β-Adrenergic Stimulation Synchronizes Intracellular Ca\textsuperscript{2+} Release During Excitation-Contraction Coupling in Cardiac Myocytes

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**Abstract**—To elucidate microscopic mechanisms underlying the modulation of cardiac excitation-contraction (EC) coupling by β-adrenergic receptor (β-AR) stimulation, we examined local Ca\textsuperscript{2+} release function, ie, Ca\textsuperscript{2+} spikes at individual transverse tubule–sarcoplasmic reticulum (T-SR) junctions, using confocal microscopy and our recently developed technique for release flux measurement. β-AR stimulation by norepinephrine plus an α\textsubscript{1}-adrenergic blocker, prazosin, increased the amplitude of SR Ca\textsuperscript{2+} release flux (J\textsubscript{SR}), its running integral (\(\int_{\text{J}_{\text{SR}}}\)), and L-type Ca\textsuperscript{2+} channel current (\(I_{\text{Ca}}\)), and it shifted their bell-shaped voltage dependence leftward by \(\approx 10\) mV, with the relative effects ranking \(I_{\text{Ca}} > J_{\text{SR}} > \int_{\text{J}_{\text{SR}}}\). Confocal imaging revealed that the bell-shaped voltage dependence of SR Ca\textsuperscript{2+} release is attributable to a graded recruitment of T-SR junctions as well as to changes in Ca\textsuperscript{2+} spike amplitudes. β-AR stimulation increased the fractional T-SR junctions that fired Ca\textsuperscript{2+} spikes and augmented Ca\textsuperscript{2+} spike amplitudes, without altering the SR Ca\textsuperscript{2+} load, suggesting that more release units were activated synchronously among and within T-SR junctions. Moreover, β-AR stimulation decreased the latency and temporal dispersion of Ca\textsuperscript{2+} spike occurrence at a given voltage, delivering most of the Ca\textsuperscript{2+} load at the onset of depolarization rather than spreading it out throughout depolarization. Because the synchrony of Ca\textsuperscript{2+} spikes affects Ca\textsuperscript{2+} delivery per unit of time to contractile myofilaments, and because the myofilaments display a steep Ca\textsuperscript{2+} dependence, our data suggest that synchronization of SR Ca\textsuperscript{2+} release represents a heretofore unappreciated mechanism of β-AR modulation of cardiac inotropy. (*Circ Res.* 2001;88:794-801.)

**Key Words:** excitation-contraction coupling ■ β-adrenergic receptor ■ L-type Ca\textsuperscript{2+} channel current ■ ryanodine receptors ■ heart cells

During cardiac excitation-contraction (EC) coupling, depolarization activates the voltage-gated L-type Ca\textsuperscript{2+} channels in the sarcoplasmic membrane encompassing the transverse (T) tubules. The ensuing Ca\textsuperscript{2+} influx triggers Ca\textsuperscript{2+} release from ryanodine receptors (RyRs) in the junctional sarcoplasmic reticulum (SR) via Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR).\(^1\) It is now widely accepted that Ca\textsuperscript{2+} sparks originating from a single RyR or a cluster of RyRs constitute elementary functional units of the SR Ca\textsuperscript{2+} release,\(^2\) and that spatial and temporal summation of discrete Ca\textsuperscript{2+} sparks gives rise to the global Ca\textsuperscript{2+} transient,\(^3,4\) which in turn, activates contractile proteins to generate a contraction. Increasing evidence also suggests that Ca\textsuperscript{2+} sparks are under the exquisite local control of single L-type Ca\textsuperscript{2+} channels.\(^3,5\) Thus, the microscopic properties of the RyR in response to L-type channel Ca\textsuperscript{2+} currents are important determinants of the efficiency of EC coupling.

β-Adrenergic receptor (β-AR) stimulation by the sympathetic neurotransmitter norepinephrine (NE) and the adrenal hormone epinephrine plays a pivotal role in modulation of cardiac function in response to stress or exercise. Major Ca\textsuperscript{2+} cycling proteins involved in EC coupling, including L-type Ca\textsuperscript{2+} channels,\(^6-8\) RyRs,\(^9,10\) and the SR Ca\textsuperscript{2+}-ATPase regulator phospholamban,\(^11,13\) are all known target proteins of β-AR–adenyl cyclase–cAMP–protein kinase A (PKA) signaling pathway. The resultant phosphorylation of these proteins increases the intracellular Ca\textsuperscript{2+} transient and contraction amplitudes, and it accelerates their kinetics. Despite extensive studies at the whole-cell level\(^14-18\) or on individual EC coupling components, such as L-type channels,\(^6-8\) and Ca\textsuperscript{2+} sparks,\(^17,19,20\) the exact microscopic mechanisms underlying β-AR–mediated modulation of EC coupling are still not well understood. For instance, although it has been well established that β-AR stimulation increases the functional availability of L-type Ca\textsuperscript{2+} channels\(^6,7\) and alters the gating pattern of the channel,\(^7,8\) little is known about how these affect the coupling between the L-type Ca\textsuperscript{2+} channels and RyRs.

By using a high-affinity Ca\textsuperscript{2+} indicator, Oregon green 488 BAPTA-5N (OG-5N), in conjunction with a slow Ca\textsuperscript{2+} buffer, EGTA, we have directly visualized local Ca\textsuperscript{2+} release...
at the single transverse tubule–sarcoplasmic reticulum (T-SR) junction level, ie, Ca\(^{2+}\) spikes, which may consist of one or a few Ca\(^{2+}\) sparks.\(^{21}\) In the present study, we intended to determine the \(\beta\)-AR–mediated effect on spatial-temporal properties of SR Ca\(^{2+}\) release and on the relationship between the triggering L-type Ca\(^{2+}\) channel current (I\(_{\text{Ca}}\)) and the SR Ca\(^{2+}\) release flux (J\(_{\text{SR}}\)). Using Ca\(^{2+}\) spikes and spatially averaged SR Ca\(^{2+}\) release fluxes (J\(_{\text{SR}}\)) as direct readouts of EC coupling, we have demonstrated microscopic mechanisms of \(\beta\)-AR modulation of excitation-induced Ca\(^{2+}\) release in cardiac myocytes. Specifically, we found that \(\beta\)-AR stimulation enhances the J\(_{\text{SR}}\) by recruiting more functional T-SR junctions, by enhancing Ca\(^{2+}\) spike amplitude, and perhaps most importantly, by synchronizing the occurrence of Ca\(^{2+}\) spikes after excitation.

Materials and Methods

**Confocal Ca\(^{2+}\) Imaging and Simultaneous Recording of I\(_{\text{Ca}}\)**

Cardiac ventricular myocytes were isolated from adult Sprague-Dawley rats by using a standard enzymatic technique.\(^{21}\) Confocal Ca\(^{2+}\) imaging microscopy and simultaneous recording of whole-cell I\(_{\text{Ca}}\) were achieved by using the techniques previously reported.\(^{21}\) Patch pipette–filling solution containing 4 mmol/L EGTA was made by mixing the following two solutions (3:2): an EGTA-free solution containing (in mmol/L) CsCl 120, MgCl\(_2\) 1.5, MgATP 5, NaCl 10, tetraethylammonium chloride 10, and HEPES 20 (pH 7.2 adjusted with CsOH); and a high-EGTA solution containing (in mmol/L): CsCl 100, MgCl\(_2\) 1.5, MgATP 5, NaCl 10, tetraethylammonium chloride 10, EGTA 10, CaCl\(_2\) 5, and HEPES 20 (pH 7.2 adjusted with CsOH). The Ca\(^{2+}\) indicator OG-5N hexapotassium salt (Molecular Probes) (1 mmol/L) was directly dissolved in the pipette solution. Tetrodotoxin (20 \(\mu\)mol/L) was added to avoid contamination of the sodium current.

**Optical Measurement of SR Ca\(^{2+}\) Release Function**

Global and localized SR Ca\(^{2+}\) release function were measured by using the OG-5N/EGTA method.\(^{21}\) Briefly, a slow, high-affinity, and nonfluorescent Ca\(^{2+}\) buffer, EGTA, was used to minimize the resident time of Ca\(^{2+}\) released into the cytosol, and a low-affinity, fast Ca\(^{2+}\) indicator, OG-5N, was chosen to optimize the detection of localized high [Ca\(^{2+}\)] in release site microdomains. Because of its slow kinetics for Ca\(^{2+}\) association, EGTA at the concentration used was not expected to disturb, to a significant extent, Ca\(^{2+}\) signaling between L-type channels and RyRs in the T-SR junctions. This, in fact, has been evidenced by the observations that intracellular dialysis of up to 10 mmol/L EGTA has little effect on the release-dependent inactivation of I\(_{\text{Ca}}\)\(^{2+}\) and that the J\(_{\text{SR}}\) determined by this method\(^{22}\) is comparable with that derived by mathematical models from conventional Ca\(^{2+}\) transients.\(^{23}\)

**Drug Delivery**

NE (10^{-6} \text{ mol/L}) plus a selective \(\alpha_1\)-adrenergic blocker, prazosin (10^{-6} \text{ mol/L}), or an L-type Ca\(^{2+}\) channel agonist, FPL64176 (FPL, 10^{-4} \text{ mol/L}), was locally delivered through a glass pipette positioned near the cell. In some experiments, caffeine (20 mmol/L, 1 second) was rapidly applied onto the cells by pressure-ejection through a pipette. In a subset of experiments, cells were loaded with fluo-3 AM (5 \(\mu\)mol/L for 15 minutes); the caffeine-releasable SR Ca\(^{2+}\) content was then assessed before and 4 minutes after \(\beta\)-AR stimulation in quiescent cells or electric field–stimulated (1.0 Hz) cells.

**Figure 1.** Measurement of local and global SR Ca\(^{2+}\) release function. A, Representative image of OG-5N/EGTA fluorescence acquired at \(-20\) mV in a rat ventricular myocyte. Left inset, Contrast-enhanced OG-5N staining at rest; bright bands correspond to Z line/T tubule regions. Discrete Ca\(^{2+}\) spikes ignited by membrane depolarization are localized to the center of Z line/T tubule regions (white lines). B, Spatially averaged OG-5N signal. The smooth line refers to the running integral of J\(_{\text{SR}}\) (\(\int J_{\text{SR}}\) dt); its steady-state level (\(\Delta F_{F_0}/F_0\)) reflects the total amount of released Ca\(^{2+}\). C, SR Ca\(^{2+}\) release function at the single T-SR junction level. Vertical dashed lines mark the beginning and end of the voltage pulse. A T-SR junction is considered to be active if its OG-5N signal exceeds a threshold set at \(\Delta F/F_0>0.2\) (top horizontal dotted lines). Open circles mark the initiation of Ca\(^{2+}\) release at individual T-SR junctions.

**Statistics**

Data were reported as mean±SEM. Student’s \(t\) test or a paired \(t\) test was applied, when appropriate, to determine statistical differences. Differences between voltage-dependent curves were assessed by using ANOVA for repeated measures. A \(P\) value less than 0.05 was considered statistically significant.

**Results**

**Measurements of Global and Single T-SR Functional Ca\(^{2+}\) Release Function**

In rat ventricular myocytes, the trigger Ca\(^{2+}\) signal, I\(_{\text{Ca}}\), was elicited by membrane depolarization under whole-cell voltage-clamp conditions, and the evoked Ca\(^{2+}\) release flux from the SR, J\(_{\text{SR}}\), was measured simultaneously with confocal microscopy by using the OG-5N/EGTA method\(^{21}\) (Figure 1A). The spatially averaged OG-5N fluorescence signal consists of two separable components: a spiky transient that is proportional to J\(_{\text{SR}}\) and a minor component that represents the running integral of J\(_{\text{SR}}\) (\(\int J_{\text{SR}}\) dt) (Figure 1B). For simplicity, a small contribution caused by the I\(_{\text{Ca}}\) influx (~6% of the peak Ca\(^{2+}\) release from the SR)\(^{21}\) was neglected in the data presentation. Linescan confocal imaging visualized discrete SR Ca\(^{2+}\) release events (Ca\(^{2+}\) spikes) at the Z line/T tubule regions that were identified by the OG-5N–stained bands separated by approximately 1.8 \(\mu\)m (Figure 1A). It is noteworthy that the voltage pulse did not always elicit a Ca\(^{2+}\) spike from a T-SR junction. At T-SR junctions that fired one or more Ca\(^{2+}\) spikes (local \(\Delta F/F_0>0.2\)), the visualization of Ca\(^{2+}\) release flux in space and time makes it possible to measure the latency and peak amplitude of Ca\(^{2+}\) spikes (Figure 1C) and to determine how these are affected by \(\beta\)-AR stimulation (see below).

**\(\beta\)-AR Stimulation Enhances SR Ca\(^{2+}\) Release Flux**

Figure 2A illustrates typical simultaneous recordings of spatially averaged J\(_{\text{SR}}\), its running integral \(\int J_{\text{SR}}\), and the
corresponding trigger $I_{Ca}$. Unlike conventional Ca\textsuperscript{2+} transients that last typically for $\approx 200$ ms, the $J_{SR}$ is rather brief ($\approx 30$ ms at 0 mV). After $\beta$-AR stimulation by NE in the presence of the $\alpha$1-adrenergic blocker, prazosin, both $I_{Ca}$ and $J_{SR}$ are markedly enhanced (Figure 2A). Figures 2B through 2D plot the average voltage dependence of peak $J_{SR}$, $\int J_{SR}$, and $I_{Ca}$ in the presence or absence of NE. Under control conditions, the peak $J_{SR}$ and $\int J_{SR}$ exhibit a bell-shaped voltage dependence, as does the trigger $I_{Ca}$. In the presence of the $\beta$-AR agonist, all three curves are shifted upward and leftward by $\approx 10$ mV, whereas the gross bell-shaped voltage dependence is retained. Interestingly, the three parameters are not equally affected by $\beta$-AR stimulation, with a prominent effect on peak $I_{Ca}$, a modest effect on $J_{SR}$, and a rather small effect on $\int J_{SR}$ (Figure 2). For example, at 0 mV, NE increased $I_{Ca}$, $J_{SR}$, and $\int J_{SR}$ by 138%, 67%, and 25%, respectively.

**Figure 2.** Effects of $\beta$-AR stimulation on global SR Ca\textsuperscript{2+} release flux. A, $\beta$-AR-mediated effects on spatially averaged $J_{SR}$, its running integral $\int J_{SR}$, and the corresponding trigger $I_{Ca}$ in a representative myocyte. The cell was depolarized from a holding potential of −60 mV to between −40 and 40 mV in the absence (left) or presence (right) of $\beta$-AR stimulation by NE (10\textsuperscript{−6} mol/L) plus an $\alpha$1-adrenergic blocker prazosin (10\textsuperscript{−6} mol/L). B, C, and D show, respectively, average effects of $\beta$-AR stimulation on $J_{SR}$, $\int J_{SR}$, and $I_{Ca}$. The rank order for the $\beta$-AR-stimulated fold-increment is $I_{Ca} > J_{SR} > \int J_{SR}$. n=9 cells in the control group (○), n=7 cells in the NE+Pra group (●). $P<0.005$ for $\beta$-AR effects on $I_{Ca}$ and $J_{SR}$, $P<0.05$ for $\int J_{SR}$.

**β-Adrenergic Modulation of Frequency and Intensity of Ca\textsuperscript{2+} Spikes**

To elucidate microscopic mechanisms underlying $\beta$-AR-mediated modulation of cardiac SR Ca\textsuperscript{2+} release, we examined the spatial and temporal recruitment of Ca\textsuperscript{2+} spikes as well as properties of individual Ca\textsuperscript{2+} spikes in response to $\beta$-AR stimulation. As shown in Figure 3A, Ca\textsuperscript{2+} spikes are discernible at all test voltages, even when the SR release is at its maximum (eg, at 0 mV). Figures 3B and 3C illustrate, respectively, a profound bell-shaped voltage dependence for the likelihood of Ca\textsuperscript{2+} spike occurrence at a given T tubule, and a more shallow voltage dependence for the amplitude of Ca\textsuperscript{2+} spikes under control conditions. Hence, the characteristic bell-shaped voltage dependence of Ca\textsuperscript{2+} transients in cardiac myocytes is mostly attributable to a smoothly graded recruitment of T-SR junctions and, to a lesser extent, to changes in local Ca\textsuperscript{2+} release flux at individual T-SR release sites. The graded nature of Ca\textsuperscript{2+} spike amplitude, however, does suggest that a single T-SR junction is capable of firing a varying number of Ca\textsuperscript{2+} sparks. In this regard, Figure 3A
shows that multiple Ca^{2+} spikes can be activated in tandem from a given T-SR site (marked by the arrows) during a 200-ms depolarizing pulse.

Based on this perspective of excitation-induced Ca^{2+} release, we hypothesized that β-AR–mediated enhancement of macroscopic SR Ca^{2+} release flux might be accounted for by the increased likelihood of Ca^{2+} spike occurrence, the intensity of individual Ca^{2+} spikes, or both. Figure 3B shows that, after β-AR stimulation, the curve for fraction of active T-SR junctions (e) is shifted upward and exhibits a plateau with e ≈ 95% between −30 and 0 mV. Figure 3C shows that the voltage dependence of the Ca^{2+} spike amplitude is also shifted upward and leftward by β-AR stimulation. Nevertheless, the duration of Ca^{2+} spikes at 50% amplitude (t_{50}), which reflects local Ca^{2+} release time, was unaltered by NE treatment (at 0 mV, t_{50} = 18.05 ± 0.79 ms, n = 9 cells, versus 18.57 ± 0.66 ms, n = 7 cells, in the absence or presence of NE, respectively).

Thus, both variations in fractional T-SR activation (synchronization among junctions) and spike amplitudes (synchronization of release units within a junction) contribute to the voltage-dependent gradation of SR Ca^{2+} release in the presence of β-AR stimulation.

**β-AR Stimulation Reduces Latency and Temporal Dispersion of the Occurrence of Ca^{2+} Spikes**

Next, we determined whether β-AR stimulation alters the temporal profile of SR Ca^{2+} release among T-SR junctions. The images in Figure 3A indicate that Ca^{2+} spike occurrence is, indeed, substantially more synchronous among T-SR junctions in the presence of NE compared with that in the control cells. To quantitate the synchrony of Ca^{2+} spikes, we measured the latency of Ca^{2+} spike occurrence from the onset of the voltage pulse. The ensembles resulting from a large number of Ca^{2+} spikes at 4 representative voltages (−40, −30, 0, and 20 mV) are shown in Figure 4A. In the absence of NE and at low voltages (eg, −40 or −30 mV), Ca^{2+} spikes were scattered over the entire 200-ms pulse. In contrast, at 0 and 20 mV, the vast majority of Ca^{2+} spikes were concentrated in the first 30 ms. To quantify the temporal pattern of spike occurrence, the mean values of spike latency (L) are plotted against membrane voltage in Figure 4C, and the mean deviations of spike latency (L_{md}), which is inversely related to the temporal togetherness of Ca^{2+} spikes, are shown in Figure 4D. Both display a curved L-shaped voltage dependence. β-AR stimulation by NE not only shortened the latency for spike activation (Figure 4C), but it also reduced the temporal dispersion of Ca^{2+} spikes, particularly at the negative voltages...
Figure 5. Effect of β-AR stimulation on SR Ca\(^{2+}\) content. Cells were voltage-clamped at −60 mV, and a caffeine puff (20 mmol/L, 1 second) was delivered by pressure-ejection through a pipette located ~100 μm away from the cell. A, Line-scan image and line plot showing caffeine-induced OG-5N signal. The smooth line depicts the integral of released Ca\(^{2+}\), ∆F/F\(_0\). B, Average data on caffeine-induced total SR Ca\(^{2+}\) release before and after β-AR stimulation. n = 5 cells. C, Caffeine-induced SR Ca\(^{2+}\) release (∆F/F\(_0\)) in a fluo-3-loaded cell in the absence of EGTA. D, Average data in quiescent (left) and field-stimulated (1.0 Hz, right) cells before and 4 minutes after NE perfusion.

(Figure 4D). Thus, in addition to modulating Ca\(^{2+}\) spike recruitment and augmenting Ca\(^{2+}\) release at a given T-SR junction, β-AR stimulation appears to front-load the system, delivering most of the Ca\(^{2+}\) at the onset of depolarization rather than spreading it out throughout depolarization.

The time courses of average I\(_{\text{Ca}}\), in the same cells are shown in Figure 4B. It is noteworthy that β-AR signaling not only increases the amplitude, but it also hastens the decay of I\(_{\text{Ca}}\), at negative potentials in particular (at −30 mV, τ\(_{\text{rel}}\) 10.25 and 8.61 ms; at 0 mV, τ\(_{\text{rel}}\) 11.96 and 9.34 ms, for control and NE, respectively). Because SR Ca\(^{2+}\) release is under tight local control by Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels,\(^3\)–\(^5\) the synchronisation of I\(_{\text{Ca}}\) explains, at least in part, the β-AR-mediated synchronization of SR Ca\(^{2+}\) release.

**Effect of β-AR Stimulation on SR Ca\(^{2+}\) Content**

The β-AR–stimulated increase in SR Ca\(^{2+}\) release might also reflect an increase in SR Ca\(^{2+}\) content. To test this possibility, we examined the possible effect of β-AR stimulation on the SR Ca\(^{2+}\) store. Figure 5A shows that, when the cell membrane potential was held at −60 mV, a pulse of caffeine (20 mmol/L, 1 second) applied by pressure-ejection rapidly empties the SR Ca\(^{2+}\) store; the I\(_{\text{Ca}}\) indexed by the ∆F/F\(_0\) at the steady state is, on average, 2-fold greater than the depolarization-elicited release at 0 mV (Figures 2C and 5B). β-AR stimulation by NE did not significantly alter the caffeine-releasable Ca\(^{2+}\) store under our experimental conditions (Figure 5B).

However, previous studies have shown that β-AR stimulation increases SR Ca\(^{2+}\) load.\(^6\)–\(^8\) This discrepancy might be attributable to the inclusion of EGTA in our experiments. Alternatively, it could be the result of different stimulation/conditioning protocols used, because in the present study cells were electrically stimulated at a low frequency (10- to 15-second intervals), whereas in most previous studies cells were paced at higher frequencies or primed by conditioning pulses. To address these issues, we performed additional experiments to assess SR Ca\(^{2+}\) content in fluo-3–loaded cells in the absence of EGTA (Figure 5C). We found that NE increases SR Ca\(^{2+}\) in electrically stimulated (1.0 Hz) cells, but it has no significant effect in quiescent cells (Figure 5D). The latter is in agreement with our data obtained in EGTA-loaded, infrequently stimulated cells, and it is consistent with the observation that in saponin-permeabilized ventricular trabeculae the SR Ca\(^{2+}\) content is unchanged after isoproterenol stimulation.\(^24\) These data thus reconcile our observations with the previous reports.\(^6\)–\(^8\) Moreover, the present results suggest that sarcolemmal Ca\(^{2+}\) influx elicited by action potentials plays an important role in loading the SR during β-adrenergic stimulation.

**Reduction of the Gain of EC Coupling by β-AR Signaling**

The ability of the SR to amplify the trigger Ca\(^{2+}\) influx has been characterized by the gain function of EC coupling, defined as the ratio of peak J\(_{\text{SR}}\) over the corresponding I\(_{\text{Ca}}\). As shown in Figure 6, the SR gain function decreased monotonically with increasing voltage, in agreement with previous observations.\(^6\)–\(^8\) Surprisingly, the gain of EC coupling was significantly reduced, rather than enhanced, after β-AR stimulation by NE, despite a net increment of SR Ca\(^{2+}\) release. One possible explanation for this unexpected β-AR–induced reduction in the gain of EC coupling may reside in local saturation of the trigger Ca\(^{2+}\) signal, caused by the increased L-type channel activity during β-AR stimulation.\(^6\)–\(^8\) To determine the possible effect of elevated L-type channel activity on the gain function, we used FPL (10\(^{-5}\) mol/L), a specific L-type channel agonist, to increase channel-open probability,\(^22\)–\(^26\) without directly affecting other β-AR target proteins involved in the EC coupling cascade. Figure 7 shows that FPL rapidly induced a 4.2-fold increment in peak I\(_{\text{Ca}}\), but only a 0.4-fold enhancement of peak J\(_{\text{SR}}\), resulting in a markedly reduced gain of EC coupling. This supports the notion that local saturation of the trigger Ca\(^{2+}\) signal could explain, at least in part, the β-AR–induced reduction in SR gain function.
Discussion

There are three major findings of the present study. First, using Ca\textsuperscript{2+} spikes and J\textsubscript{SR} as the immediate readouts of I\textsubscript{Ca}-elicited SR Ca\textsuperscript{2+} release, we showed that \( \beta \)-AR stimulation by NE increases the functional availability of T-SR junctions (\\( e \)'), ie, the fraction of T-SR junctions that fire Ca\textsuperscript{2+} spikes during a 200-ms depolarization pulse. Second, we demonstrated that \( \beta \)-AR stimulation increases the likelihood of RyRs firing synchronously at a given T-SR junction, augmenting Ca\textsuperscript{2+} spike amplitude without affecting its duration. Third, we identified a heretofore unappreciated mode of \( \beta \)-AR modulation of EC coupling, ie, synchronization of SR Ca\textsuperscript{2+} release to the onset of depolarization at a given voltage. Specifically, \( \beta \)-AR stimulation abbreviates the latency of Ca\textsuperscript{2+} spikes and markedly reduces the temporal dispersion of spike events among T-SR junctions. The \( \beta \)-AR–mediated effects on Ca\textsuperscript{2+} spikes provide a subcellular perspective for the well documented \( \beta \)-AR modulation of the whole-cell Ca\textsuperscript{2+} transients.\textsuperscript{14-18}

\( \beta \)-AR–Mediated Synchronization of SR Ca\textsuperscript{2+} Release and Its Physiological Significance

As we reported previously, synchronization of intracellular Ca\textsuperscript{2+} release provides a novel mechanism to enhance cardiac inotropic performance.\textsuperscript{21} The unusually steep Ca\textsuperscript{2+}–dependence of cardiac muscle force generation (Hill coefficient 4.87 as in Gao et al\textsuperscript{27}) implies that any slight change in Ca\textsuperscript{2+} delivery per unit of time would be amplified in the output of force generation and cell shortening. Because SR release occurs in a discrete manner, spatial synchronization of Ca\textsuperscript{2+} release also plays an important role. In an extreme scenario, it has been shown that solitary Ca\textsuperscript{2+} sparks fail to produce any local movement.\textsuperscript{28} For multiple Ca\textsuperscript{2+} sparks not overlapping in space and time, they may also be ineffective in initiating local or global cell shortening. Indeed, at \(-30\) mV, when spikes are dyscoordinated in space and time (\\( \epsilon =0.57, L_{\text{md}}=37\) ms, Figure 4), cell shortening is barely detectable.\textsuperscript{29,30} Unsynchronized Ca\textsuperscript{2+} release at more negative voltages is totally futile in terms of activating cell shortening. At \(-10\) mV, however, when a similar amount of \( f_{\text{SR}} \) is discharged in a more uniform (\\( \epsilon =0.87 \)) and synchronous (\\( L_{\text{md}}=14\) ms) manner (Figure 4), cell contraction amplitude reaches its maximum, as shown in previous studies.\textsuperscript{29,30} Finally, asynchronous SR Ca\textsuperscript{2+} release might create microdomains of refractoriness, hindering the development of a forceful contraction. A slow and inhomogeneous Ca\textsuperscript{2+} release might also be counterbalanced by Ca\textsuperscript{2+} clearance from the cytosol as a result of SR Ca\textsuperscript{2+} reserquestration and local Ca\textsuperscript{2+} buffering, limiting the peak Ca\textsuperscript{2+} and peak contraction attained.

The present study demonstrated, for the first time, that \( \beta \)-AR stimulation provides a physiological mechanism to modulate the degree of synchronization of SR Ca\textsuperscript{2+} release (Figures 3 and 4). By enhancing the synchrony of Ca\textsuperscript{2+} release, \( \beta \)-AR stimulation may facilitate the highly cooperative action of the released Ca\textsuperscript{2+} on the contractile apparatus, eliciting a greater contraction for a given Ca\textsuperscript{2+} release. Thus, a given level of force development would require less SR Ca\textsuperscript{2+} cycling and its associated energy utilization.

Alterations in the synchrony of SR Ca\textsuperscript{2+} release and its physiological modulation by \( \beta \)-AR stimulation may have important pathophysiological relevance. In failing hearts of humans and animal models, diminished Ca\textsuperscript{2+} transient and contraction are often characterized by sluggish onset and relaxation.\textsuperscript{19,31,32} In this regard, it has been recently reported that dysynchronous Ca\textsuperscript{2+} sparks are elicited by action potentials in myocytes from infarcted canine hearts.\textsuperscript{33} Furthermore, the ability of \( \beta \)-AR stimulation to restore the synchrony of SR Ca\textsuperscript{2+} release may also be diminished, as a result of the receptor downregulation and desensitization.\textsuperscript{34} Thus, the possible contribution of dyssynchronization of intracellular Ca\textsuperscript{2+} release to cardiac contractile dysfunction in the failing heart merits future investigation.

Possible Mechanisms Underlying \( \beta \)-AR–Mediated Synchronization of SR Ca\textsuperscript{2+} Release

One possible mechanism for \( \beta \)-AR–mediated synchronization of SR Ca\textsuperscript{2+} release is related to \( \beta \)-AR modulation of the trigger I\textsubscript{Ca}. At the single-channel level, \( \beta \)-AR modulation increases the functional availability of the L-type channel,\textsuperscript{6} redistributes the channel from mode 0 gating (characterized by infrequent brief openings) toward mode 1 (bursts of brief openings) and mode 2 (very long-lasting openings) gating, without changing I\textsubscript{Ca} amplitude.\textsuperscript{7,8} At the whole-cell level, these effects translate into an increased I\textsubscript{Ca} amplitude and accelerated activation and inactivation (Figure 4B). Interestingly, the time course of J\textsubscript{SR} essentially mirrors the waveform of I\textsubscript{Ca}, suggesting that the change in I\textsubscript{Ca} kinetics largely accounts for \( \beta \)-AR–induced synchronization of Ca\textsuperscript{2+} spikes.

The second possible mechanism may be related to a use-dependent inactivation of RyRs in intact cardiac myocytes.\textsuperscript{35} During a single pulse, unfired RyRs are expected to be exhausted in a time-dependent manner. In response to \( \beta \)-AR stimulation, increased trigger I\textsubscript{Ca} would enhance the early SR Ca\textsuperscript{2+} release, which in turn, suppresses the late occurrence of Ca\textsuperscript{2+} spikes, reducing both L and L\textsubscript{md} of Ca\textsuperscript{2+} spikes. It is noteworthy that RyR inactivation per se may not be altered by \( \beta \)-AR stimulation, because the duration of individual Ca\textsuperscript{2+} spikes (\( \approx 18 \) ms) remains constant in the
absence and presence of NE. The recovery of RyRs from inactivation, assessed by restitution of J_{SR}, is also unaffected by β-AR stimulation by isoprotrenol.36

In principle, an increase in CICR sensitivity to I_{cL} would also promote an early activation of Ca^{2+} spikes and a leftward shift of the spike latency histogram, which would contribute to the synchronization effect. However, it remains controversial whether and how β-AR– or PKA-dependent phosphorylation of RyRs modulates the gating properties of the Ca^{2+} release channel in vitro and in vivo.10,17,20,37

**β-AR Modulation of the Coupling Efficiency Between L-Type Channels and RyRs**

Unexpectedly, we documented a reduction of the gain of EC coupling in response to β-AR stimulation (Figure 6). This observation is in contrast to previous observations that a small I_{cL} elicits a maximal Ca^{2+} transient in isoprotrenol-stimulated cells.6 This discrepancy may be explained by different experimental conditions. In the present study, a train of conditioning pulses (300 ms, 0 mV) was used to load the SR, resulting in an essentially all-or-none behavior. In the present experimental setting, however, the SR Ca^{2+} load is unaltered by NE, and the inclusion of EGTA makes it possible to dissect the J_{SR} directly triggered by I_{cL}, and to examine its response to β-AR stimulation.

Because cardiac RyRs are under tight local control of single L-type Ca^{2+} channels, a subtle change in microscopic properties of the L-type channel may have profound consequences on the efficiency of EC coupling. The increase in L-type channel availability and the shift of the channel gating to high-activity mode by β-AR stimulation6–8 would increase the cumulative L-type channel-open duration in a given T-SR junction, augmenting Ca^{2+} release properties of the Ca^{2+} channel in vitro and in vivo.10,17,20,37

In summary, the present study demonstrates that β-AR stimulation in cardiac myocytes synchronizes SR Ca^{2+} release among T-SR junctions, reducing the latency and the temporal dispersion of Ca^{2+} spike occurrence. β-AR stimulation also promotes synchronous activation of more release units within a given T-SR junction, augmenting Ca^{2+} spike amplitude without altering the SR Ca^{2+} load or spike duration. Both contribute to the increase in J_{SR} amplitude. The more synchronous delivery of Ca^{2+} to myofilaments in the presence of β-AR stimulation likely facilitates the cooperative interaction of Ca^{2+} to activate contraction. Thus, synchronization of intracellular Ca^{2+} release constitutes a newly discovered mechanism underlying β-adrenergic modulation of cardiac SR Ca^{2+} release and EC coupling.

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β-Adrenergic Stimulation Synchronizes Intracellular Ca\(^{2+}\) Release During Excitation-Contraction Coupling in Cardiac Myocytes
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