} slows conduction and decreases conduction safety. I_{to1} (16) and I_{Ca,L} (22, 33, 34) have also been accorded a role in conduction safety in uncoupled tissue. The present study demonstrates that I_{Ca,L} facilitates and I_{to1} inhibits conduction in poorly coupled NZ strands, but remodeling of these currents has no effect on conduction in the EBZ. The participation of I_{Ca,L} and I_{to1} in conduction requires 1) prominent I_{Na}, so that a robust AP upstroke strongly activates I_{Ca,L} and I_{to1}, and 2) highly uncoupled gap junctions, so that long intercellular delays allow time for I_{Ca,L} and I_{to1} to make large charge contributions during propagation. The model suggests that conduction in pathophysiological conditions in which I_{Na} is compromised, such as the healing infarct, will not be directly affected by I_{Ca,L} and I_{to1} expression. This result also suggests that cell pair experiments (16, 22), in which direct stimulation leads to a more robust AP upstroke than that observed during multicellular propagation, are likely to overestimate the role of these currents in propagation. In addition, the antiarrhythmic effects of enhanced I_{Ca,L} on the healing infarct (32) are likely attributable to effects other than direct facilitation of conduction by I_{Ca,L}. Our simulation results do, however, show that I_{K1} reduction in the EBZ increases conduction safety and allows conduction at more severe degrees of gap junction uncoupling than would otherwise be possible.

The reduction in inducibility of tachycardia and fibrillation by SkM1-I_{Na} injection postinfarction reported experimentally (24) suggests an exciting new antiarrhythmic approach. The authors attribute this antiarrhythmic effect of SkM1-I_{Na} to the promotion of propagation at normal conduction velocity in depolarized tissue. Our simulations suggest several desirable effects of SkM1-I_{Na} in the EBZ. In regions of normal gap junction coupling, EBZ conduction safety and velocity are near normal, despite reduced I_{Na} and increased V_{rest}. SkM1-I_{Na} is likely to play its most important role in regions of uncoupled tissue, where its effects on conduction safety and velocity are most pronounced. In addition, the simulations suggest that SkM1-I_{Na} normalizes EBZ APD restitution and refractoriness and eliminates the VW. Potentially undesirable side effects, such as intracellular Na^{+} concentration overload, were not observed. Taken together, these results suggest that SkM1-I_{Na} treatment of EBZ tissue is a promising antiarrhythmic approach.

Limitations. Repolarizing current densities in the EBZ model were selected based on experimentally observed ion...
current remodeling and APD in the EBZ. While the EBZ-to-NZ $I_{Kr}$ density ratio in the model is larger than the experimental average, it remains within the range of experimental error. More severe reductions in model $I_{Kr}$ resulted in APD$_{50}$ and APD$_{95}$ beyond the range observed in EBZ cell experiments. A previous computational study (3) suggested that EBZ remodeling results in decreased sensitivity of APD to $I_{Kr}$ and $I_{Kr}$ reduction and block, but this decreased sensitivity was not observed in our model. If the model indeed overestimates the density of EBZ $I_{Kr}$, this current is likely to play less of a role in abnormal EBZ restitution in vivo than the model predicts. It is important to note that single cell experiments show ~50 ms prolongation of APD (26) at full repolarization in the EBZ ([K$^+$]$_o$ = 4 mM), whereas EBZ tissue experiments showed an ~50 ms decrease in APD (36). The reason for this large difference in the effects of remodeling on APD in cell and tissue is unclear. Our simulations suggest that elevated resting $V_m$ in EBZ tissue due to elevated [K$^+$]$_o$ partially accounts for the decrease in APD, as a previous modeling study (4) has also concluded.

Although the present work provides several novel mechanistic insights into ionic mechanisms of remodeling and arrhythmogenesis postinfarction, the EBZ is a complex substrate where many additional factors may promote the initiation and maintenance of arrhythmia. Our study examines the vulnerability to and underlying mechanisms of the formation of the initial line of block, a necessary prerequisite for the initiation of reentry. Studies of reentry dynamics will require simulations of reentry. Future work should consider how spatial heterogeneity in tissue structure on reentrant circuits in the epicardial border zone of the healing canine infarct: a computational study. Am J Physiol Heart Circ Physiol 284: H372–H384, 2003.


ACKNOWLEDGMENTS
The authors thank Dr. Gregory Faber, Dr. Jonathan R. Silva, Dr. Thomas J. Hund, Thomas O’Hara, Dr. Leonid Livshitz, Namit Gaur, Dr. Ali Nekouzadeh, and Jordi Heijman for helpful discussions.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grants RO1-HL-33343 and RO1-HL-49054 and by Fondation Leducq Grant 08CDVD01 (to Y. Rudy). This material is based on work supported by the National Science Foundation under Grant CBET-0929633. Y. Rudy is the Fred Saigh Distinguished Professor at Washington University (St. Louis, MO).

DISCLAIMER
Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


3. Baba S, Dun W, Cabo C, Boyden PA. Modeling studies and will depend on the regulation of CaMKII activation by intracellular Ca$^{2+}$ and oxidation state and the effect of the CaMKII activation level on Ca$^{2+}$–handling proteins (27), Na$^+$ (37), and Cu$^{2+}$ and K$^+$ currents (38).


27. Maier LS. Role of CaMKII for signaling and regulation in the heart. *Front Biosci* 14: 486–496, 2009.


