Ionic bases for electrical remodeling of the canine cardiac ventricle

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1The Heart and Vascular Research Center and Department of Biomedical Engineering, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio; 2Washington University School of Medicine, Washington University, St. Louis, Missouri; 3Case Cardiovascular Research Institute, Case Western Reserve University, Cleveland, Ohio; and 4Department of Biomedical Engineering, Washington University, St. Louis, Missouri

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Jeyaraj D, Wan X, Ficker E, Stelzer JE, Deschenes I, Liu H, Wilson LD, Decker KF, Said TH, Jain MK, Rudy Y, Rosenbaum DS. Ionic bases for electrical remodeling of the canine cardiac ventricle. Am J Physiol Heart Circ Physiol 305: H410–H419, 2013.—Emerging evidence suggests that ventricular electrical remodeling (VER) is triggered by regional myocardial strain via mechanoelectric feedback mechanisms; however, the ionic mechanisms underlying strain-induced VER are poorly understood. To determine its ionic basis, VER induced by altered electrical activation in dogs undergoing left ventricular pacing (n = 6) were compared with un-paced controls (n = 4). Action potential (AP) durations (APDs), ionic currents, and Ca2+ transients were measured from canine epicardial myocytes isolated from early-activated (low strain) and late-activated (high strain) left ventricular regions. VER in the early-activated region was characterized by minimal APD prolongation, but marked attenuation of the AP phase 1 notch attributed to reduced transient outward K+ current. In contrast, VER in the late-activated region was characterized by significant APD prolongation. Despite marked APD prolongation, there was surprisingly minimal change in ion channel densities but a twofold increase in diastolic Ca2+. Computer simulations demonstrated that changes in sarcromelal ion channel density could only account for attenuation of the AP notch observed in the early-activated region but failed to account for APD remodeling in the late-activated region. Furthermore, these simulations identified that cytosolic Ca2+ accounted for APD prolongation in the late-activated region by enhancing forward-mode Na+/Ca2+ exchanger activity, corroborated by increased Na+/Ca2+ exchanger protein expression. Finally, assessment of skinned fibers after VER identified altered myofilament Ca2+ sensitivity in late-activated regions to be associated with increased diastolic levels of Ca2+. In conclusion, we identified two distinct ionic mechanisms that underlie VER: 1) strain-independent changes in early-activated regions due to remodeling of sarcromelal ion channels with no changes in Ca2+ handling and 2) a novel and unexpected mechanism for strain-induced VER in late-activated regions in the canine arising from remodeling of sarcromelal Ca2+ handling rather than sarcromelal ion channels.

electrical remodeling; calcium cycling; ion channels; mechanical strain; T-wave memory

Ventricular electrical remodeling (VER) is a persistent change in the electrophysiological properties of the myocardium in response to a change in the pattern of ventricular electrical activation. Numerous human (22, 32, 33) and animal (6, 14) studies have shown that an alteration of ventricular activation by pacing produces persistent and marked ECG T-wave changes termed “T-wave memory,” providing clear evidence that the electrophysiological properties of the human ventricular myocardium exhibit the plasticity needed to induce VER. Altered electrical activation is a common sequelae of a variety of cardiac pathologies, including conduction system disease, ischemia, hypertrophy, and heart failure (30). Similarly, alteration of electrical activation by cardiac pacing reduces the mechanical efficiency of contraction and increases mortality (34). In contrast, restoration of synchronized electrical activation by biventricular pacing improves mechanical function and reduces mortality (4). However, the ionic mechanisms that govern VER remain poorly understood.

Interestingly, unlike atrial electrical remodeling, VER is characterized by prolongation rather than shortening of the action potential (AP) duration (APD) (14, 25). Another characteristic feature of VER is attenuation of the AP phase 1 notch due to reduced expression of transient outward K+ current (Ito) and its α (Kv4.3)/β (KChIP2)-subunits (21). Using transmural optical imaging from multiple left ventricular (LV) regions, we identified two distinct types of AP remodeling induced by altered activation (14). Interestingly, the type of AP remodeling observed was dependent on whether myocytes were located in regions proximal to (early activated) or remote from (late activated) the site of pacing. Specifically, the early-activated region (i.e., region adjacent to the site of altered activation) exhibited minimal changes in APD. In contrast, the most significant APD prolongation occurred in the late-activated region (i.e., region farthest from the site of altered activation), which accounted for the repolarization changes underlying VER, including T-wave memory (14). Notably, marked APD remodeling in the late-activated region was attributed to focally increased mechanical strain (14), whereas remodeling of the AP shape but, to a lesser extent, APD in early-activated regions occurred without increased strain. A similar response was later reported in the dysynchronous pacing model of heart failure, which exhibits marked AP prolongation in the late-activated lateral LV (1).

Despite its implications to cardiac function in health and disease, the ionic basis for both strain-dependent and strain-independent long-term VER remains unclear. Based on the aforementioned discussion, a key to understanding the mechanisms for VER is ascertaining the distinct ionic bases for AP remodeling in early-activated (low strain) versus late-activated (high strain) ventricular regions. These mechanisms were investigated in the present study by comparing ion channel densities and Ca2+ transients between regions of the myocardium isolated from early- and late-activated regions.

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METHODS

Experimental model of VER. The Institutional Animal Care and Use Committee of Case Western Reserve University (Cleveland, OH) approved all animal protocols used in the present study. A previously validated canine model of VER induced by anterior LV epicardial pacing for 4 wk (n = 6) was compared with unpaced controls (n = 4) (14). Briefly, adult male mongrel dogs were anesthetized with propofol (10 mg/kg), intubated, ventilated, and maintained on inhaled isoflurane. After a lateral thoracotomy, a unipolar lead was implanted from the right atrium and on the epicardial surface of the LV (isoflurane. After a lateral thoracotomy, a unipolar lead was implanted from the right atrium and on the epicardial surface of the LV (n = 6). Atrial and ventricular leads were connected to a pulse generator (Discovery II, Guidant, Minneapolis, MN), which was implanted subcutaneously. We used a model of VER induced by a change in ventricular activation independent of the heart rate [VDD mode pacing, i.e., atrial sensing was used to trigger ventricular pacing using a short atrioventricular (AV) delay] for 4 wk. This resulted in 99% ventricular paced beats at the animal’s intrinsic sinus rate. The pacemaker was implanted outside (5-10 mm) of the electrode wire and data were collected weekly interval to monitor the development of VER as evidenced by changes in the amplitude of the second QRS complex on the ECG.

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Table 1. Summary data of AP duration, ionic currents, and Ca\textsuperscript{2+} transient parameters from the early- and late-activated regions of control and VER hearts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Early-Activated Region</th>
<th>Late-Activated Region</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP remodeling</td>
<td>Control</td>
<td>VER</td>
<td></td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>271 ± 7 (7)</td>
<td>270 ± 20 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Ionic current remodeling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(I_{\text{b}}), pA/pF</td>
<td>11.36 ± 0.1 (8)</td>
<td>-5.88 ± 0.7 (12)</td>
<td>0.001</td>
</tr>
<tr>
<td>(I_{\text{Ca,L}}), pA/pF</td>
<td>-2.62 ± 0.2 (10)</td>
<td>-2.11 ± 0.2 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>(I_{\text{K,R}}), pA/pF</td>
<td>0.69 ± 0.09 (10)</td>
<td>0.43 ± 0.08 (12)</td>
<td>0.03</td>
</tr>
<tr>
<td>(I_{\text{NCX}},\text{ pA/pF}</td>
<td>0.26 ± 0.07 (6)</td>
<td>0.28 ± 0.07 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>(I_{\text{Kt}},\text{ pA/pF}</td>
<td>0.24 ± 0.09 (4)</td>
<td>0.22 ± 0.13 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>(I_{\text{Kf}}), pA/pF</td>
<td>-19.7 ± 3 (4)</td>
<td>-17.4 ± 5 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+} transient remodeling</td>
<td>Diastolic Ca\textsuperscript{2+}, mM</td>
<td>372 ± 39 (7)</td>
<td>454 ± 30 (8)</td>
</tr>
<tr>
<td>Amplitude, nM</td>
<td>153 ± 19 (7)</td>
<td>186 ± 55 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Time constant, ms</td>
<td>272 ± 22 (7)</td>
<td>262 ± 16 (8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 2\) independent control and remodeled animals, with parentheses showing the number of cells recorded. AP, action potential; VER, ventricular electrical remodeling; \(I_{\text{b}}\), transient outward K\textsuperscript{+} current; \(I_{\text{Ca,L}}\), L-type Ca\textsuperscript{2+} current; \(I_{\text{K,R}}\), slowly activated delayed rectifying K\textsuperscript{+} current; \(I_{\text{NCX}}\), Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger current; \(I_{\text{Kt}}\), inward rectifying K\textsuperscript{+} current; NS, not significant.

Western immunoblot analysis. Western blots were performed on membrane preparations made from rapidly frozen tissues obtained from myocardial regions of the late-activated epicardium and controls. Briefly, tissues were homogenized in 5 volumes of 0.3 M sucrose and 10 mM sodium phosphate (pH 7.4) in the presence of protease and phosphatase inhibitors. Samples were centrifuged at 2,800 g for 10 min to pellet nuclei and debris. The supernatant was collected and centrifuged at 8,800 g for 10 min to pellet mitochondria. The supernatant was collected and centrifuged at 20,000 g for 60 min to pellet the membranes. The membrane pellet was then solubilized 10 min on ice in 1% Triton X-100 lysis buffer with protease and phosphatase inhibitors.

All samples were run on 10% bis-Tris precast gels (Bio-Rad, Hercules, CA). The following primary antibodies were used: ryanodine receptor (RyR; MA3-916, Affinity BioReagents), sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase 2a (SERCA2a; ab28681, Abcam), phospholamban (GT222865, Gene Tex), and NCX (R3F1, Swant). Relative band densities were normalized for protein loading, we used \(\beta\)-actin (A4700, Sigma).

Apparatus and experimental protocols for in vitro muscle experiments. Solution compositions for mechanical experiments were calculated using a computer program (11) and known stability constants (12) corrected to pH 7.0 and 22°C. All solutions contained (in mM) 100 N,N-bis-(2 hydroxy-ethyl)-2-aminoethanesulfonic acid, 15 creatine phosphate, 5 DTT, 1 free Mg\textsuperscript{2+}, and 4 MgATP. pCa 9.0 solution contained 7 mM EGTA and 0.02 mM Ca\textsuperscript{2+}, pCa 4.5 solution contained 7 mM EGTA and 7.01 mM Ca\textsuperscript{2+}, and preactivating solution contained 0.07 mM EGTA. The ionic strength of all solutions was adjusted to 180 mM with potassium propionate. Solutions containing different amounts of free [Ca\textsuperscript{2+}] ([Ca\textsuperscript{2+}]\textsubscript{free}) were prepared by mixing the appropriate volumes of pCa 9.0 and 4.5 solutions.

On the day of the experiment, myocardial tissue from control and VER regions were isolated for preparation of the multicellular myocardium for mechanical experiments (27). Multicellular preparations were skinned in a solution containing Triton X-100 for 30 min, and the ends of the preparations were then attached to the arms of a position motor and force transducer as previously described (27). Motor position and force signals were sampled using SL Control software (11) and saved to computer files for later analysis.

Force-pCa analysis. Each myocardial preparation was set to a sarcomere length of 2.25 \(\mu\)m before activation and allowed to develop steady force in solutions of varying [Ca\textsuperscript{2+}]\textsubscript{free}. The difference between steady-state force and the force baseline obtained after the 20% slack step was measured as the total force at that [Ca\textsuperscript{2+}]\textsubscript{free}. Active force was then calculated by subtracting Ca\textsuperscript{2+}-independent force in solution of pCa 9.0 from the total force and was normalized to the cross-sectional area of the preparation, which was calculated from the width of the preparations assuming a cylindrical cross section. Force-pCa relationships were constructed by expressing submaximal force (P) at each pCa as a fraction of maximal force (Po) determined at pCa 4.5, i.e., P/Po. The apparent cooperativity in the activation of force development was inferred from the steepness of the force-pCa relationship and was quantified using a Hill plot transformation of the force-pCa data (27).

Myofilament gel electrophoresis. Myofilament protein preparation from frozen heart tissue was performed as previously described (15). Profiling of total and phosphorylated myofilament proteins by gel electrophoresis and phosphoprotein-specific staining were performed as previously described (39). Briefly, purified myofilament proteins were separated by one-dimensional SDS-PAGE using 4–12% gradient gels (Invitrogen). Gels were fixed in a 10% acetic acid and 10% methanol solution. Phosphorylated proteins were detected by Pro-Q Diamond staining (Invitrogen) according to the manufacturer’s instructions. Pro-Q Diamond-stained gels were imaged with a Typhoon scanner (GE Healthcare). Subsequently, gels were stained with Coomassie blue (Bio-Rad) to reveal total proteins.

Statistical analysis. Data are plotted as means ± SE. The nonparametric Wilcoxon rank-sum test was used to assess significance between groups. \(P\) values of <0.05 were considered significant.

Results

\(AP\) changes underlying VER in early- versus late-activated regions. Figure 1 shows the two distinct types of \(AP\) remodeling observed at early- versus late-activated regions after a 4-wk period of pacing-induced altered ventricular activation. In the early-activated region, there was attenuation of the \(AP\) phase 1 notch with minimal changes in APD [control: 271 ± 7 ms vs. VER: 270 ± 20 ms, \(P =\) not significant (NS)]. In contrast, VER in the late-activated region was characterized by marked APD prolongation (325 ± 13 ms) compared with control (248 ± 22 ms, \(P < 0.01\)). These data are consistent with our previous report (14) and reaffirm the diverse mani-
festation of VER determined by anatomic proximity of the myocardium to the origin of altered activation.

Ionic changes underlying VER in early-activated regions. \(I_{\text{to}}\) and its molecular determinants (Kv4.3/KChIP2) have been previously suggested to be critical to the development of cardiac memory (21). Therefore, to examine if \(I_{\text{to}}\) changes could underlie the AP changes observed in VER (Fig. 1A), we assessed \(I_{\text{to}}\) in early- and late-activated VER regions. In agreement with a previous report (38), the AP phase 1 notch amplitude in the early-activated region was significantly reduced (control vs. early-activated regions: 55.7 ± 5.8 vs. 30.3 ± 3.3 mV, \(P = 0.008\)). Consistent with this observation, \(I_{\text{to}}\) density was reduced by ~50% in the early-activated region (Fig. 1B and Table 1) and not in the late-activated region (Fig. 1, A and B). To confirm the focal remodeling of \(I_{\text{to}}\), we also assessed mRNA expression levels of the \(\alpha\) (Kv4.3)- and \(\beta\) (KChIP2)-subunits. There was an ~5-fold reduction in Kv4.3 (control: 1.03 ± 0.14 vs. VER: 0.27 ± 0.08, \(P = 0.02\)) and a >10-fold reduction in KChIP2 (control: 1.02 ± 0.12 vs. VER: 0.09 ± 0.02, \(P = 0.02\)); however, expression levels of the transcripts were unchanged compared with controls in the late-activated region.

Next, to determine if changes in other ionic currents could account for the AP remodeling, we measured \(I_{\text{Kr}}, I_{\text{Ks}}, I_{\text{K1}}, I_{\text{Ca}},\) and \(I_{\text{NCX}}\) from control and VER hearts. Table 1 shows ion channel densities measured from early- and late-activated regions of control (unpaced) and VER hearts. After VER, in the early-activated region, in addition to changes in \(I_{\text{to}}\), there was a 19% reduction in \(I_{\text{Ca}}\) that did not reach significance and a 38% reduction in \(I_{\text{Ks}}\) (Table 1). No significant changes in other ionic currents were observed (Table 1). To examine if the measured ionic current densities could account for the AP remodeling, we incorporated the ionic current changes in a computational model of the canine AP. In the early-activated region, consistent with our experimentally measured attenuation of the AP phase 1 notch, we observed a simulated epicardial AP with a reduced phase 1 notch amplitude (Fig. 2). Therefore, the measured ionic current changes, and \(I_{\text{to}}\) in particular, could account for the AP morphological remodeling observed in the early-activated region.

Ionic changes underlying VER in late-activated regions. In contrast to the early-activated region, the AP phase 1 notch was not significantly different from controls in the late-activated region (control vs. late-activated regions: 70.4 ± 4.5 vs. 51.4 ± 13.1 mV, \(P = \text{NS}\)). Furthermore, the 37% reduction in \(I_{\text{Ks}}\) was the only ionic change in the late-activated region that was close to being significant (Table 1). Incorporating this change of \(I_{\text{Ks}}\) into the canine epicardial AP model failed to recapitulate the measured AP prolongation observed in the late-activated region (see Fig. 4, left). This suggested that sarcolemmal ionic current changes could not account for the AP remodeling in the late-activated region.

We (14) have previously reported an important mechanistic role for mechanical strain in AP remodeling in the late-activated region. Since mechanoelectrical coupling is mediated through changes in intracellular \([Ca^{2+}]\), we reasoned that \(Ca^{2+}\) transient changes could account for the AP remodeling in VER. To measure \(Ca^{2+}\) transient changes independent of the

![Fig. 1. Action potential (AP) changes after ventricular electrical remodeling (VER). A: representative APs from early- and late-activated VER regions compared with unpaced controls. B: representative transient outward K+ current (\(I_{\text{to}}\)) recordings from VER and control hearts.](http://ajpheart.physiology.org/)

![Fig. 2. Ionic remodeling underlies the AP remodeling in the early-activated region. Computational simulations were based on measured ionic current changes in the canine epicardial AP model. \(I_{\text{Ca}}\), L-type Ca2+ current; \(I_{\text{to}}\), rapidly activated delayed rectifying K+ current.](http://ajpheart.physiology.org/)
This APD prolongation was due to enhanced forward-mode Ca2+. In these experiments, we did not observe significant changes in the ionic AP clamp from control and VER myocytes (Fig. 3). Specifically, there were significantly elevated levels of diastolic Ca2+ and increased amplitude of the Ca2+ transient (data shown in Table 1). Although a trend for a slower Ca2+ reuptake was observed, this did not reach statistical significance (Table 1).

To examine whether remodeling of Ca2+ transients could account for the AP remodeling, we measured Ca2+ transients during an identical AP clamp from control and VER myocytes (Fig. 3). In these experiments, we did not observe significant changes in the Ca2+ transient amplitude or diastolic levels of Ca2+ in the early-activated region. In sharp contrast, there were profound changes in Ca2+ transients from the late-activated region (Fig. 3). Specifically, there were significantly elevated levels of diastolic Ca2+ and increased amplitude of the Ca2+ transient (data shown in Table 1). Although a trend for a slower Ca2+ reuptake was observed, this did not reach statistical significance (Table 1).

To examine whether remodeling of Ca2+ transients could account for the AP remodeling, we measured Ca2+ transient changes in the canine epicardial AP model. Interestingly, simulation of the Ca2+ transient remodeling (Fig. 4, middle) caused ~30% APD prolongation, which was congruent with the measured APD prolongation of ~25% in the late-activated region (Fig. 1A). This APD prolongation was due to enhanced forward-mode I_{NaC} as changes in other ionic currents (I_{CaL}, I_{To}, or I_K) could not account for the APD prolongation (Fig. 4, middle). When both measured ionic (reduced I_K) and Ca2+ transient changes were incorporated in the canine epicardial AP model (Fig. 4, right), APD was further prolonged by ~35%. To examine if altered Ca2+ handling in the late-activated region arises from altered expression of Ca2+-handling proteins, we measured the expression of several components of the sarcoplasmic reticulum Ca2+-handling machinery. As shown in Fig. 5, there were no significant changes in the expression of RyR, SERCA, or phospholamban. However, a modest increase in NCX expression was noted in the late-activated region (Fig. 5).

Finally, since there were minimal alterations in the expression of Ca2+-handling proteins, we examined if changes in myofilament Ca2+ sensitivity were evident after remodeling in the late-activated region. To examine this, we measured myofilament Ca2+ sensitivity from skinned fibers at a fixed sarcomere length by measuring force development at various extracellular [Ca2+]. Interestingly, the force-pCa relationship was shifted to the left in late-activated VER regions, consistent with an increase in the sensitivity of myofilaments to Ca2+ (Fig. 6). Since these changes could be either a direct response to stretch or due to an alteration in the phosphorylation status of myofilaments, we measured gross changes in myofilament phosphorylation using Pro-Q Diamond stain. Interestingly, we did not observe significant changes in myofilament phosphorylation, which suggests a primary change in myofilament Ca2+ sensitivity (Fig. 7). In summary, these data support the notion that changes in myofilament Ca2+ may trigger changes in intracellular Ca2+ transients that underlie AP remodeling in the late-activated ventricular region.

**DISCUSSION**

Altered cardiac electrical activation from heart disease or pacing is an important but poorly understood marker for morbidity and mortality. In a previous study (14), we identified regionally heterogeneous AP remodeling after altered activation. Specifically, the most significant AP remodeling occurred in myocardial regions that were far from site of pacing where mechanical strain was greatest (14). However, the cellular and ionic mechanisms underlying this form of mechanoelectrical remodeling of the ventricular myocardium were heretofore unknown. In the present investigation, we performed detailed electrophysiological assessment in conjunction with computer simulations in a clinically relevant animal model. Using this approach, we identified two distinct remodeling responses that occurred after altered activation, both providing insights into mechanisms that underlie regionally heterogeneous remodeling of myocardial repolarization. In summary, ionic currents play a central role in AP remodeling in the early-activated, low-strain region, whereas remodeling in the late-activated, high-strain region was attributable to alterations in myocyte Ca2+ handling.

**Mechanism of VER in the early-activated region.** We found that AP remodeling in the early-activated region was characterized by attenuation of the epicardial phase 1 notch, as previously reported (Fig. 1A) (9, 38). This was due to a localized reduction in I_to and its molecular components in this...
Fig. 4. Ca\textsuperscript{2+} transient remodeling underlies the AP remodeling in late-activated VER regions. Measured ionic and Ca\textsuperscript{2+} transients from late-activated regions were incorporated into the canine epicardial AP model. The incorporation of ionic changes only (left) had minimal effects on the AP. In contrast, simulation of the Ca\textsuperscript{2+} transient changes (middle) caused AP prolongation due to a marked increase in inward Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) current (I\textsubscript{NCX}) despite a compensatory increase in I\textsubscript{Ks}. Right: exaggerated AP duration (APD) prolongation when both ionic and Ca\textsuperscript{2+} transient changes were simulated. Cai, intracellular Ca\textsuperscript{2+}. 
region (Fig. 1B) (19, 38). These findings are consistent with a previous study (18) in both long and short-term models of cardiac memory. Two key observations from previous studies have led to the hypothesis that changes in \( I_{\text{lo}} \) are central to the development of cardiac memory. First, in isolated canine myocardial preparations, pharmacological blockade of \( I_{\text{lo}} \) inhibits the accumulation of memory (9). Second, neonatal dogs with reduced expression of \( I_{\text{lo}} \) subjected to ventricular pacing for 2 h have a diminished accumulation of memory (20). In the present study, we extend prior observations by demonstrating that remodeling of \( I_{\text{lo}} \) and its molecular components are limited to the early-activated region (Fig. 1B). Hence, VER in response to altered electrical activation is not homogeneous throughout the ventricle but rather involves several distinct, but consistent, electrophysiological responses.

In addition to remodeling of \( I_{\text{lo}} \), there were modest reductions in \( I_{\text{Ca}} \) and \( I_{\text{Ks}} \) in the early-activated region with no significant changes in \( Ca^{2+} \) transients (Table 1). The reduction in \( I_{\text{Ca}} \) in the early-activated region could arise from a reduction in KChIP2, the \( \beta \)-subunit for \( I_{\text{lo}} \), which has recently been identified to interact with the L-type \( Ca^{2+} \) channel and enhance its activity (29). In contrast to changes in \( I_{\text{lo}} \) and \( I_{\text{Ca}} \), which were limited to the early-activated region, changes in \( I_{\text{Ks}} \) were common to both early and late-activated regions. Thus, \( I_{\text{Ks}} \) changes may reflect a broader mechanism operative in VER. In summary, remodeling of sarcolemmal ionic currents in the early-activated region account for the AP morphological remodeling that occurs after altered activation.

**Mechanism of VER in the late-activated region.** AP remodeling in the late-activated region was most notably characterized by marked APD prolongation (Fig. 1A). In contrast to the early-activated region characterized by ion channel remodeling, the electrogenic driving force for AP remodeling in the late-activated region was enhanced forward-mode \( I_{\text{NCX}} \) (Fig. 4) due to changes in the diastolic levels of \( Ca^{2+} \) and \( Ca^{2+} \) transient amplitudes. The enhanced protein expression of \( I_{\text{NCX}} \) in the late-activated region also provides support for this observation (Fig. 5). Increased \( I_{\text{NCX}} \) is a well-described compensatory response during mechanical remodeling of the heart (2). A common mechanism is thought to arise from the increased forward-mode \( I_{\text{NCX}} \) in a compensatory attempt to counteract the \( Ca^{2+} \) overload, thereby generating an inward depolarizing current causing AP prolongation. For example, the chronic AV-block dog develops significant AP remodeling

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**Fig. 5.** \( Ca^{2+} \)-handling proteins after VER in late-activated regions. A: representative Western immunoblots of \( Ca^{2+} \)-handling proteins from late-activated regions of control and VER hearts. B: quantitative analysis of \( Ca^{2+} \)-handling proteins using densitometry (\( n = 4/\text{group} \)). RyR, ryanodine receptor; SERCA2a, sarco(endo)plasmic reticulum \( Ca^{2+}\)-ATPase 2a.

**Fig. 6.** Increased myofilament \( Ca^{2+} \) sensitivity in late-activated VER regions. A: skinned myocardium isolated from paced, late-activated segments displayed increased \( Ca^{2+} \) sensitivity of force compared with skinned myocardium isolated from control segments (\( n = 4 \) control segments and 4 VER segments). Forces measured at submaximal free \( Ca^{2+} \) concentration ([\( Ca^{2+} \])_{\text{free}} \) were expressed relative to the maximal force obtained at pCa 4.5. The smooth lines were fit using the following Hill equation: \( P/P_0 = [Ca^{2+}]^{nH}/[Ca^{2+}]^{nH} + [Ca^{2+}]^{nH} \), where \( P \) is the force measured at submaximal \( [Ca^{2+}]_{\text{free}} \), \( P_0 \) is the force measured at maximal \( [Ca^{2+}]_{\text{free}} \) (pCa 4.5), \( n_H \) is the Hill coefficient, and \( k \) is the \( [Ca^{2+}] \) required for half-maximal activation (i.e., pCa5). B: summary data of steady-state mechanical properties of fibers isolated from control and VER hearts. F_{\text{min}}: minimal force; F_{\text{max}}: maximal force.

<table>
<thead>
<tr>
<th></th>
<th>F_{\text{min}} (mN/mm²)</th>
<th>F_{\text{max}} (mN/mm²)</th>
<th>pCa_{50}</th>
<th>n_H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0 ± 0.6</td>
<td>24.9 ± 2.6</td>
<td>5.68 ± 0.003</td>
<td>3.86 ± 0.39</td>
</tr>
<tr>
<td>VER (LateActivated)</td>
<td>1.1 ± 0.7</td>
<td>26.7 ± 2.9</td>
<td>5.73 ± 0.003</td>
<td>3.77 ± 0.41</td>
</tr>
</tbody>
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* P=0.03
secondary to increased intracellular Ca\textsuperscript{2+} and enhanced NCX expression (26). Similarly, the dyssynchronous model of canine heart failure (LBBB with rapid pacing at 190–200 beats/min) also exhibits significant AP prolongation and increased NCX expression in the late-activated lateral LV (1). In contrast to the AV-block dog and our model, the dyssynchronous heart failure model develops reduced Ca\textsuperscript{2+} transient amplitudes resembling the Ca\textsuperscript{2+} transients observed in the failing myocardium. Interestingly, in contrast to both models with significant structural remodeling, i.e., hypertrophy in the AV-block canine model and heart failure in dyssynchrony model, we (14) previously reported that the physiological pacing model used in our study at 1 mo exhibits no overt evidence of structural remodeling. Finally, a recent transmural optical imaging study (17) in human heart failure reported heterogenous transmural remodeling of Ca\textsuperscript{2+} transients as well as the expression of SERCA2a. It would be of interest to examine the relative contribution of transmural versus regional changes in AP and Ca\textsuperscript{2+} transient remodeling in modulating the electrophysiological changes in various disease states. In summary, our present study identifies remodeling of myocyte Ca\textsuperscript{2+} handling in late-activated, high-strain regions to play a central role in driving the AP remodeling after long-term altered activation.

The degree of Ca\textsuperscript{2+} remodeling in our study, in particular the diastolic levels of Ca\textsuperscript{2+} that are higher than systolic levels of controls, is of concern as it could cause contracture. Interestingly, prior studies under physiological conditions (24) and pathological conditions such as heart failure (36) have reported similar changes in the diastolic levels of Ca\textsuperscript{2+}. Furthermore, all measurements in the present study were from viable myocytes with normal resting membrane potentials with no overt signs of cell shortening. Despite marked changes in myocyte Ca\textsuperscript{2+} handling, we were surprised to find minimal changes in sarcomeric Ca\textsuperscript{2+}-handling proteins in the late-activated region. In search for alternate mechanisms that could link mechanical strain to myocyte Ca\textsuperscript{2+} handling (5), we explored myofilament Ca\textsuperscript{2+} sensitivity, a well-described adaptive response to changes in sarcomere length, which underlies the Frank Starling response (3). Thus, the enhanced myofilament Ca\textsuperscript{2+} sensitivity in late-activated regions observed in our study is reflective of an increased affinity for Ca\textsuperscript{2+} to troponin C (Fig. 6). Interestingly, a recent murine model of troponin mutation with increased myofilament Ca\textsuperscript{2+} sensitivity also exhibited increased diastolic levels of Ca\textsuperscript{2+} (23). Myofilament Ca\textsuperscript{2+} sensitivity has been reported to demonstrate similar changes in a canine model of heart failure (35). Furthermore, the absence of changes in myofilament phosphorylation changes (Fig. 7) are suggestive of primary remodeling in myofilament Ca\textsuperscript{2+} sensitivity. Future studies are needed to examine if myofilament Ca\textsuperscript{2+} sensitivity is a cause of cardiac electrical remodeling or a consequence of the AP/Ca\textsuperscript{2+} transient remodeling. Finally, indepth genomic and proteomic changes in the early- and late-activated regions are likely to provide insights into the upstream signaling and transcriptional mechanisms that underlie the ionic basis for cardiac electrical remodeling. These experiments may eventually pave the way for the development of novel therapies for the prevention and treatment of cardiac electrical remodeling.

Limitations. In the present study, we focused on analyses of repolarization, as prior clinical and experimental models of remodeling or T-wave memory have demonstrated minimal changes in conduction as evidenced by QRS changes on the surface ECG (14). Next, we principally studied early- and late-activated basal epicardial LV regions to obtain insights into the role of mechanical strain in cardiac electrophysiology. However, remodeling of important gradients such as transmural, apical to basal, or interventricular gradients was not evaluated in the present study. Thus, future studies that analyze
remodeling in a more global fashion are likely to provide a better perspective on regional VER. Furthermore, we did not conduct detailed mechanical activation and strain mapping in the present study, but this has been previously reported (14). With regard to major ionic currents, we studied all major cardiac membrane currents with only two exceptions (Table 1): the Ca\(^{2+}\)-dependent component of the \(I_{Na}\) (\(h_{Na}\)) and the late Na\(^{+}\) current. Possible interactions of the sympathetic system in modulating \(I_{Ks}\) were not evaluated in the present study. Furthermore, we measured whole cell changes in [Ca\(^{2+}\)]\(_{i}\) but did not measure Ca\(^{2+}\) changes in other myocyte microdomains, such as the sarcoplasmic reticulum or mitochondria. Since Ca\(^{2+}\) transient changes were only observed in late-activated regions, our Ca\(^{2+}\)-handling protein and myofilament analyses were limited to these regions. In the present study, we have proposed altered myofilament Ca\(^{2+}\) sensitivity as one plausible mechanism in causing the accumulation of cytosolic Ca\(^{2+}\). However, we do not provide evidence showing that the changes in myofilament Ca\(^{2+}\) sensitivity could fully account for the Ca\(^{2+}\) transient changes or changes in the AP observed in the late-activated region. Other possible mechanisms, such as ANG II-mediated signaling, activation of stretch-activated ion channels, or posttranslational modifications of proteins involved in sarcomeric Ca\(^{2+}\)-handling, were not analyzed and are potential avenues for future investigation. Future studies that examine these mechanisms may provide insights into the novel mechanisms mediating stretch-mediated VER.

**Clinical implications.** In the present study, we report two distinct forms of VER that are highly dependent on the pattern of mechanical strain during altered activation. Remodeling in the early-activated, low-strain region is largely due to sarcolemmal ion channel changes altering AP morphology. In contrast, remodeling in the late-activated, high-strain region is due to increased levels of cytosolic Ca\(^{2+}\) causing AP prolongation by enhancing depolarizing \(I_{scx}\). One important implication to such distinctly differing electrophysiological responses is that it explains how altered electrical activation can induce electrophysiological heterogeneities. For example, selective APD prolongation distal but not proximal to the site of pacing amplifies repolarization gradients in the heart (14). This may explain, in part, why clinical evidence of aberrant activation (10, 28, 37) from heart disease is independently associated with cardiovascular mortality. Furthermore, our study also provides the first insights into how alterations of myofilament Ca\(^{2+}\) sensitivity may affect cytosolic levels of Ca\(^{2+}\), thus serving as an interface between changes in mechanics and cardiac electrophysiology. Consequently, future experimental and modeling studies should incorporate effects of mechanical stretch as an important dimension to the electrophysiological function of the heart.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


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