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. First published August 13, 2010; doi:10.1152/ajpheart.00362.2010.—The increased incidence of 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 (I_{Na}) and L-type Ca²⁺ current (I_{Ca,L}) promoted increased effective refractory period and prolonged APD at a short diastolic interval. While postrepolarization refractoriness due to remodeled EBZ I_{Na}, 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 I_{Na}, 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 I_{Ca,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-I_{Na} 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.

METHODS

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.

Transitions between successful conduction, formation of the initial line of block, reentry, and fibrillation depend on the heterogeneous response of healthy and diseased cells to complex myocardial activation patterns. Our recently published computational model (9) accurately reproduces the response of the canine ventricular epicardial cell to a range of relevant pacing protocols. Cell and one-dimensional strand models were used to test several hypotheses relevant to the effects of remodeling on action potential (AP) duration (APD), conduction, and vulnerability to conduction block postinfarction. Specifically, we extended previous studies (4, 17) on the effect of remodeling on EBZ repolarization by focusing on how Na⁺ current (I_{Na}) and L-type Ca²⁺ current (I_{Ca,L}) remodeling lead to abnormal APD restitution, rate-dependent APD heterogeneity, and increased vulnerability to conduction block. Previous simulation studies have shown that reductions in I_{Na}, (34) and I_{Ca,L}, (33, 34) impaire conduction, that reductions in transient outward K⁺ current (I_{to}) facilitate conduction (16), and that reductions in gap junction coupling can facilitate or impair conduction depending on severity (34). Considering these observations, the combined effect of remodeling of I_{Na},, I_{Ca,L},, I_{to}, and gap junction coupling on conduction in the EBZ is complex. Strand simulations were therefore used to explore the ionic determinants of conduction in the EBZ. The potential of gene therapy using a skeletal muscle Na⁺ channel isoform (SkM1-I_{Na},) in the prevention of arrhythmia postinfarction (24) was also explored.

Address for reprint requests and other correspondence: K. Decker, Washington Univ., Campus Box 1097, Whitaker Hall, Rm. 260, One Brookings Dr., St. Louis, MO 63130-4899 (e-mail: kfd1@cec.wustl.edu).

1 Supplemental Material for this article is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.
The effective refractory period (ERP) was defined as the smallest CIS2 for which AP propagation was successful. Postrepolarization refractoriness (PRR) was defined as ERP to 1, 4-aminopyridine-sensitive transient outward K current; 

\[ \text{Charge received for excitation} = \int_{A} I_{C} \, dt + \int_{A} I_{out} dt \]

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 \( Q_{m} \) is the time integral of transmembrane current. The integration limit \( A (Q_{m} > 0) \) 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.

**RESULTS**

**EBZ cell model.** Figure 2 shows a comparison between model-generated and experimentally recorded EBZ remodeling data. The reduction of \( I_{Na} \) density and leftward shift in \( I_{Na} \) steady-state inactivation resulted in a reduced maximum AP upstroke velocity (Fig. 2A, left) and increased time constant of recovery of AP upstroke velocity (Fig. 2A, right), which were quantitatively consistent with experimental data (26). The model reproduced the reduced \( I_{Ca,L} \) (Fig. 2B, left) and faster \( I_{Ca,L} \), 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 (\( I_{to1} \) and \( I_{to2} \)) 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 (\( I_{Ks} \) and \( I_{Kr} \), respectively) (21), reduced inward rectifier K+ current (\( I_{Kr} \)) (26), and more rapid \( I_{Kr} \) activation (21) were reproduced by the EBZ model.

**APD heterogeneity and conduction block.** Figure 3A shows a comparison of steady-state APs [CL_{S1}, 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 \( I_{to1} \)) and AP triangulation (due to the reduction in \( I_{Kr} \)) observed experimentally (26). Both model and experiment data showed similar APD_{S0} 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, (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-EBZ_{HET} strands were paced from the NZ end, and conduction was characterized as a function of CL_{S1} or CL_{S2}. 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 CL_{S1}, the VW was defined as the range of CL_{S2} for which EBZ conduction block was observed. For restitution protocols, VW was defined as the range of CL_{S2} 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:

\[ \text{Charge received for excitation} = \int_{A} I_{C} \, dt + \int_{A} I_{out} dt \]

\[ \text{SF} = \frac{1}{A} \int_{A} Q_{m} \, dt \]

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

**RESULTS**

**EBZ cell model.** Figure 2 shows a comparison between model-generated and experimentally recorded EBZ remodeling data. The reduction of \( I_{Na} \) density and leftward shift in \( I_{Na} \) steady-state inactivation resulted in a reduced maximum AP upstroke velocity (Fig. 2A, left) and increased time constant of recovery of AP upstroke velocity (Fig. 2A, right), which were quantitatively consistent with experimental data (26). The model reproduced the reduced \( I_{Ca,L} \) (Fig. 2B, left) and faster \( I_{Ca,L} \), 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 (\( I_{to1} \) and \( I_{to2} \)) 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 (\( I_{Ks} \) and \( I_{Kr} \), respectively) (21), reduced inward rectifier K+ current (\( I_{Kr} \)) (26), and more rapid \( I_{Kr} \) activation (21) were reproduced by the EBZ model.

**APD heterogeneity and conduction block.** Figure 3A shows a comparison of steady-state APs [CL_{S1}, 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 \( I_{to1} \)) and AP triangulation (due to the reduction in \( I_{Kr} \)) observed experimentally (26). Both model and experiment data showed similar APD_{S0} 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 $[K^+]_o = 4$ mM were consistent with previous single cell modeling results (4, 17). Figure 3C shows a comparison of simulated APs from the middle cell of NZHOMO and EBZHOMO strands. An elevation of EBZ $[K^+]_o$ was required to reproduce the marked elevation of EBZ resting $V_m$ observed in tissue experiments (36) (model: NZ, $[K^+]_o$: 4.5 mM and $V_m$: −91.5 mV and EBZ, $[K^+]_o$: 7.6 mM and $V_m$: −78.6 mV; and experiment: NZ, $V_m$: −90 mV and EBZ, $V_m$: −79 mV). The degree of elevation of $[K^+]_o$ required to reproduce the elevation of resting membrane potential ($V_{rest}$) observed experimentally was a critical determinant of EBZ repolarization and refractoriness. An EBZHOMO strand paced at CLS1 = 1,000 ms with $[K^+]_o$: 4.5 mM had APD95 = 306 ms, PRR = 12 ms, and ERP = 318 ms, whereas at elevated $[K^+]_o$ = 7.6 mM, EBZ APD95 = 253 ms, PRR = 74 ms, and ERP = 327 ms. Increasing $[K^+]_o$ 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 $[K^+]_o$ thus leads to a net increase in ERP of 9 ms, with slowing of $I_{Na}$ recovery due to resting $V_m$ 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...
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 APD after showed similar behavior to that of APD (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 $I_{Na}$ 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 $I_{Na}$ from inactivation (Fig. 4C, right). Note that for restitution in an NZHOMO strand, $I_{Na}$ recovered rapidly with distance from the stimulus site, resulting in near-complete $I_{Na}$ activation at the downstream cell for which restitution is shown (cell 48). In the EBZ HOMO strand, reduced $I_{Na}$ availability and slow recovery from inactivation led to $I_{Na}$ activation that remained severely reduced at the stimulus site and the downstream cell (cell 48) (see Supplemental Material, Supplemental Fig. S2 for further details). The weak $I_{Na}$ 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 $I_{Ca,L}$ activation (Fig. 4D, right) and slow $I_{Ca,L}$-dependent depolarization (Fig. 4B, right). In the NZ, positive $V_m$ promoted robust $I_{Ca,L}$ activation at short DI (Fig. 4D, left), in contrast to the slow, weak activation of $I_{Ca,L}$ observed in the EBZ. In addition, positive $V_m$ led to rapid $I_{Kr}$ activation (Fig. 4E, left) and promoted short APD at short DI. The critical role of slow $I_{Kr}$ recovery from inactivation and weak $I_{Ca,L}$ activation in leading to abnormal EBZ restitution was confirmed via additional simulations. The restoration of normal $I_{Na}$ or normal $I_{Ca,L}$ 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 $I_{Na}$ activation (Fig. 5C, middle), a weakened AP upstroke (Fig. 5C, middle), $I_{Ca,L}$ activation (Fig. 5C, bottom), and prolonged APD relative to the NZ (Fig. 5C, top). At CLS1 = 1,000 ms, $I_{Na}$ was more fully recovered (Fig. 5D, middle), $I_{Ca,L}$ 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
shows the VW in an NZ-EBZHET strand for CLS1 < 300 ms. Pacing over a CLS1 range from 190 to 260 ms resulted in 2:1 block, pacing at CLS1 = 270 ms resulted in 3:2 block, and pacing at CLS1 = 280 ms resulted in 5:4 block at the NZ-to-EBZ transition. To explore the ionic mechanisms underlying this large VW and abnormal periodic block patterns, Fig. 6, B–D, shows \( V_m \), \( I_{Na} \), and \( I_{Ca,L} \) from central NZ (cell 24) and central EBZ (cell 72) at CLS1 = 270, 250, and 200 ms, respectively. At CLS1 = 270 ms, 3:2 block at the NZ-to-EBZ transition occurred (Fig. 6B). Block was preceded by a series of two successful EBZ APs in which APD progressively increased (208 to 244 ms) and DI shortened (62 to 26 ms) until the EBZ ERP was exceeded and block occurred. The increasing APD was accompanied by decreasing \( I_{Na} \) activation (Fig. 6B, middle) and increasing slowly 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 CLS1 = 280 ms followed a similar \( I_{Na} \)- and \( I_{Ca,L} \)-dependent pattern of increasing APD with decreasing DI until block occurred. At CLS1 = 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 CLS1 = 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 \( I_{to1} \), 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 NZHOMO and EBZHOMO 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, \( V_m \), \( I_{Na} \), and \( I_{Ca,L} \), from NZ (cell 24) and EBZ (cell 72) cells at CLS1 = 300 ms (C) and 1000 ms (D). APD was calculated at 95% repolarization.

**Fig. 5.** A: rate dependence in heterogenous NZ-EBZ (NZ-EBZHET) strand simulations. Strands were composed of 48 NZ and 48 EBZ cells, with stimulation at the NZ end. APD is shown for central NZ (cell 24) and central EBZ (cell 72) cells. B: vulnerable window (VW) as a function of S2 coupling interval (CLS2) for a range of CLS1. C and D: \( V_m \), \( I_{Na} \), and \( I_{Ca,L} \), from NZ (cell 24) and EBZ (cell 72) cells at CLS1 = 300 ms (C) and 1000 ms (D). APD was calculated at 95% repolarization.
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 $V_m = -13$ mV; Fig. 8B, right). Intercellular conduction delay (3.2 ms) at the larger critical EBZ $g_i$ 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 NZHOMO and EBZHOMO 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 $\mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$) and $I_{\text{to1}}$ ($-5.3 \, \mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$) were significant but smaller than that of $I_{\text{Na}}$ (47.5 $\mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$). However, charge contributions of $I_{\text{Ca,L}}$ (1.3 $\mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$) and $I_{\text{to1}}$ ($-0.005 \, \mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$) in the EBZ were even smaller relative to $I_{\text{Na}}$ (25.1 $\mu \text{A} \cdot \text{ms} \cdot \mu \text{F}^{-1}$). EBZ remodeling thus increased intercellular delay and suppressed $I_{\text{Ca,L}}$ 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 antiarrhythmic 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 EBZHOMO strand simulations, the increase in APD at short DI observed during EBZ restitution was attenuated (Fig. 9B), and PRR was dramatically reduced (EBZHOMO PRR = 74 ms and EBZHOMO + $SkM1-I_{\text{Na}}$ PRR = 2 ms). Figure 9C 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-EBZHET 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. At
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 $>$ 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 $I_{Na}$ 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-\(I_{\text{Na}}\) 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 \(I_{\text{Na}}\) remodeling in ERP prolongation (4, 8), slowed conduction (4, 8), and formation of the VW (8). The present study demonstrates that remodeling of \(I_{\text{Na}}\) has several additional important consequences, including a reduction in SF, alteration of the roles of \(I_{\text{o}1}\) and \(I_{\text{Ca,L}}\) 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 \(I_{\text{Na}}\) recovery (due to \(I_{\text{Na}}\) remodeling and elevated \(V_{\text{rest}}\)) and decreased \(I_{\text{Ca,L}}\) density. EBZ APs where a weak upstroke (\(V_{\text{m}} \sim 15\) mV) results in slow and delayed \(I_{\text{Ca,L}}\) activation and AP prolongation are analogous to those observed in the epicardium in response to \(I_{\text{Na}}\) block (23) or increased \(I_{\text{o}1}\) (7). The results of the present study suggest that a sufficiently negative peak AP upstroke can lead to delayed \(I_{\text{Ca,L}}\) activation and AP prolongation, even in the absence of large \(I_{\text{o}1}\).

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 CLS\(_1\) 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. \(V_{\text{rest}}\) (36), \(I_{\text{Na}}\) remodeling (3), and \(I_{\text{Ca,L}}\) 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 CLS\(_1\).

The healing infarct is characterized by compromised \(I_{\text{Na}}\) (30), localized gap junction uncoupling (6, 40), and depolarized \(V_{\text{rest}}\) (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 \(I_{\text{Na}}\) slows conduction and decreases conduction safety. \(I_{\text{o}1}\) (16) and \(I_{\text{Ca,L}}\) (22, 33, 34) have also been accorded a role in conduction safety in uncoupled tissue. The present study demonstrates that \(I_{\text{Ca,L}}\) facilitates and \(I_{\text{o}1}\) inhibits conduction in poorly coupled NZ strands, but remodeling of these currents has no effect on conduction in the EBZ. The participation of \(I_{\text{Ca,L}}\) and \(I_{\text{o}1}\) in conduction requires \(I_{\text{o}1}\) prominent \(I_{\text{Na}}\), so that a robust AP upstroke strongly activates \(I_{\text{Ca,L}}\) and \(I_{\text{o}1}\), and 2) highly uncoupled gap junctions, so that long intercellular delays allow time for \(I_{\text{Ca,L}}\) and \(I_{\text{o}1}\) to make large charge contributions during propagation. The model suggests that conduction in pathophysiological conditions in which \(I_{\text{Na}}\) is compromised, such as the healing infarct, will not be directly affected by \(I_{\text{Ca,L}}\) and \(I_{\text{o}1}\) 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 \(I_{\text{Ca,L}}\) on the healing infarct (32) are likely attributable to effects other than direct facilitation of conduction by \(I_{\text{Ca,L}}\). Our simulation results do, however, show that \(I_{\text{K1}}\) 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-\(I_{\text{Na}}\) injection postinfarction reported experimentally (24) suggests an exciting new antiarrhythmic approach. The authors attribute this antiarrhythmic effect of SkM1-\(I_{\text{Na}}\) to the promotion of propagation at normal conduction velocity in depolarized tissue. Our simulations suggest several desirable effects of SkM1-\(I_{\text{Na}}\) in the EBZ. In regions of normal gap junction coupling, EBZ conduction safety and velocity are near normal, despite reduced \(I_{\text{Na}}\) and increased \(V_{\text{rest}}\). SkM1-\(I_{\text{Na}}\) 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-\(I_{\text{Na}}\) 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-\(I_{\text{Na}}\) treatment of EBZ tissue is a promising antiarrhythmic approach.

Limitations. Repolarizing current densities in the EBZ model were selected based on experimentally observed ion

---

**Fig. 9.** A–C: effects of skeletal muscle Na\(^{+}\) channel (SkM1-\(I_{\text{Na}}\)) addition on steady-state \(I_{\text{Na}}\) availability (A). APD restitution (B) and SF (C) in NZ/\(I_{\text{Na}}\)-HET, EBZ/\(I_{\text{Na}}\)-HET, and EBZ/\(I_{\text{Na}}\)+ SkM1-\(I_{\text{Na}}\) strands. D: effect of SkM1-\(I_{\text{Na}}\) addition on VW during S1-S2 protocols for CLS\(_1\) = 300, 500, and 1,000 ms in NZ-EBZ/\(I_{\text{Na}}\) strands after SkM1-\(I_{\text{Na}}\) addition. E: VW at fast CLS\(_1\) in NZ-EBZ/\(I_{\text{Na}}\) strands after SkM1-\(I_{\text{Na}}\) addition to the EBZ region.
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 $\sim50$-ms prolongation of APD (26) at full repolarization in the EBZ ($[K^+]_o = 4$ mM), whereas EBZ tissue experiments showed an $\sim50$-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 $V_{Na}$ 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 in two- or three-dimensional models of the EBZ. Tissue anisotropy is likely to contribute to the formation of lines of block and lines of apparent block during reentry (10) in the EBZ and will require a detailed description of cell orientation (36) and gap junction distribution (28, 41) for accurate modeling. A previous study (34) using strand models of propagation provided valuable insights into the ionic mechanisms and SF of propagation. This study makes several important predictions about the role of $g_y$, $I_{Na}$, $I_{Ca,L}$, $I_{bol}$, and $I_{K1}$ in conduction and block after infarction. These strand simulation results provide hypotheses that can be tested and refined in future studies of two-dimensional anisotropic tissue. The variable thickness of the EBZ (28) may also contribute to the formation of stable reentrant circuits, a phenomenon best studied in three-dimensional models of the EBZ. Arrhythmogenesis in the EBZ may also depend on spatial heterogeneity of both electrophysiological and structural remodeling (3, 5, 6). The present study examined spatially homogeneous gap junction uncoupling in NZ and EBZ strands to focus on how the interplay of various remodeling processes ($I_{Na}$, $I_{Ca,L}$, $I_{bol}$, $I_{K1}$, and gap junctions) alters the safety and ionic mechanisms of conduction in the EBZ. Future work should consider how spatial heterogeneity in coupling (NZ vs. EBZ, longitudinal vs. transverse, and interior vs. exterior regions of the EBZ) affects the initiation and maintenance of arrhythmia. Recent modeling studies have shown that increased levels of oxidized CaMKII (8) may contribute to slowed conduction and prolonged ERP in the EBZ through effects of CaMKII on the steady-state availability of $I_{Na}$ (37). The arrhythmogenic effects of $I_{Na}$ remodeling characterized here may depend on CaMKII hyperactivity and oxidation state, although this dependence is not explicitly included in the present model. The role of CaMKII in the EBZ is an important subject for future experimental and 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).

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 08CVD001 (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


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


