Modulation of Ca\(^{2+}\) Release in Cardiac Myocytes by Changes in Repolarization Rate

Role of Phase-1 Action Potential Repolarization in Excitation-Contraction Coupling

Rajan Sah, Rafael J. Ramirez, Peter H. Backx

Abstract—The early rate of action potential (AP) repolarization varies in the mammalian heart regionally, during development, and in disease. We used confocal microscopy to assess the effects of changes in repolarization rate on spatially resolved sarcoplasmic reticulum (SR) Ca\(^{2+}\) release. The kinetics and peak amplitude of Ca\(^{2+}\) transients were reduced, and the amplitude, frequency, and temporal synchronization of Ca\(^{2+}\) spikes decreased as the rate of repolarization was slowed. The first latencies and temporal dispersion of Ca\(^{2+}\) spikes tracked closely with the time to peak and the width of the L-type Ca\(^{2+}\) current (I_{Ca,L}), suggesting that the effects of repolarization on excitation-contraction coupling occur primarily via changes in I_{Ca,L}. Next, we examined the effect of changes in the rapid early repolarization rate (phase 1) of a model human AP on SR Ca\(^{2+}\) release by varying the amount of transient outward K\(^{+}\) current. Slowing of phase-1 repolarization also caused a loss of temporal synchrony and recruitment of Ca\(^{2+}\)-release events, associated with a reduced amplitude and lengthened time to peak of I_{Ca,L}. Isoproterenol application enhanced and largely resynchronized SR Ca\(^{2+}\) release, while it increased the magnitude and shortened the time to peak of I_{Ca,L}. Our data demonstrate that membrane repolarization modulates the recruitment and synchronization of SR Ca\(^{2+}\) release via I_{Ca,L} and illustrate a physiological role for the phase-1 notch of the AP in optimizing temporal summation and recruitment of Ca\(^{2+}\)-release events. The effects of slowing phase-1 repolarization can be overcome by β-adrenergic stimulation. (Circ Res. 2002;90:165-173.)

Key Words: confocal microscopy ■ electrophysiology ■ calcium sparks ■ heart disease

In cardiac myocytes, the intracellular Ca\(^{2+}\) transient is mediated by Ca\(^{2+}\)-induced Ca\(^{2+}\) release via the close interaction of voltage-gated L-type Ca\(^{2+}\) channels (VLCCs) and SR Ca\(^{2+}\)-release channels (RyRs), followed by Ca\(^{2+}\) removal from the cytosol, predominantly by sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase and the Na\(^{+}\)-Ca\(^{2+}\) exchanger.1 Altered expression, function, and interaction of these Ca\(^{2+}\)-handling proteins have been associated with the characteristically slowed kinetics and blunted amplitude of the Ca\(^{2+}\) transient observed in failing cardiac myocytes from animals2-4 and humans.5 Despite extensive investigation, the precise mechanisms responsible for such alterations in the intracellular Ca\(^{2+}\) transient remain incompletely understood, although changes in SR Ca\(^{2+}\) content,6 coupling between RyRs and VLCCs,4 and VLCC density3 are likely to be contributing factors.

Several studies have also shown that heart disease is associated with reductions in repolarizing K\(^{+}\) currents, which cause alterations in the action potential (AP) profile.7-9 Although the typical electrophysiological consequences of increased AP duration in heart disease include QT prolongation10 and an enhanced propensity for cardiac arrhythmias,11 alterations in AP profile, particularly when caused by reductions in transient outward K\(^{+}\) current (I_{to}), are also known to influence L-type Ca\(^{2+}\) current (I_{Ca,L}),12 intracellular Ca\(^{2+}\) transients,9,13 and excitation-contraction coupling.14 Therefore, reductions in I_{to} and the associated loss of the early repolarization notch might contribute significantly to changes in the kinetics and amplitude of SR Ca\(^{2+}\) release in heart disease.

Consistent with a link between altered AP repolarization and SR Ca\(^{2+}\) release, recent studies have shown that slowed AP repolarization15 and impaired SR Ca\(^{2+}\) release occur in rabbit myocytes after myocardial infarction.3 Furthermore, the depressed kinetics and amplitudes of Ca\(^{2+}\) transients from failing rabbit myocytes were linked to asynchronous Ca\(^{2+}\) release and poorly coordinated summation of Ca\(^{2+}\) sparks.3 Other studies have also suggested that temporal synchronization of Ca\(^{2+}\) release is an important determinant of peak systolic [Ca\(^{2+}\)], and cardiac inotropy in rat ventricular myo-
cytes, independent of SR Ca\textsuperscript{2+} load, under resting conditions\textsuperscript{16} and after β-adrenergic stimulation.\textsuperscript{17} Therefore, modifying the time course of SR Ca\textsuperscript{2+} release in response to changes in the AP profile may indeed provide an additional mechanism whereby the Ca\textsuperscript{2+} transient can be altered in normal and diseased myocardium.

In the present study, confocal Ca\textsuperscript{2+} imaging was combined with patch-clamp recordings of myocytes to investigate the connection between the rate of membrane repolarization and time course of SR Ca\textsuperscript{2+} release. We find that the rate of repolarization influences the time course and magnitude of the whole-cell Ca\textsuperscript{2+} transient, independent of SR Ca\textsuperscript{2+} load, by modulating the recruitment and synchronization of fundamental Ca\textsuperscript{2+} release events via changes in $I_{\text{Ca,L}}$. Extending these results to physiological waveforms, we find early phase-1 repolarization of the AP to be critical for maintaining optimal firing and synchronization of release events. Asynchronous Ca\textsuperscript{2+} release after the slowing of phase-1 repolarization could be largely overcome by β-adrenergic stimulation. Thus, we propose a novel paradigm in which altered AP morphology contributes to reduced as well as asynchronous SR Ca\textsuperscript{2+} release, which can be reversed by β-adrenergic stimulation.

Materials and Methods

Cardiomyocyte Isolation
Cardiac ventricular myocytes were isolated and electrophysiological recordings were performed as previously described.\textsuperscript{14,16}

Confocal Ca\textsuperscript{2+} Imaging
Myocytes were imaged with a Fluoview confocal microscope (Olympus) and scanned by using the 488-nm spectrum line.

Measurement of Whole-Cell Ca\textsuperscript{2+} Transients
Ca\textsuperscript{2+} transients were measured in myocytes dialyzed with (mmol/L) KCl 140, HEPES 10, MgCl\textsubscript{2} 1, NaCl 7, MgATP 7, and fluo 3 pentapotassium salt 0.060, adjusted to pH 7.2 with KOH, and superfused at room temperature (20°C to 23°C at ~1 mL·min\textsuperscript{-1}) with extracellular solution of the following composition (mmol/L): NaCl 140, KCl 4, HEPES 10, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, and D-glucose 10, adjusted to 7.4 with NaOH (solution A).

Measurement of Ca\textsuperscript{2+} Spikes
Ca\textsuperscript{2+} spikes were measured in myocytes superfused with solution A (20°C to 23°C) and dialyzed with the following solution (mmol/L): KCl 140, HEPES 10, MgCl\textsubscript{2} 1, NaCl 10, MgATP 7, fluo 3 0.75, EGTA 3, and CaCl\textsubscript{2} 1.55, adjusted to pH 7.2 with KOH. The stimulus waveforms were applied every 15 seconds. In some experiments, Ca\textsuperscript{2+} spikes were measured after ~4 minutes of exposure to solution A plus isoproterenol (ISO, 200 nmol/L, Sigma Chemical Co).

Measurement of $I_{\text{Ca,L}}$
$I_{\text{Ca,L}}$ was measured in a separate group of experiments by using a pipette solution containing (mmol/L) aspartic acid 120, CsOH 120, HEPES 10, MgCl\textsubscript{2} 1, MgATP 7, tetraethylammonium chloride 10, EGTA 4, and NaCl 7, adjusted to pH 7.2 with CsOH, and sodium-free extracellular solution containing (mmol/L) choline chloride 135, HEPES 10, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, and D-glucose 10, adjusted to 7.4 with CsOH (solution B). Stimulus waveforms were applied every 15 seconds, and the effects of β-adrenergic stimulation were tested after ~4 minutes of exposure to solution B plus 200 nmol/L ISO.

Statistical Analysis
Data are presented as mean±SEM; n refers to the sample size. A value of $P<0.05$ was considered to be statistically significant. An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results
Changes in [Ca\textsuperscript{2+}], With Ramp Repolarization
After a loading train of eight 100-ms voltage steps to 10 mV, myocytes were stimulated by use of ramp depolarizations (Figure 1B, broken line; see Materials and Methods). Figure

![Figure 1](http://circres.ahajournals.org/)

Figure 1. Effect of repolarization rate on Ca\textsuperscript{2+} release. A, Line scans (1.6 seconds) were taken during 50-, 200-, and 500-ms voltage ramps after a train of eight 100-ms steps to 10 mV. In line scans taken during 200- and 500-ms ramps, Ca\textsuperscript{2+} release was sufficiently asynchronous to resolve individual Ca\textsuperscript{2+} sparks (arrows). B and C, Intracellular Ca\textsuperscript{2+} transients (solid line, B) triggered by the superimposed voltage ramps (dotted line, B) were derived from the line scans above and show a significant ($P<0.005$) decreasing relationship (C) between $\Delta[Ca^{2+}]$ and ramp duration. D and E, Time to peak (D) and time to 50% decay (E) of Ca\textsuperscript{2+} transients were correlated strongly ($R=0.999$) and increased significantly ($P<0.005$) with decreasing repolarization rate. *$P<0.05$ vs both 50- and 200-ms ramp data; †$P<0.05$ vs both 50- and 200-ms ramp data.
1 shows 1.6-second confocal line scans (Figure 1A) and corresponding fluo 3 transients (Figure 1B) derived from these line scans in response to the voltage-ramp stimulus. As summarized in Figure 1C, peak fluorescence and Ca\textsuperscript{2+} transient amplitudes progressively decreased (P<0.005) as the repolarization period was lengthened from 50 to 500 ms. The time course of Ca\textsuperscript{2+} release also depended on the repolarization rate. As repolarization rates were decreased, the leading edge of the line scans became increasingly diffuse, indicating a more asynchronous pattern of Ca\textsuperscript{2+} release and more slowly rising Ca\textsuperscript{2+} transients (Figures 1A and 1B). A plot of the time to peak of the Ca\textsuperscript{2+} transient versus repolarization time (Figure 1D) revealed a strong and significant (P<0.001) positive correlation between these parameters. Prolonged repolarization times were also associated with slowed rates of decay of the Ca\textsuperscript{2+} transients. Figure 1E shows that the time from peak systolic [Ca\textsuperscript{2+}], to 50% decline of the transient increased progressively and significantly (P<0.005) with longer ramps. Collectively, these results show that slowed repolarization influences the amplitude and temporal profile of Ca\textsuperscript{2+} transients.

Repolarization Rate Modulates Ca\textsuperscript{2+} Release via Changes in $I_{\text{Ca,L}}$

The basis for the effect of the repolarization rate on the intracellular Ca\textsuperscript{2+} transient was further investigated by measuring the frequency, amplitude, and synchronization of Ca\textsuperscript{2+} spikes, because the magnitude and shape of the whole-cell Ca\textsuperscript{2+} transient is determined by the number and timing of such fundamental Ca\textsuperscript{2+}-release events.\textsuperscript{3,16,17} For these experiments, myocytes were dialyzed with a K\textsuperscript+-based solution with high concentrations of fluo 3 (0.75 mmol/L) and EGTA (3 mmol/L), similar to those in previous studies,\textsuperscript{16,19} whereas $I_{\text{Ca,L}}$ was isolated in a separate set of experiments by dialysis with a Cs\textsuperscript+-based solution (see Materials and Methods). Figure 2 shows that slower repolarization rates increase the temporal dispersion of Ca\textsuperscript{2+} spikes in line scans (Figure 2C) and surface plots (Figure 2D) while they also decrease the peak amplitude and slow the time course of $I_{\text{Ca,L}}$ (Figure 2B, Table 1). Similar results were also observed with the use of voltage ramps peaking at 40 mV (data not shown), establishing that these effects are also observed at more physiological membrane potentials. As summarized in Figures 3A and 3B, both the amplitude and total number of triggered Ca\textsuperscript{2+} spikes decreased significantly (P<0.05) and progressively as the repolarization time was lengthened, with a pattern similar to that observed with the Ca\textsuperscript{2+} transient amplitudes (Figure 1C). Because the amplitude of Ca\textsuperscript{2+}-release events under these experimental conditions reflects the number of activated Ca\textsuperscript{2+}-release units within the active SR junction,\textsuperscript{17} these data demonstrate that slower repolarization rates reduce the numbers of SR-release units that contribute to Ca\textsuperscript{2+} spikes as well as the total number of Ca\textsuperscript{2+} spikes.

Efficient temporal summation of individual Ca\textsuperscript{2+}-release events can be just as important as the frequency of triggered events in determining the peak Ca\textsuperscript{2+} transient amplitude;\textsuperscript{17} therefore, we also analyzed the temporal distribution and coherence of Ca\textsuperscript{2+} spikes triggered by different voltage ramps by using the first latency of Ca\textsuperscript{2+} spikes and standard deviation of latency as described previously.\textsuperscript{17} To assess the time profile of $I_{\text{Ca,L}}$ during voltage ramps, the time to peak and full width at half-
maximum \(I_{\text{Ca,L}}\) were also measured. Both the first latency of Ca\(^{2+}\) spikes and time to peak of \(I_{\text{Ca,L}}\) increased linearly and significantly \((P<0.001)\) with the repolarization rate (data not shown), and as shown in Figure 3C, they were strongly correlated \((R=0.99)\). These results are entirely expected, inasmuch as the peak of the whole-cell \(I_{\text{Ca,L}}\) reflects the product of the open probability of Ca\(^{2+}\) channels and the unitary Ca\(^{2+}\) current \((i)\), both of which vary complexly with voltage during ramps and are tightly linked to SR Ca\(^{2+}\) release.20 This tight connection is further established in Figure 3D, which shows a strong linear correlation \((R=0.99, \text{Figure 3D})\) between the standard deviation of Ca\(^{2+}\) spike latency and the width of \(I_{\text{Ca,L}}\), which were also shown to increase significantly \((P<0.001)\) with the repolarization rate (data not shown). Nevertheless, the Ca\(^{2+}\) transient amplitude and profile depends in a complex manner on Ca\(^{2+}\)-release processes (ie, Ca\(^{2+}\) spikes) combined with Ca\(^{2+}\) reuptake. Taken together, our data indicate that the whole-cell Ca\(^{2+}\) transient profile can be altered through changes in repolarization rate by influencing the recruitment and temporal coherence of fundamental Ca\(^{2+}\)-release events via corresponding changes in the \(I_{\text{Ca,L}}\) profile.

### Effect of Altered Phase-1 AP Repolarization on SR Ca\(^{2+}\) Release

\(I_{\text{Ca,L}}\) is a critical determinant of early phase-1 AP repolarization. To examine the consequences of \(I_{\text{Ca,L}}\) changes and phase-1 repolarization on SR Ca\(^{2+}\) release, APs were generated by using a computer model in which \(I_{\text{Ca,L}}\) density was varied to alter early AP repolarization, as seen in human myocardium.21,22 Two AP waveforms with different rates of phase-1 repolarization were then used as command waveforms on rat (Figure 4) and rabbit (Figure 5) ventricular myocytes. The early repolarization rates of fast (high \(I_{\text{Ca,L}}\) and slow (low \(I_{\text{Ca,L}}\) phase-1 APs (AP duration at 30% repolarization) were comparable to those of the 50- and 200-ms ramps, respectively (see online Table 1 in the data supplement available at http://www.circresaha.org). As expected from the ramp data, with slowed phase-1 AP repolarization, peak \(I_{\text{Ca,L}}\) was reduced \((P<0.001)\), whereas the time to peak and duration of \(I_{\text{Ca,L}}\) were each increased \((P<0.01)\) (Table 2 and Figure 4B). As shown in Figures 4 and 5, there was also a reduction \((P<0.005)\) in amplitude

### TABLE 1. L-Type Ca\(^{2+}\) Current Properties During Voltage Ramps

<table>
<thead>
<tr>
<th>Waveform</th>
<th>50-ms Ramp</th>
<th>200-ms Ramp</th>
<th>500-ms Ramp</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i_{\text{Ca,L}}), PA/pF (n=6)</td>
<td>8.3±0.5</td>
<td>3.9±0.2*</td>
<td>2.4±0.4†</td>
</tr>
<tr>
<td>ttp, ms (n=9)</td>
<td>17.7±0.7</td>
<td>52.6±2.3*</td>
<td>115.9±4.4†</td>
</tr>
<tr>
<td>Fwhm, ms (n=9)</td>
<td>17.2±0.6</td>
<td>68.4±3.2*</td>
<td>152.8±7.5†</td>
</tr>
<tr>
<td>Q, pC/pF (n=6)</td>
<td>0.16±0.01</td>
<td>0.25±0.01*</td>
<td>0.32±0.02†</td>
</tr>
</tbody>
</table>

\*Denotes statistical difference, \(P<0.05\), between 50- and 200-ms ramps; 
†\(P<0.05\), between 500-ms ramp and both 50- and 200-ms ramps.
(A_{fast}=2.24\pm0.19, \ A_{slow}=1.80\pm0.15; \ n=10) \ and \ total \ number \ (69.4\pm4.2\%, \ n=9) \ of \ Ca^{2+} \ spikes \ triggered \ by 
slow \ versus \ fast \ phase-1 \ repolarization. \ In \ addition, \ the 
mean \ first \ latency \ of \ firing \ of \ Ca^{2+} \ spikes \ (Lat) \ was 
lengthened \ (P<0.005) \ 2.7-fold \ by \ slowed \ phase-1 \ repolar-
ization \ (Lat_{fast}=8.41\pm0.72 \ ms, \ Lat_{slow}=22.4\pm3.7 \ ms; 
\ n=10), \ along \ with \ a \ 3.8-fold \ increase \ (P<0.05) \ in 
the \ standard \ deviation \ of \ latency \ (L_{SD,fast}=5.0\pm0.7 \ ms, 
L_{SD,slow}=19.1\pm5.0 \ ms; \ n=10; \ Figures \ 5C \ and \ 5D). \ The 
similarities \ in \ the \ trend \ of \ I_{Ca,L} \ and \ SR \ Ca^{2+} \ release \ with 
Figure 4.

**Figure 4.** Effect of fast and slow phase-1 AP repolarization and β-adrenergic stimulation on Ca^{2+} spikes. A, AP waveforms applied to rat ventricular myocytes in the absence (left and center) and presence of 200 nmol/L ISO (right). B, Representative I_{Ca,L} traces in myocytes under the conditions shown directly above (see Table 2). C and D, Line scans (600 ms, C) and surface plots (D) showing loss of recruitment and temporal synchronization of Ca^{2+}-release events in myocytes stimulated with slow phase-1 AP (center), which was effectively reversed by β-adrenergic stimulation (right). Note that I_{Ca,L} was measured in a separate set of experiments from Ca^{2+} spike measurements (see Materials and Methods).

**Figure 5.** Slowing of phase-1 repolarization and ISO alters microscopic properties of Ca^{2+} release. A and B, Ca^{2+} spike amplitude (A) and frequency (B) were significantly (P<0.05) reduced by slowing of phase-1 AP repolarization and were restored by ISO application. C, First latency of Ca^{2+} spike firing and time to peak of trigger I_{Ca,L} were significantly (P<0.05) lengthened when stimulated with slow phase-1 AP and largely restored with β-adrenergic activation. D, Standard deviation of Ca^{2+} spike latency and full width at half maximum of I_{Ca,L} were increased significantly (P<0.05) on stimulation with slow phase-1 AP. ISO application significantly (P<0.05) reduced the standard deviation of Ca^{2+} spike firing, whereas the full width at half maximum of I_{Ca,L} actually increased. *P<0.05 for slow vs fast phase-1 AP data; †P<0.05 for slow phase-1 AP in the absence vs presence of ISO application.
prolonged voltage ramps and the slowing of phase-1 AP repolarization suggest that the rate of early repolarization is critical for the recruitment and synchronization of Ca\textsuperscript{2+} release.

Although freshly isolated rat cardiac myocytes are commonly used to study E-C coupling, it is possible that fundamental species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to larger species differences in E-C coupling\textsuperscript{23,24} may exist between rodents, larger mammals, and humans. To assess whether our observations in rodents are applicable to large...
and 4D; Figure 6C, right), as illustrated by the reduced mean latency (Lat slow+/ISO = 11.53 ± 1.6 ms in rats and 10.0 ± 1.1 ms in rabbits) and standard deviation of latency (Lat dev slow+/ISO = 6.4 ± 0.8 ms in rats and 4.5 ± 0.6 ms in rabbits; Figures 5C and 5D, Figures 6F and 6G). These results demonstrate that β-adrenergic stimulation is capable of enhancing E-C coupling sufficiently to reverse the asynchronous Ca²⁺ release that occurs when early repolarization is slowed.

**Discussion**

**Membrane Repolarization Modulates Ca²⁺ Release via Alterations in I_{Ca,L}**

AP repolarization varies regionally in normal myocardium⁸⁻¹⁰ and is impaired in diseased myocardium.⁷⁻⁹ Although normal AP repolarization is believed to be important primarily for maintaining coordinated electrical activity and reducing the propensity for cardiac arrhythmias,¹¹ alterations in AP profile are also known to influence I_{Ca,L},¹² intracellular Ca²⁺ transients,⁹¹³ and E-C coupling.¹⁴ Our studies show that altering the rate of repolarization profoundly affects both the magnitude and time course of SR Ca²⁺ release. A profound and monotonic decrease in the amplitude (Figure 1C) and an increase in the time to peak of Ca²⁺ transients (Figure 1D) were observed in response to reductions in the repolarization rate. Visualization of the fundamental Ca²⁺-release events that constitute the Ca²⁺ transient revealed that the delayed time to peak of the Ca²⁺ transient with longer ramps (Figures 1B and 1D) resulted from increases in first latency of Ca²⁺-release events (Figure 3C), whereas the reduced Ca²⁺ transient amplitudes were due to reductions in the recruitment (Figures 3A and 3B) and in the temporal summation of Ca²⁺-release events (Figure 3D). Underlying these changes in the synchronization of SR Ca²⁺ release was a close correlation between the time to peak of I_{Ca,L} and the first latency of Ca²⁺ spikes. This correlation is expected because the probability of spark occurrence has been shown to be proportional to I_{Ca,L} · t (ie, open probability multiplied by t).²⁰ Thus, alterations in the time course and magnitude of I_{Ca,L} via changes in the repolarization rate will produce complementary changes in the recruitment and temporal summation of elementary Ca²⁺-release events, which ultimately influence the amplitude and kinetics of the whole-cell Ca²⁺ transient, albeit in a complex manner.

Longer ramps were also associated with mild slowing of the decay rate of Ca²⁺ transients in rat myocytes (Figure 1E). This observation was initially surprising because relaxation of the transient is traditionally considered to be dependent primarily on reuptake via the SR Ca²⁺-ATPase and extrusion by the Na⁺-Ca²⁺ exchanger.¹ When Ca²⁺ release is rapid and synchronous, these Ca²⁺-removal processes likely dominate the decay of the intracellular Ca²⁺ transient, as observed with the 50-ms ramp. On the other hand, with longer ramps (500 ms), the time periods of SR Ca²⁺ release and reuptake partially overlap (data not shown), such that very-late-release events effectively slow the early decay of the Ca²⁺ transient (Figures 2C and 2D). However, slowed repolarization will also decrease forward-mode activity of the voltage-dependent Na⁺-Ca²⁺ exchanger, thereby also contributing to slowed relaxation. The connection between AP prolongation and intracellular Ca²⁺ handling has been suggested previously to account for impaired intracellular Ca²⁺ relaxation in human heart failure³ and clearly warrants further study.

Our results are consistent with a recent report showing a connection between trigger I_{Ca,L} and the recruitment and synchronization of Ca²⁺ release in rat ventricular myocytes by use of voltage steps in the presence and absence of either β-adrenergic stimulation or I_{Ca,L} agonist (FPL).¹⁷ In failing rabbit myocytes, Litwin et al¹³ also demonstrated that a reduction of I_{Ca,L} was associated with asynchronous late-firing Ca²⁺ sparks, resulting in blunted Ca²⁺ transients with slowed kinetics similar to our results in rat ventricular myocytes stimulated with slowly repolarizing ramps (Figures 1A and 1B, 200- and 500-ms ramps). Because changes in AP repolarization have also been shown previously to alter trigger I_{Ca,L},⁹¹²⁻¹⁴ we speculated that the rate of phase-1 repolarization of the AP may also influence SR Ca²⁺ release in a manner similar to that observed with ramps.

**Phase-1 AP Repolarization Is Critical for Optimizing SR Ca²⁺ Release**

We extended the modulatory role of membrane repolarization rate by examining the effects of different rates of phase-1 repolarization of the AP on Ca²⁺ release. Using computer-generated APs like those recorded in human epicardial and endocardial myocytes⁷⁻¹⁰ or in failing versus nonfailing human ventricular myocytes,² we observed a significant loss in the amplitude, number, and temporal synchronization of Ca²⁺ spikes after stimulation with the slow phase-1 AP compared with the rapid phase-1 AP, which is associated with a reduction in peak I_{Ca,L} and a shift in the time to peak of I_{Ca,L}. Although changes in overall AP duration after I_{Ca,L} reduction in human heart disease is somewhat controversial, it is generally agreed that there is loss of the phase-1 notch in failing myocytes (see review by Nabauer and Kaab²⁸). Furthermore, the role of I_{Ca,L} in setting the rate of early repolarization has been well established in model studies,¹²⁻²² in studies in vitro,²⁹ and in studies in vivo.³⁰ Our findings suggest an important physiological role for I_{Ca,L} and the phase-1 notch of the human AP in optimizing the synchronization and recruitment of Ca²⁺-release events by increasing the driving force for Ca²⁺ influx and enhancing trigger I_{Ca,L}. This relationship between I_{Ca,L} density, phase-1 AP repolarization, and I_{Ca,L} has also been shown theoretically in a recent canine AP computer model,¹² in which selective reductions in K,4.3-based I_{Ca,L} result in elevation of the AP notch and a decrease in peak I_{Ca,L}. Thus, reductions in I_{Ca,L} and in the rate of early repolarization might contribute to the slowed, blunted Ca²⁺ transient observed in failing human myocardium³ by decreasing trigger I_{Ca,L} and impairing SR Ca²⁺ release. Indeed, this effect will further exacerbate the impairment in SR Ca²⁺ handling observed in heart failure that has been linked to reductions in SR Ca²⁺ content,⁶ β-adrenergic receptor desensitization,⁵¹ RyR and VLCC coupling,⁴ and VLCC density.³⁵

Interestingly, the early repolarization rates of the fast and slow phase-1 APs were comparable to those of the 50- and 200-ms ramps, respectively, as indicated by the AP duration at 30% repolarization (see online Table 1), and resulted in a
similar trend toward desynchronization of Ca\(^{2+}\) release with slowed repolarization. On the other hand, late repolarization of both APs, as indicated by AP duration at 50% and 90% repolarization, differed markedly from either the 50- or 200-ms ramp and also showed no correlation with the magnitude or kinetics of SR Ca\(^{2+}\) release. Collectively, this suggests that early AP repolarization mediated by \(I_{\text{Ca}}\) is more important with respect to E-C coupling than is late repolarization of the AP.

**β-Adrenergic Stimulation Promotes Synchronous Firing of Ca\(^{2+}\)-Release Events**

Recent studies have demonstrated that β-stimulation enhances E-C coupling\(^2\) and promotes synchronization of Ca\(^{2+}\) release in normal\(^17\) and failing\(^3\) myocardium. We also found that β-adrenergic stimulation effectively restores temporal synchrony and enhances the recruitment of Ca\(^{2+}\)-release events triggered by the slow phase-I AP. Similarly, the magnitude and kinetics of \(I_{\text{Ca,L}}\) triggered by the slow phase-I AP was partially restored on β-adrenergic stimulation, with the exception of the width at half-maximum \(I_{\text{Ca,L}}\) and the total integrated \(I_{\text{Ca,L}}\), which actually increase. β-Adrenergic stimulation may enhance SR Ca\(^{2+}\) release via increases in the \(I_{\text{Ca,L}}\) trigger,\(^17\) increases in SR Ca\(^{2+}\) load,\(^26\) or enhancements in cross signaling between L-type Ca\(^{2+}\) channels and RyRs.\(^27\) Although direct effects of ISO on \(I_{\text{Ca,L}}\) may contribute in part to the enhanced recruitment and synchronization of Ca\(^{2+}\) release observed in the present study, the modest increase in \(I_{\text{Ca,L}}\) amplitude and the discrepancy between the increase in width of \(I_{\text{Ca,L}}\) and Ca\(^{2+}\) release after β-adrenergic stimulation suggest that other mechanisms may also contribute to the observed effect. Regardless, the ability of β-adrenergic stimulation to restore normal SR Ca\(^{2+}\) release to a failing myocyte may represent an ideal compensatory mechanism by which the desynchronizing effect of slow phase-I AP repolarization can be countered. However, in the setting of heart failure, the ultimate onset of β-receptor desensitization\(^31\) may minimize this compensation, effectively uncovering the latent deficiency in E-C coupling resulting from impaired early repolarization.

In summary, we have demonstrated that the rate of membrane repolarization modulates the recruitment and synchronization of SR Ca\(^{2+}\)-release events primarily via alterations in \(I_{\text{Ca,L}}\) and that this influences the amplitude as well as the rise and decay of the intracellular Ca\(^{2+}\) transient. Furthermore, our results suggest a putative physiological role for the phase-I notch of the AP in optimizing the temporal summation and recruitment of Ca\(^{2+}\)-release events. Our results also suggest that asynchronous Ca\(^{2+}\) release resulting from slowed phase-I repolarization after \(I_{\text{Ca}}\) reduction in failing myocytes can be acutely overcome by β-adrenergic stimulation but may ultimately lead to impaired systolic and diastolic function due to β-receptor desensitization. According to this paradigm, therapeutic agents designed to accelerate phase-I repolarization and reestablish the AP notch may prove to be beneficial by possibly improving both systolic and diastolic dysfunction.

**Acknowledgments**

This study was supported by a Canadian Institutes of Health Research (CIHR) grant to Dr Backx. R. Sah is the recipient of an MD/PhD Studentship from the CIHR, and R.J. Ramirez is the recipient of a Research Traineeship Award from the Heart and Stroke Foundation of Canada. Dr Backx is a Career Investigator of the Heart and Stroke Foundation of Ontario. We are grateful for equipment support from the Tiffin Trust Fund, the Centre for Cardiovascular Research at the University of Toronto, and the Heart and Stroke/ Richard Lewar Centre of Excellence. We also thank Robert Tsushima for providing myocytes for some of these studies.

**References**


Modulation of Ca\textsuperscript{2+} Release in Cardiac Myocytes by Changes in Repolarization Rate: Role of Phase-1 Action Potential Repolarization in Excitation-Contraction Coupling

Rajan Sah, Rafael J. Ramirez and Peter H. Backx

_Circ Res_. 2002;90:165-173; originally published online December 6, 2001;
doi: 10.1161/hh0202.103315

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/90/2/165

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2002/02/01/90.2.165.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/