Dyssynchronous Ca$^{2+}$ Sparks in Myocytes From Infarcted Hearts

Sheldon E. Litwin, Dongfang Zhang, John H.B. Bridge

Abstract—The kinetics of contractions and Ca$^{2+}$ transients are slowed in myocytes from failing hearts. The mechanisms accounting for these abnormalities remain unclear. Myocardial infarction (MI) was produced by ligation of the circumflex artery in rabbits. We used confocal microscopy to record spatially resolved Ca$^{2+}$ transients during field stimulation in left ventricular (LV) myocytes from control and infarcted hearts (3 weeks). Compared with controls, Ca$^{2+}$ transients in myocytes adjacent to the infarct had lower peak amplitudes and prolonged time courses. Control myocytes showed relatively uniform changes in [Ca$^{2+}$] throughout the cell after electrical stimulation. In contrast, in MI myocytes [Ca$^{2+}$] increased inhomogeneously and localized increases in [Ca$^{2+}$] occurred throughout the rising and falling phases of the Ca$^{2+}$ transient. Ca$^{2+}$ content of the sarcoplasmic reticulum did not differ between MI and control myocytes. Peak L-type Ca$^{2+}$ current density was reduced in MI myocytes. The macroscopic gain function was not different in control and MI myocytes when calculated as the amplitude of the Ca$^{2+}$ transient/peak I$_{Ca}$. However, when calculated as the peak rate of rise of the Ca$^{2+}$ transient/peak I$_{Ca}$, the gain function was modestly decreased in the MI myocytes. Application of isoproterenol (100 nmol/L) improved the synchronization of Ca$^{2+}$ release in MI myocytes at both 0.5 and 1 Hz. The poorly coordinated production of Ca$^{2+}$ sparks in myocytes from infarcted rabbit hearts likely contributes to the diminished and slowed macroscopic Ca$^{2+}$ transient. These abnormalities can be largely overcome when phosphorylation of Ca$^{2+}$ cycling proteins is enhanced by β-adrenergic stimulation. (Circ Res. 2000;87:1040-1047.)

Key Words: myocardial infarction ■ calcium channels ■ heart failure ■ sarcoplasmic reticulum ■ sparks

The rate of contraction and relaxation are decreased in tissue and myocytes from hypertrophied and failing hearts. Substantial evidence suggests that alterations in intracellular Ca$^{2+}$ cycling contribute to the slowing of contractions.1,2 Despite extensive investigation, the mechanisms accounting for the abnormalities of cellular Ca$^{2+}$ cycling continue to be debated. Decreased expression of the sarcoplasmic reticulum Ca$^{2+}$-ATPase (SERCA 2a) in the hypertrophied or failing heart has been proposed as a major cause of abnormal Ca$^{2+}$ signaling in these conditions.3 Such a change could potentially explain both the slowing of relaxation and impaired contractility. Although early studies seemed to clearly support this hypothesis,4 several more recent studies have shown unchanged levels of SERCA 2a protein expression in failing myocardium from both animals and humans.5-7 Furthermore, some studies suggest that Ca$^{2+}$ uptake in sarcoplasmic reticulum (SR) vesicles from failing hearts is not depressed.8 Finally, SR Ca$^{2+}$ content is not necessarily decreased in myocytes from failing hearts.9 Therefore, a simple decrease in SR Ca$^{2+}$ uptake may not fully explain the prolongation of Ca$^{2+}$ transients in failing hearts. Ca$^{2+}$ transients in cardiac myocytes are thought to result from the temporal and spatial summation of localized Ca$^{2+}$ release events, or sparks.10 The coordinated production of sparks during the early portion of an action potential results in homogeneous, early peaking Ca$^{2+}$ transients. We tested the hypothesis that alterations of SR Ca$^{2+}$ release may contribute to the slowed kinetics of contractions and Ca$^{2+}$ transients in myocytes from diseased hearts. We used a model of left ventricular (LV) dysfunction attributable to myocardial infarction (MI) in the rabbit. This model has the advantages of strong clinical relevance and a higher degree of similarity to human myocyte physiology than is seen in smaller rodents.2,11

Materials and Methods
Animals used in the present study received care according to the guidelines of the American Physiological Society. MI was produced in male New Zealand White rabbits (R&R Research & Development, Stanwood, Wash) by ligating the circumflex artery.12,13 Echocardiographic and hemodynamic studies were performed 3 to 4 weeks after surgery.13 Immediately thereafter, LV myocytes from control and infarcted hearts were isolated, as described previously.12,13 In the infarcted hearts, myocytes were selectively taken from a 2- to 3-mm...
sparks appear long after the stimulus. Episodic sparks continue to appear even as the average [Ca\textsuperscript{2+}] is declining. Integrated pixel intensity versus time plots (Ca\textsuperscript{2+} transients) corresponding to the line scan images for each cell are shown below. The Ca\textsuperscript{2+} transients in the MI myocyte have a lower peak and slower rates of rise and decline.

rim of surviving myocardium surrounding the clearly demarcated scar.

**Confocal Microscopy**

Myocytes were incubated with 10 \mu mol/L fluo-3 AM and then perfused with a modified Tyrode solution (in mmol/L: NaCl 138, MgCl\textsubscript{2} 1.0, KCl 4.4, dextrose 11, CaCl\textsubscript{2} 1.2, HEPES 12, and probenecid 0.5; pH 7.4; 22°C to 23°C). Confocal images (Biorad 1024) were recorded with the scan line oriented along the long axis of the cell. Fluo-3 was excited at 488 nm, with emitted fluorescence measured at 515 nm. Ca\textsuperscript{2+} transients were reconstructed by stacking 512 consecutive line scans and performing a time-intensity plot using NIH image software. [Ca\textsuperscript{2+}] was calculated using a pseudoratio method, as detailed by Satoh et al.\textsuperscript{14} The K\textsubscript{c} and resting [Ca\textsuperscript{2+}] were assumed to be 1.1 \mu mol/L and 150 nmol/L, respectively.\textsuperscript{15} Ca\textsuperscript{2+} transients were elicited by field stimulation (4-ms pulses). Recordings were made during steady-state stimulation at 0.5 Hz and 1 Hz. In a separate group of MI myocytes, measurements were made after 5 minutes of exposure to isoproterenol (100 nmol/L; Sigma).

**Measurement of the Macroscopic Gain Function**

Myocytes were voltage-clamped using borosilicate micropipettes (resistance 1 to 2 M\textohm), which contained (in mmol/L) CsCl 130, dextrose 5.5, K\textsubscript{2}ATP 5, HEPES 10, EGTA 0.02, MgCl\textsubscript{2} 0.5, and NaCl 10; pH 7.1. Ten conditioning pulses (−80 to +10 mV) were applied to load the SR. A 100-ms prepulse to −40 mV was applied to inactivate Na\textsuperscript{+} current before each test pulse (400-ms steps from −40 mV to +60 mV in 10-mV increments). I\textsubscript{Ca} was expressed relative to membrane capacitance (C\textsubscript{m}). I\textsubscript{Ca} inactivation kinetics were analyzed by fitting the decaying phase of the currents with a second order exponential. Epifluorescence was measured in these studies to allow comparison of whole-cell fluorescence and currents. The macroscopic gain function at each membrane potential was defined to be 1.1 \mu mol/L and 150 nmol/L, respectively.\textsuperscript{15} Ca\textsuperscript{2+} transients were elicited by field stimulation (4-ms pulses). Recordings were made during steady-state stimulation at 0.5 Hz and 1 Hz. In a separate group of MI myocytes, measurements were made after 5 minutes of exposure to isoproterenol (100 nmol/L; Sigma).

**Statistics**

Data are shown as mean±SEM. Comparisons of data from control and MI myocytes were performed using a 2-tailed Student’s unpaired t test. A value of P<0.05 was considered to be significant.

**Results**

A total of 12 control and 13 MI rabbits were studied. The number of cells used in each protocol are shown in the table and figure legends. Infarcts occupied ≈20% of the total LV weight (see online data supplement available at http://www.circresaha.org). Rabbits with MI showed morphological evidence of chronic LV dysfunction, including increased atrial, right ventricular, and lung weights. Echocardiographic measurements revealed significant LV dilatation and systolic dysfunction in the MI rabbits. Intracardiac pressure measurements showed moderate elevation of LV end-diastolic pressure after MI.

**Line Scan Imaging in Control and MI Myocytes**

Electrically stimulated control myocytes exhibited a rapid and uniform increase in [Ca\textsuperscript{2+}] (Figure 1, top left). After reaching a peak, [Ca\textsuperscript{2+}] declined homogeneously throughout control cells. In contrast, in the majority of MI myocytes, the leading edge of the Ca\textsuperscript{2+} transient was quite irregular, with some areas showing immediate increases in [Ca\textsuperscript{2+}] and other regions showing slow or delayed rises in [Ca\textsuperscript{2+}] (Figure 1, top right). Furthermore, discrete and abrupt Ca\textsuperscript{2+} rises (presumably sparks) appeared diffusely throughout the MI myocytes during the entire Ca\textsuperscript{2+} transient, including the declining phase. The spatially integrated Ca\textsuperscript{2+} transients in the MI myocytes had slower upstrokes and declines (Figure 1, bottom; Table). When the stimulation rate was increased from 0.5 Hz to 1 Hz in control myocytes, the leading edge of the Ca\textsuperscript{2+} transient visualized by confocal imaging remained

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**Figure 1.** Stacked line scan images (6-ms intervals) in single LV myocytes from control and MI rabbits at a frequency of 0.5 Hz (above). Distance is displayed on the y-axis (0.2 \mu m/pixel). The contraction of the control myocyte is seen as inward deflections of the cell leading edge at the top and bottom portions of each scan beginning approximately 30 ms after the rise in Ca\textsuperscript{2+}. The time course of the contraction parallels that of the Ca\textsuperscript{2+} transient. In this example, there is little cellular shortening along the scan line in the MI myocyte. Fluorescence intensity is displayed on a linear color scale (see inset). In the control myocyte (left), electrical stimulation causes a synchronous increase in fluo-3 fluorescence intensity across the length of the myocyte. Fluorescence intensity then declines consistently along the entire scan line. In contrast, the leading edge of the Ca\textsuperscript{2+} transient is appreciably fragmented in the MI myocyte and individual Ca\textsuperscript{2+} sparks appear long after the stimulus. Episodic sparks continue to appear even as the average [Ca\textsuperscript{2+}] is declining. Integrated pixel intensity versus time plots (Ca\textsuperscript{2+} transients) corresponding to the line scan images for each cell are shown below. The Ca\textsuperscript{2+} transients in the MI myocyte have a lower peak and slower rates of rise and decline.

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**SR Ca\textsuperscript{2+} Content**

After a train of 6 conditioning pulses, cells were held at −60 mV and then rapidly superfused with a caffeine-containing solution (20 mmol/L).\textsuperscript{13} The caffeine-induced inward current was integrated to give an estimate of the total amount of Ca\textsuperscript{2+} released from the SR.\textsuperscript{16} The integral of the caffeine-induced inward current was normalized to membrane capacitance.
Intracellular Ca\(^{2+}\) Transients in Myocytes From Control and Infarcted Rabbit Hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI</th>
<th>MI + Isoproterenol</th>
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<tbody>
<tr>
<td>Peak [Ca(^{2+})], µmol/L</td>
<td>0.89±0.09†</td>
<td>0.56±0.05*</td>
<td>1.11±0.11†</td>
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<tr>
<td>Time to peak [Ca(^{2+})], ms</td>
<td>160±8</td>
<td>205±12*</td>
<td>132±10†</td>
</tr>
<tr>
<td>T50 [Ca(^{2+})], ms</td>
<td>354±11</td>
<td>461±23*</td>
<td>276±11†</td>
</tr>
</tbody>
</table>

Myocytes were field-stimulated at 0.5 Hz, as described in Materials and Methods. n indicates number of myocytes studied from each group; T50 [Ca\(^{2+}\)], time from peak of Ca\(^{2+}\) transient to 50% decline in [Ca\(^{2+}\)]. Isoproterenol concentration=100 nmol/L.

†P<0.05 vs control.
*P<0.05 vs MI + isoproterenol vs MI.

sharp, and the rise and decline in [Ca\(^{2+}\)] became more rapid (Figure 2, left). The irregular nature of the Ca\(^{2+}\) transient in MI myocytes became even more pronounced at 1 Hz than at 0.5 Hz (Figure 2, right). This pronounced pattern of a fragmented leading edge with clearly evident late sparks was seen in 39 of 67 MI myocytes. A total of 14 of 67 MI myocytes had a milder pattern of dyssynchrony (rare late sparks seen only intermittently), and 14 had normal appearing transients (smooth leading edges with no late sparks). Three of 50 control myocytes had clear dyssynchrony, 8 were classified as mild, and 39 were normal.

The regional variations in Ca\(^{2+}\) signaling within a single MI myocyte are depicted graphically in Figure 3. Sections of line scan images free from movement artifact were cropped and expanded (Figure 3A). Plots of fluorescence intensity versus time at discrete points in each cell (regional Ca\(^{2+}\) transients) are displayed in a pseudo 3-dimensional format, which highlights the marked spatial heterogeneity in Ca\(^{2+}\) signaling within the MI myocyte (Figure 3B). In comparison, the regional Ca\(^{2+}\) transients are similar at all locales within the control myocyte (Figure 3C). An example of a single regional transient (taken from the point indicated by the arrow in panel A) in the MI myocyte demonstrates the recurrence of nonpropagated sparks at a single location.

Measurement of SR Ca\(^{2+}\) Content

We assessed the hypothesis that reduced SR Ca\(^{2+}\) content in the MI myocytes might result in focal failures of \(I_{Ca}\) to trigger SR release events. We found that the SR Ca\(^{2+}\) content was not different in MI compared with control myocytes (online Figure 1; see online data supplement available at http://www.circresaha.org). Similar findings have been reported in other models of heart failure. Therefore, decreased SR Ca\(^{2+}\) stores seem unlikely to be a major cause of the abnormal Ca\(^{2+}\) transients in MI myocytes. Although the integral of the caffeine-induced currents were not different in the control and MI myocytes, the peak amplitude of these currents tended to be larger in the MI myocytes (-0.71±0.04 versus 0.61±0.04 pA/pF, \(P=0.1\)). This finding is compatible with a modest increase in Na\(^+-\)Ca\(^{2+}\) exchange activity in the MI myocytes.

Measurement of \(I_{Ca}\)

Small but detectable inhomogeneities in [Ca\(^{2+}\)] during the early portion of action potentials in normal myocytes results from the stochastic nature of L-type Ca\(^{2+}\) channel opening and the resultant stochastic production of sparks. The inhomogeneity can be markedly accentuated by the application of both organic and inorganic Ca\(^{2+}\) channel-blocking agents. Therefore, we postulated that the fragmented appearance of the leading edge of the Ca\(^{2+}\) transient in the MI myocytes might result if there were a sufficient decrease in the number of functional L-type Ca\(^{2+}\) channels so that adjacent sparks failed to fuse together. In support of this hypothesis, we found that \(I_{Ca}\) density was significantly decreased (~20%) in MI myocytes compared with controls (Figure 4). The fast and slow time constants of \(I_{Ca}\) inactivation were not different in control and MI myocytes; however, the amplitudes of both components were decreased in the MI myocytes. The relative proportion of fast inactivation was not different in control and MI myocytes.

Figure 2. When the stimulation frequency was increased to 1 Hz, Ca\(^{2+}\) transients were enhanced in the control myocyte (compare with Figure 1). In contrast, the prolonged and inhomogeneous subcellular Ca\(^{2+}\) release pattern in the MI myocyte became more pronounced and the integrated Ca\(^{2+}\) transients became smaller.
Macrosopic Gain Function Measured Under Voltage Clamp

Work in other models of cardiac hypertrophy or heart failure suggests that the functional coupling of sarcoplasmic and SR Ca\(^{2+}\) channels is impaired.\(^9\) To determine whether such a change occurred in our model, we simultaneously measured macroscopic Ca\(^{2+}\) currents and Ca\(^{2+}\) transients. Under these conditions, Ca\(^{2+}\) transients were reduced in amplitude in MI myocytes compared with controls (Figures 5 and 6). The differences between control and MI transients were greatest at +10 mV, the potential at which \(I_{\text{Ca}}\) amplitude is maximal (Figures 5 and 6A). The difference between the groups was less pronounced but still significant at +60 mV. This finding suggests a greater contribution of Ca\(^{2+}\) influx by the reverse mode of Na\(^{+}\)-Ca\(^{2+}\) exchange in the MI myocytes. The effectiveness of \(I_{\text{Ca}}\) in producing Ca\(^{2+}\) transients (gain_peak) was not different between control and MI myocytes (ie, \(I_{\text{Ca}}\) and the peak amplitude of the Ca\(^{2+}\) transients were proportionally reduced in the MI myocytes [Figure 6B]). However, when the gain function was defined as the rate of rise of the Ca\(^{2+}\) transient/\(I_{\text{Ca}}\), the MI myocytes showed a modest reduction in gain (gain_ri; Figure 6D).

Effects of Isoproterenol

To determine whether the alterations in Ca\(^{2+}\) cycling could be overcome by activation of the β-adrenergic signaling cascade, we recorded line scan images in MI myocytes after treatment with isoproterenol. Isoproterenol markedly improved the kinetics and synchrony of Ca\(^{2+}\) release, as evidenced by the smoother contour of the leading edge of the Ca\(^{2+}\) transient and the absence of late-appearing sparks (Figure 7). The rate of rise of the Ca\(^{2+}\) transient, time to peak Ca\(^{2+}\), and rate of Ca\(^{2+}\) decline were also improved by isoproterenol (Figure 7, Table).

Discussion

In normal cardiac myocytes, membrane depolarization produces a rapid rise in cytosolic [Ca\(^{2+}\)] because of the highly coordinated production and summation of many localized sparks.\(^10\) Individual Ca\(^{2+}\) sparks are not resolved during action potentials unless the probability of opening of individual L-type Ca\(^{2+}\) channels is significantly reduced.\(^11\) SR Ca\(^{2+}\) release is terminated primarily by the inactivation or adaptation of SR Ca\(^{2+}\) release channels.\(^20\) After a stimulated Ca\(^{2+}\) release, a finite amount of time is required before ryanodine receptors can be activated again.\(^20\) A breakdown of the timing of this highly orchestrated sequence of events may underlie the abnormalities of Ca\(^{2+}\) transients in myocytes from infarcted hearts.

Mechanisms Accounting for Inhomogeneities of the Early Ca\(^{2+}\) Transient

The irregularities in the leading edge of the Ca\(^{2+}\) transients in the MI myocytes implies that there are localized regions where synchronized Ca\(^{2+}\) release either does not occur or occurs with a substantial delay. This phenomenon could be most easily explained by focal areas where components of the Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) apparatus are missing or regional variations within a single myocyte in the function of Ca\(^{2+}\) release units (eg, local depletion of SR Ca\(^{2+}\) content, desensitization of ryanodine receptors, abnormal ryanodine receptor gating, or delayed recovery from inactivation of L-type Ca\(^{2+}\) channels, SR Ca\(^{2+}\) release channels, or both).

The scalloped appearance of the early transient might result simply from localized reductions in the density of L-type Ca\(^{2+}\) channels. In support of this hypothesis, we found evidence of a decrease in whole-cell \(I_{\text{Ca}}\) density in myocytes from infarcted hearts.\(^12\) The beginning of the Ca\(^{2+}\) transient in the MI myocytes has an appearance similar to that seen...
during the onset of Ca\(^{2+}\) channel blockade in normal myocytes. Thus, a decrease in the number of functional L-type channels remains a possible explanation for the silent areas seen during the early transient. Decreased \(I_{\text{Ca}}\) density is seen in some but not all models of heart failure. Therefore, a simple decrease in the number of channels is unlikely to be the fundamental cause of abnormal Ca\(^{2+}\) signaling in failing hearts. On the other hand, even with a full complement of L-type Ca\(^{2+}\) channels, dephosphorylation of some channels could theoretically produce a heterogeneous pattern of channel availability. Hence, localized abnormalities of L-type Ca\(^{2+}\) channel function may be important, even if differences in whole-cell current density are not seen. A loss of functional ryanodine receptors seems less likely, because the caffeine-induced SR Ca\(^{2+}\) releases (as estimated by the inward currents) were unchanged in MI myocytes.

**Figure 4.** A, Examples of \(I_{\text{Ca}}\) during voltage clamp steps to +10 and +60 mV in a control myocyte and an MI myocyte. Note smaller inward current at +10 mV but larger outward current at +60 mV in MI myocyte compared with control. B, Summary data for current-voltage relationships (control, \(n=40\); MI, \(n=35\)). Peak Ca\(^{2+}\) current density was reduced in MI myocytes versus controls (\(*P<0.05\)). C, Fast and slow time constants of current inactivation are shown with the corresponding amplitudes of each component shown in panel D. There were no differences in the kinetics of \(I_{\text{Ca}}\) inactivation between control and MI myocytes; however, the amplitude of the fast component was reduced in the MI myocytes.

**Figure 5.** Ca\(^{2+}\) transients recorded during voltage clamp steps to +10 and +60 mV in a control (left) and an MI myocyte (right). At +10 mV, the amplitude of the Ca\(^{2+}\) transient is reduced and the rate of rise of the transient is slower in the MI myocyte compared with the control. At +60 mV, the differences between the control and MI transients are still present but are less pronounced. Moreover, in the MI myocyte, the peak amplitude of the transient at +60 mV is only slightly lower than that at +10 mV. All measurements were made just before the repolarizing step (timing of clamp step shown below MI tracings).
total increase in cytosolic $[\text{Ca}^{2+}]$ as in control myocytes (no change in gain peak). However, it takes longer for this same increase to occur. An increase in volume of the diadic cleft would be expected to reduce the magnitude and rate of rise of $[\text{Ca}^{2+}]$ in the vicinity of a ryanodine receptor cluster when an L-type $\text{Ca}^{2+}$ channel was activated. This, in turn, could cause episodic failures to reach the threshold necessary for opening of the ryanodine receptor cluster.

Reductions in $\text{Ca}^{2+}$ content of the SR in MI myocytes could create areas that are incapable of producing sparks. Our finding of similar caffeine-induced currents in control and MI myocytes makes this explanation fairly unlikely unless our experimental conditions obscured true differences in SR content.

**Mechanisms Accounting for the Late Appearance of $\text{Ca}^{2+}$ Sparks**

The late-appearing sparks in the MI myocytes may be triggered events (ie, CICR). Normally, $I_{\text{Ca},L}$ peaks rapidly after depolarization and then undergoes both voltage- and...
Ca\(^{2+}\)-dependent inactivation.\(^{24}\) There is typically a small, noninactivating component of \(I_{Ca}\) that could theoretically serve as a trigger for later opening of ryanodine receptors. However, delayed SR releases occur very infrequently under normal circumstances, because ryanodine receptors activate during the earliest openings of the adjacent L-type channel and then quickly enter an inactivated state that is responsible for the termination of individual sparks.\(^{20,25}\) Furthermore, the driving force for Ca\(^{2+}\) entry through open channels during the plateau of the action potential is low. Release events initiated by late openings of L-type channels might occur if action potential duration was prolonged to an extent that allowed ryanodine receptors to recover from inactivation. We have previously reported that action potentials are prolonged in ventricular myocytes in the rabbit model of postinfarction heart failure.\(^{12}\) Thus, the late appearance of sparks that we have observed in the MI myocytes could result simply from a longer duration of time in which cells remain in a depolarized state.

Ca\(^{2+}\) entry via reverse-mode Na\(^{-}\)-Ca\(^{2+}\) exchange during the later portions of the action potential may directly or indirectly contribute to localized SR Ca\(^{2+}\) releases. In this animal model, Na\(^{-}\)-Ca\(^{2+}\) exchanger current density is increased in myocytes from the infarct border zone, action potentials are prolonged, and reverse mode Na\(^{-}\)-Ca\(^{2+}\) exchange influences the duration of contractions and SR Ca\(^{2+}\) content.\(^{12}\) In the present study, we found that the peak amplitude of Ca\(^{2+}\) transients at +60 mV tended to be enhanced relative to those at +10 mV in MI myocytes (Figures 5 and 6B). Moreover, outward current was more evident in the MI myocytes at positive potentials (Figure 4A). Thus, it seems likely that Ca\(^{2+}\) entry via Na\(^{-}\)-Ca\(^{2+}\) exchange occurs to a greater extent in the MI myocytes. Ca\(^{2+}\) entry via the Na\(^{-}\)-Ca\(^{2+}\) exchanger may have a significant influence on cellular Ca\(^{2+}\) cycling, particularly during the later portions of the action potential.\(^{28}\) Goldhaber et al\(^{27}\) have shown that simply decreasing Ca\(^{2+}\) extrusion via forward Na\(^{-}\)-Ca\(^{2+}\) exchange can dramatically increase the probability of spontaneous Ca\(^{2+}\) sparks in rat myocytes. They proposed that Na\(^{-}\)-Ca\(^{2+}\) exchange locally regulates the resting [Ca\(^{2+}\)] in the diadic cleft and thereby modulates the threshold for triggering Ca\(^{2+}\) sparks. Thus, enhanced reverse Na\(^{-}\)-Ca\(^{2+}\) exchange in MI myocytes may produce slow increases in cytosolic [Ca\(^{2+}\)] in regions where SR Ca\(^{2+}\) release does not occur, and, during the prolonged action potential plateau, reverse exchange may increase the probability that occasional openings of L-type channels will induce a local release event.

Late Ca\(^{2+}\) sparks may represent spontaneous openings of SR release units. Spontaneous Ca\(^{2+}\) sparks or waves typically occur in the setting of SR Ca\(^{2+}\) overload.\(^{28}\) We think that SR overload in the MI myocytes is unlikely, because we found no difference in SR Ca\(^{2+}\) content between MI and control myocytes and isoproterenol greatly reduced the number of late sparks in the MI myocytes. Because isoproterenol usually increases SR Ca\(^{2+}\) content, we would have expected isoproterenol to increase rather than decrease the late sparks if they were attributable to Ca\(^{2+}\) overload.

Finally, altered ryanodine receptor gating could theoretically produce a heterogeneous release pattern. Recently, Marx et al\(^{29}\) proposed that hyperphosphorylation of ryanodine receptors in failing hearts caused dissociation of the accessory protein FKBP 12.6 from the ryanodine receptor complex. They hypothesized that the dissociation of FKBP 12.6 causes increased sensitivity to Ca\(^{2+}\)-induced activation. Such a change might account for the erratic pattern of sparks seen in many of the MI myocytes. Furthermore, Marx et al\(^{26}\) previously reported that FKBP 12.6 is responsible for coupled gating between ryanodine receptors. Therefore, dissociation or loss of FKBP 12.6 might also cause variations in spark size at the same location.

**Mechanisms Possibly Accounting for the Improved Synchronization of Ca\(^{2+}\) Sparks After Isoproterenol Treatment**

A relative decrease in phosphorylation of L-type Ca\(^{2+}\) channels might cause an increased latency to opening of some channels or a different mode of channel gating that could produce poor synchronization of Ca\(^{2+}\) releases.\(^{24}\) Likewise, dephosphorylation of ryanodine receptors could reduce the sensitivity of the channels to activating Ca\(^{2+}\) and, thus, cause some channels to fail to reach the threshold for opening.\(^{31}\) The beneficial effects of isoproterenol could accrue by reversing either of these abnormalities. In addition, by increasing the total Ca\(^{2+}\) influx into a diadic junction, protein kinase A–mediated Ca\(^{2+}\) channel phosphorylation might overcome the limitation in excitation-contraction coupling caused by a physical expansion of the diadic cleft. Lastly, phosphorylation of phospholamban is likely to increase SR Ca\(^{2+}\) content. Increased SR content is known to increase the fractional release of Ca\(^{2+}\) for similarly sized Ca\(^{2+}\) currents.\(^{32}\) The finding of improved spark coordination after isoproterenol treatment does not seem to fit well with the hyperphosphorylation hypothesis put forth by Marx et al.\(^{29}\) However, it is certainly possible that protein kinase A activity may be compartmentalized within a cell and that various targets may be phosphorylated with different kinetics or affinities.

**Limitations**

The pseudoratio method for calculation of [Ca\(^{2+}\)], requires assumptions about the diastolic [Ca\(^{2+}\)] and, therefore, may produce errors in the true systolic [Ca\(^{2+}\)]. However, this method is widely accepted for use in confocal microscopy because of the advantages of fluo-3 as a Ca\(^{2+}\) indicator, and errors in the quantitation of [Ca\(^{2+}\)], if present, will not alter our main conclusions about the temporal and spatial abnormalities of Ca\(^{2+}\) sparks in MI myocytes.

**Conclusions**

These results show for the first time, to our knowledge, that the smaller size and slowed kinetics of Ca\(^{2+}\) transients in myocytes from diseased hearts may be attributable, at least in part, to reduced and dysynchronous production of Ca\(^{2+}\) sparks rather than a simple slowing of the decline in cytosolic [Ca\(^{2+}\)]. Because the same pathophysiological mechanism may affect both the rising and falling phases of the Ca\(^{2+}\) transient in diseased myocytes, it may not be appropriate to consider systolic and diastolic dysfunction as distinct disease processes.
Acknowledgments

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References


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Supplementary material:

**Supplementary Table 1 (for online use).** Cardiac chamber weights in control rabbits and rabbits with myocardial infarction (MI).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 12)</th>
<th>MI (n = 13)</th>
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<td>Atrial weight (g)</td>
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<td>2.44±0.18 *</td>
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<tr>
<td>RV weight (g)</td>
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<td>LV weight (g)</td>
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<td>Lung weight (g)</td>
<td>11.9±0.4</td>
<td>14.1±0.8 *</td>
</tr>
</tbody>
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Chamber weights were obtained after perfusion with collagenase. Rabbits were studied 3 weeks after myocardial infarction (MI) or sham surgery (Control). LV = left ventricular; RV = right ventricular. Data are mean±S.E.M. * p < 0.05 vs. control.
**Supplementary Table 2 (for online use).** Echocardiographic and hemodynamic measurements in control rabbits and rabbits with healed myocardial infarction (MI).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 12)</td>
<td>(n = 13)</td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>147±6</td>
<td>136±7</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>1.69±0.04</td>
<td>1.98±0.03 *</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>1.08±0.04</td>
<td>1.39±0.05 *</td>
</tr>
<tr>
<td>FS (%)</td>
<td>36.3±1.8</td>
<td>29.8±2.0 *</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>78±3</td>
<td>64±3 *</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>78.6±2.9</td>
<td>71.3±2.1</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.3±0.7</td>
<td>9.4±1.7 *</td>
</tr>
</tbody>
</table>

HR = heart rate; LVIDd = LV internal diastolic dimension; LVIDs = LV internal systolic dimension; FS = fractional shortening; CI = cardiac index; LVSP = LV systolic pressure; LVEDP = LV end-diastolic pressure. Data are mean±S.E.M. * p < 0.05 vs. control.
**Supplementary Figure (for online use).** Examples of sarcoplasmic reticulum (SR) $\text{Ca}^{2+}$ content measurement in a control (A) and an MI (B) myocyte. SR $\text{Ca}^{2+}$ content was estimated by integrating the inward current produced during caffeine exposure (see methods). Mean data are shown in C ($n = 11$ control myocytes, $n = 16$ MI myocytes; $p = \text{NS}$). Data are mean ± S.E.M.