Alternans of cardiac calcium cycling in a cluster of ryanodine receptors: a simulation study


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Alternans of cardiac calcium cycling in a cluster of ryanodine receptors: a simulation study. Am J Physiol Heart Circ Physiol 295: H598–H609, 2008. First published May 30, 2008; doi:10.1152/ajpheart.01086.2007—Mechanical alternans in cardiac muscle is associated with intracellular Ca2+ alternans. Mechanisms underlying intracellular Ca2+ alternans are unclear. In previous experimental studies, we produced alternans of systolic Ca2+ under voltage clamp, either by partially inhibiting the Ca2+ release mechanism, or by applying small depolarizing pulses. In each case, alternans relied on propagating waves of Ca2+ release. The aim of this study is to investigate by computer modeling how alternans of systolic Ca2+ is produced. A mathematical model of a cardiac cell with 75 coupled elements is developed, with each element contains L-type Ca2+ current, a subspace into which Ca release takes place, a cytoplasmic space, sarcoplasmic reticulum (SR) release channels [ryanodine receptor (RyR)], and uptake sites (SERCA). Interelement coupling is via Ca2+ diffusion between neighboring subspaces via cytoplasmic spaces and network SR spaces. Small depolarizing pulses were simulated by step changes of cell membrane potential (20 mV) with random block of L-type channels. Partial inhibition of the release mechanism is mimicked by applying a reduction of RyR open probability in response to full stimulation by L-type channels. In both cases, systolic alternans follow, consistent with our experimental observations, being generated by propagating waves of Ca2+ release and sustained through alternation of SR Ca2+ content. This study provides novel and fundamental insights to understand mechanisms that may underlie intracellular Ca2+ alternans without the need for refractoriness of L-type Ca or RyR channels under rapid pacing.

sarcoplasmic reticulum; computer modeling; diffusion; computer model

UP TO 50% OF DEATHS SEEN in heart failure are sudden and likely, therefore, to be due to cardiac arrhythmias. In heart failure, up to 40% of patients exhibit a phenomenon known as mechanical alternans (19), when the force of contraction of the heart alternates between strong and weak. A related phenomenon is microvolt T-wave alternans, detectable in the ECG (1) and considered to be especially arrhythmogenic. Many cardiac arrhythmias are thought to be due to irregularity of Ca2+ handling (11, 30, 36) by the sarcoplasmic reticulum (SR), the main source of Ca2+ for cardiac contraction. Systolic Ca2+ release occurs via Ca2+-induced Ca2+ release (CICR), triggered by Ca2+ entry on L-type Ca2+ channels (3). In mechanical alternans, large and small contractions follow each other (10, 13, 27) due to alternation of systolic Ca2+ (21, 40). In isolated cardiac cells, alternans of systolic Ca2+ is associated with alternans of cell shortening (16). Systolic Ca alternans are also associated with alternans of action potential duration (6, 22, 31) and alternans of the T-wave of the ECG (31).

Changes in amplitude and timing of Ca release during systole can influence the action potential shape by acting on Ca-sensitive currents, e.g., Na/Ca exchange (NCX) causing ECG abnormalities and the arrhythmogenicity of systolic Ca alternans.

Previous work in our laboratory has focused on intracellular Ca2+ concentration ([Ca2+]i) alternans and suggests that SR Ca content fluctuation is key to cardiac alternans (7). We found that, in rat ventricular myocytes, small (20 mV) depolarizing pulses produced alternans of the amplitude of the Ca2+ transient. Confocal measurements of [Ca2+]i showed the larger transients involved waves of CICR. In addition, SR Ca2+ content alternated in phase with the alternans of Ca2+ transient amplitude. During alternans of systolic release, we found that the relationship between Ca2+ transient amplitude and SR Ca2+ content was extremely steep. We attributed this steeper relationship to the dependence on Ca wave propagation, as this requires a “threshold” SR Ca2+ content (8).

The work presented here attempts to build a theoretical framework to describe the observations of Ca2+ release profiles measured by confocal microscopy and to investigate detailed mechanisms underlying the emergence of Ca2+ alternans. The simulation results support conclusions from experiments that alternans can arise due to propagation of waves of Ca2+ release, and that this results from a large increase in the gain of the feedback controlling SR Ca2+ content. This study provides a novel and fundamental insight into a mechanism underlying the genesis of systolic Ca2+ alternans that does not require refractoriness of L-type Ca or ryanodine receptor (RyR) channel.

METHODS

Our model consists of a cluster of coupled RyRs for a cardiac cell (see Fig. 1A). The cell has a length of 150 μm, which is discretized into 75 units along the longitudinal direction. Each unit encompasses 2 μm in the longitudinal dimension, and the entire cell depth and height in other dimensions. The spatial resolution of 2 μm is similar to the longitudinal distance between adjacent Ca2+ release units of cardiac cells (1.94 ± 0.31 μm) (5) and is fine enough to produce stable numerical solutions for Ca2+ waves in the model. Each element has a cluster of unitary voltage-gated L-type Ca2+ channels (contributing to global L-type Ca2+ channel current in each element; hereafter referred to as L-type Ca2+ channel), a subspace under the sarco-

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lemma, a cytoplasmic space, and a cluster of SR RyR channels (contributing global SR RyR channel release in each element; hereafter referred to as RyR channel) (see Fig. 1B). For each element, mathematical equations (see APPENDIX) were developed to model Ca\(^{2+}\) cycling. Inter-element coupling is via Ca\(^{2+}\) diffusion from subspaces to cytoplasmic spaces and via network SR spaces. In simulations, we have taken two approaches: 100-ms depolarizing pulses at 1 Hz from a holding potential of \(-40\) mV were used to activate L-type channels in each element. In the first approach, the depolarizing pulse was from \(-40\) to \(0\) mV, and we partially inhibited the Ca\(^{2+}\) release mechanism by increasing the threshold of RyR Ca\(^{2+}\) release to mimic the decreased sensitivity of RyR by tetracaine, as suggested by a previous study (15). In the second approach, the depolarizing pulse was small, from \(-40\) to \(-20\) mV. To simulate the decreased global L-type Ca\(^{2+}\) current in the cell in response to such a small stimulus pulse (7), the L-type Ca\(^{2+}\) current was blocked (setting to zero) in some elements randomly chosen from the cell, but remained the same as in the control for the non-blocked elements. Thus Ca\(^{2+}\) release was activated at only a few elements, with L-type Ca\(^{2+}\) current being fully activated. In simulations, the population of blocked elements varies (say 40 out of 75 elements) to produce different levels of L-type Ca\(^{2+}\) current amplitude reduction in the cell.

**Development of equations: table and choices of parameters.** We assume the global L-type Ca channel current (produced by a cluster of
unitary L-type channels in each element) and intracellular Ca\(^{2+}\) cycling of RyRs have the same kinetics for all elements and are described by whole cell equations similar to the approach used in the study of Shiferaw and Karma (34). The equations of Ca\(^{2+}\) cycling were modified from the model developed by Kurata et al. (20) with parameters and equations derived from experimental data of ventricular myocytes and used in the Luo-Rudy (26) model of guinea pig ventricular myocytes. In the model, some parameters, such as the volumes of cell, subspace, cytoplasm, network SR, and junctional SR, were adjusted (3) to fit the rat ventricular cell in our experiments. Despite being simple, the model captures most general features of cardiac Ca cycling necessary for CICR wave propagation, which enables us to unravel the major determinants responsible for systolic Ca\(^{2+}\) alternans, i.e., propagation of CICR waves and steep relationship between RyR release and the SR Ca content. Detailed glossary and equations are given in the APPENDIX.

The cardiac myocyte is a spatially extended object, within which Ca\(^{2+}\) diffuses. Our previous experimental results suggest Ca\(^{2+}\) diffusion is crucial during systolic Ca\(^{2+}\) alternans (7, 9). Spatially extended models have been developed to study Ca\(^{2+}\) sparks and waves (38). To simulate Ca\(^{2+}\) wave propagation and Ca\(^{2+}\) release alternans, we developed a more detailed model of Ca\(^{2+}\) spatial diffusion. It includes Ca\(^{2+}\) diffusion from the subspaces to myoplasmic spaces and between network SR spaces of neighboring elements. The diffusion parameters were chosen in such a way that the propagation of CICR waves matches our experimental data; these parameters are close to those used in previous studies (14, 15, 17, 20, 23, 26, 28, 32, 34, 35, 37, 38). Detailed comparisons of model parameter values with those of literature-based and other models are given in Table 1.

### Table 1. Comparison of parameters used in the model with experimental data and other models

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model Value</th>
<th>Literature-Based or Other Model Values</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(t_{\text{Ca, diff}}) (time constant for Ca diffusion from subspace to cytoplasmic space)</td>
<td>0.04 ms</td>
<td>0.04 ms</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05–0.6 ms</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04 ms</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0007 ms</td>
<td>32</td>
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<tr>
<td></td>
<td></td>
<td>0.0007 ms</td>
<td>38</td>
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<td></td>
<td></td>
<td>0.005 ms</td>
<td>14</td>
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<td></td>
<td></td>
<td>2 ms</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.7 ms</td>
<td>28</td>
</tr>
<tr>
<td>(t_{\text{Ca, diff}}) (time constant for Ca diffusion between neighboring cytoplasmic spaces)</td>
<td>4 ms</td>
<td>5 ms</td>
<td>34</td>
</tr>
<tr>
<td>(t_{\text{Ca SD, diff}}) (time constant for Ca diffusion between neighboring network SR spaces)</td>
<td>600 ms</td>
<td>100 ms</td>
<td>34</td>
</tr>
<tr>
<td>Estimated Ca diffusion coefficient in the cytoplasmic space</td>
<td>(10.0 \times 10^{-6}) cm(^2)/s</td>
<td>(1.0 \times 10^{-6}) cm(^2)/s</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.0 \times 10^{-6}) cm(^2)/s</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.0 \times 10^{-7}) to (7.9 \times 10^{-6}) cm(^2)/s</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5 \times 10^{-6}) to (3.0 \times 10^{-6}) cm(^2)/s</td>
<td>34</td>
</tr>
<tr>
<td>Measured Ca propagation velocity (t_{\sigma}) (time constant for Ca diffusion from network SR to junctional SR)</td>
<td>0.212 mm/s</td>
<td>0.05–15 mm/s</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>1–100 ms</td>
<td>35</td>
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<td></td>
<td></td>
<td>50 ms</td>
<td>34</td>
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<tr>
<td></td>
<td></td>
<td>60 ms</td>
<td>20</td>
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<tr>
<td></td>
<td></td>
<td>180 ms</td>
<td>26</td>
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<td></td>
<td></td>
<td>34.48 ms</td>
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<td></td>
<td></td>
<td>10 ms</td>
<td>38</td>
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<td></td>
<td></td>
<td>3 ms</td>
<td>14</td>
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<td></td>
<td></td>
<td>0.5747 ms</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.005 ms</td>
<td>32</td>
</tr>
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SR, sarcoplasmic reticulum.

**RESULTS**

**Spatially uniform model.** Several models of Ca handling in cardiac muscle have been shown to produce systolic Ca alternans (18, 35, 37). Typically, these models lack a spatial dimension, and alternans is produced by raising the pacing frequency. We thought it important to show that our model can also produce similar behavior. Thus the traces in Fig. 2A show simulations run at 1 and 2 Hz in a single element of our model, i.e., there are now no other elements into which Ca can diffuse. At 1-Hz pacing, the Ca transient is uniform, with no variability from pulse to pulse. However, when the pacing frequency is raised to 2 Hz, clear, large alternans of systolic Ca are present. The full frequency dependence is shown in Fig. 2B, where the Ca transient amplitude is plotted as a function of the frequency of stimulation (the stimulus pulse is the same for all stimulus frequency, e.g., from \(-40\) to \(0\) mV, with a duration of 100 ms).

At a frequency of 2 Hz, alternans is maximal, but, as the stimulation frequency increases further, the ratio of alternans decreases until it reaches 1 again at 4 Hz. The plot (Fig. 2C) below shows how the SR Ca content varies with stimulation frequency. It decreases dramatically with the increase of stimulus rate, due to more frequent Ca release from the SR and less time interval for Ca being taken back to the SR between two successive stimuli.

The other maneuver we have used in this study to elicit alternans is an increase of the half-maximal subspace [Ca\(^{2+}\)] for Ca\(^{2+}\) release from junctional SR \((K_{\text{rel}})\) (the subspace...
[Ca\textsuperscript{2+}] required to stimulate RyR to open is raised by 20% to mimic inhibition of CICR. In the uniform model (functionally equivalent to a single release site version of the model: Fig. 3A), there are two control stimuli, and raising $K_{\text{rel}}$ leads to alternans following two very small systolic transients. Junctional SR Ca\textsuperscript{2+} content (below) shows each control stimulus depletes the SR, followed by a refilling period. After raising $K_{\text{rel}}$, the period with very small transients allows the SR to fill to the point where alternans can occur. Before small releases, SR content is less than before large releases. The plot in Fig. 3B shows how the Ca transient amplitude (at 1 Hz) changes with increasing $K_{\text{rel}}$. The 1:1 alternans that we illustrate throughout this study is, in fact, only seen in a narrow range of $K_{\text{rel}}$ values, around 1.2 times control: at lower values, two large releases accompany one small, and, at higher values, there are two small releases for each large. Examples of the Ca transients at each of the three values of $K_{\text{rel}}$ plotted (1.15, 1.2, and 1.3) are shown above.

The effect of adding a spatial dimension. Introduction of a spatial dimension allows us to increase $K_{\text{rel}}$ in a random pattern across the 75 release unit array. On average, the increase of $K_{\text{rel}}$ is the same as in Fig. 3, but now it varies among the 75 release units randomly. This pattern of raised threshold also changes with each stimulus, to mimic in a simplified manner the random nature of CICR inhibition in RyR clusters. The line

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Fig. 2. Simulations from a single Ca\textsuperscript{2+} release unit showing the effects of pacing frequency. For all stimulus frequency, the stimulus pulse has the same amplitude (from $-40$ to 0 mV) and duration (100 ms). A: time traces of cytoplasmic Ca\textsuperscript{2+} transient at 1 Hz (left) and 2 Hz (right). Ca\textsuperscript{2+} release is uniform from pulse to pulse at 1 Hz, but shows clear alternans at 2 Hz. B: Ca transient amplitude as a function of frequency; bifurcation leads to cytoplasmic Ca\textsuperscript{2+} transient alternans. C: associated SR Ca\textsuperscript{2+} content. [Ca\textsuperscript{2+}], intracellular Ca\textsuperscript{2+} concentration.

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Fig. 3. Model-generated Ca\textsuperscript{2+} alternans by increasing half-maximal Ca\textsuperscript{2+} subspace concentration for Ca\textsuperscript{2+} release from junctional SR ($K_{\text{rel}}$) to mimic tetracaine. Ca transients were produced by a sequence of 1-Hz, 100-ms depolarizing pulses from a holding potential of $-40$ to 0 mV. $K_{\text{rel}}$ was increased uniformly by 20% after the first 2 pulses. Ca\textsuperscript{2+} alternans developed after a period of Ca release inhibition after $K_{\text{rel}}$ was increased. A: traces of cytoplasmic Ca\textsuperscript{2+} transients (top) and SR Ca\textsuperscript{2+} (bottom). B: relationship between SR Ca content and systolic Ca in the cytoplasmic space for control (C) and increased $K_{\text{rel}}$ (E) conditions as SR Ca recovers from empty state. Solid line was produced by curve fitting of systolic Ca $= a + b(SR \text{Ca})^n$, where $n = 3.5$ for control and rises to 15.0 for increased $K_{\text{rel}}$. C: systolic Ca as a function of $K_{\text{rel}}$. Bifurcation leads to cytoplasmic Ca\textsuperscript{2+} alternans. Increase in $K_{\text{rel}}$ results in various patterns of cytoplasmic Ca\textsuperscript{2+} alternans, such as 2:1 (i), 1:1 (ii), or 1:2 (iii) as shown in the inset.
scan in Fig. 4A shows two control stimuli followed by two very small transients after \( K_{\text{rel}} \) is raised. Raising \( K_{\text{rel}} \) by an average of 20% is indicated by the line above the line scan. The line is initially dashed to indicate randomly changing \( K_{\text{rel}} \); thereafter, the solid line indicates a uniformly raised value. Raising \( K_{\text{rel}} \) fragments the release profile, and local alternans follows. In agreement with our experimental data (9), large releases are associated with miniwaves of \( \text{Ca}^{2+} \) release (see inset A). Previously we have suggested that, associated with such behavior, there would be local variations in SR \( \text{Ca}^{2+} \) content caused by variations of local release and efflux. The model can give us this information; Fig. 4B shows local alternans of systolic \( \text{Ca} \) at the point marked by the arrow in Fig. 4A, the middle panel shows SR \( \text{Ca}^{2+} \) content from this area. During local alternans, SR \( \text{Ca} \) is relatively higher locally before large releases than before small releases. As the extent of alternans varies across the cell, the degree of alternans averaged over the whole cell is somewhat smaller that that shown in B (c.f., Fig. 4, B and C).

Cytoplasmic \( \text{Ca}^{2+} \) transients produced by small depolarizations. Another experimental method that produces alternans is the use of small depolarizations (7). In the model, a series of small stimulus pulses lead to sustained alternans of cytoplasmic \( \text{Ca}^{2+} \) transients (e.g., Fig. 5A). However, the degree of alternans averaged over the whole cell is much greater than in Fig. 4. This is because either none of the cell releases \( \text{Ca}^{2+} \) (a in Fig. 5A), or all of the cell responds (b in Fig. 5A), i.e., there is no out-of-phase alternation taking place to reduce the overall degree of alternans. However, unlike Figs. 2 and 3, where similarly large alternans is present in the dimensionless model, the large release involves \( \text{Ca}^{2+} \) wave propagation (Figs. 5A and 7A), very similar to our experimental data (7). The steepness of the relationship between systolic \( \text{Ca}^{2+} \) and SR \( \text{Ca}^{2+} \) is also increased in this alternans. The fitted lines in Fig. 5B have \( n \) set to 3.5 in control (open symbols) and 18.4 during small depolarizations (solid symbols).

In Fig. 5, A and B, 40 out of 75 elements in the cell are chosen randomly to have their L-type Ca current blocked. The remained current in the whole cell is 46% of control. Further studies have also been conducted to explore the general relationship between \( \text{Ca}^{2+} \) release and the reduction in the amplitude of L-type channel current in the whole cell via varying the number of blocked elements. The graph in Fig. 5C shows the full relationship between the remained averaged global L-type current (as percentage of control) in the whole cell and \( \text{Ca} \) release. A decrease of L-type channel current means the number of sites where CICR can take place decreases. At small current values (<60% of control), there is large alternans. At the current values between 30 and 50% of control, there is 1:1 alternans. With L-type currents <30% and between 50 and 60%, the alternans pattern produced is complicated and does not follow the 1:1 pattern shown in Fig. 5A (somewhat similar to the situation in Fig. 3C). Finally, >60% of L-type current amplitude alternans disappears.

Two phases of \( \text{Ca}^{2+} \) release. The model also reproduces the biphasic release we see experimentally when using small pulses. The trace of systolic \( \text{Ca}^{2+} \) in Fig. 6A shows two successive stimuli each demonstrating a small, initial release (marked by small arrow). A second phase of release (produced by wave propagation; marked by big arrow) follows in the first stimulus. Again, this fits well with data from cells under similar experimental conditions. Both phases are due to CICR: the first represents release stimulated by L-type Ca channel activity, and the second, propagating CICR.

**SR \( \text{Ca}^{2+} \) content, NCX current, and \( \text{Ca}^{2+} \) transient.** Alternans following small depolarizations also relies on changes of SR \( \text{Ca} \) content; Fig. 6 shows SR \( \text{Ca}^{2+} \) content (averaged over all release units) and efflux via NCX current during large and
small Ca\(^{2+}\) transients. A large Ca\(^{2+}\) transient was associated with a large SR Ca\(^{2+}\) release (i.e., junctional SR content falls more; Fig. 6B) and more Ca\(^{2+}\) removal by NCX (Fig. 6C), while a small Ca\(^{2+}\) transient was associated with less Ca\(^{2+}\) release and less Ca\(^{2+}\) removal by NCX. The dashed line in Fig. 6B illustrates that, before the small release, SR Ca\(^{2+}\) is lower than before the large release. This, too, is consistent with experimental observations (7). The L-type currents in Fig. 6D indicate that changes in SR Ca\(^{2+}\) are solely responsible for changes in systolic release as trigger Ca\(^{2+}\) is unchanged between large and small releases.

One question is: where is release triggered by L-type channel openings and where is it propagated following small depolarizations? Obviously, triggered release will only be possible where L-type Ca current is activated. The solid bars in Fig. 7A show the elements at which the L-type Ca channels open on this particular stimulus; where release is initiated (sites 28 and 29 and 31 and 32), there is full opening of L-type channels in the element. However, such L-type openings do not always trigger Ca release, e.g., sites 48, 49, and 50 and 56 and 58 have L-type openings but no CICR follows. These sites had to wait for the arrival of the wave initiated at sites 31 and 32 to release Ca.

One possible explanation for failure of L-type channels to trigger CICR is suggested by Fig. 7, B and C. Figure 7B shows changes in SR Ca at two different sites from the line scan, i.e., sites 32 and 56. At site 32, there is an early release of Ca due to activation of L-type Ca channels; at site 56, Ca release fails initially but is activated later by the arrival of the wave. Although sites 32 and 56 seem to have the same initial SR Ca content, Fig. 7C shows the initial values in more detail for each site, and it is clear that site 32 has a marginally higher content, making it more likely to release.

DISCUSSION

Our previous work has shown two maneuvers that produce local and global alternans of systolic Ca\(^{2+}\) release in ventricular cells (7, 9). Reducing RyR open probability in voltage-clamped cells with, e.g., tetracaine or acidic intracellular pH, produces alternans of systolic release that remains local, i.e., different parts of the cell show alternans out of phase with each other. Decreasing the amplitude of the L-type Ca current produces alternans that involve the whole cell. We have suggested that alternans is due to increased steepness of the relationship between SR Ca\(^{2+}\) content and release of Ca\(^{2+}\) (a measure of the gain of Ca release). Increased steepness in both local and global alternans is due to the appearance of waves, as these rely on the SR reaching a threshold Ca\(^{2+}\) content (8). This ought to lead to an essentially infinitely steep relationship: above threshold, waves occur and release is large; below threshold, there are no waves and release is small. In this paper, we present simulations of this behavior to determine the important factors underlying generation of alternans of systolic Ca release. Our major finding is that the fragmented Ca\(^{2+}\) wave propagation and steep relationship between SR Ca\(^{2+}\) content and release of Ca\(^{2+}\) play a major role in the genesis of Ca\(^{2+}\) alternans at low-stimulus frequency (1 Hz) without the need for refractoriness of L-type Ca or RyR channels. The alternans sustains through alternation of SR Ca\(^{2+}\) content. This is different from the mechanism underlying the genesis of Ca\(^{2+}\) alternans at rapid pacing, as reported by Shiferaw and Karma (34), where refractoriness of ion channels and electrical action potentials are involved to produce instable Ca diffusion, leading to symmetry breaking.

The simulations we have done here can reproduce all features of systolic Ca\(^{2+}\) alternans, local and global, that we have
seen in cells. The two types of alternans we find share several features in common: on a large Ca transient, initial release is stimulated by L-type Ca current in only a few sites; the released Ca\(^{2+}\)/H\(^{+}\) initiates a propagating wave by inducing further SR Ca\(^{2+}\) release in regions that did not respond to the initial depolarization. Such a large Ca release locally ensures that the SR remains depleted at the next stimulus.

In the model, the reduced open probability of RyR can be produced by increasing the parameter \(K_{rel}\) (the subspace Ca required to open the RyR, i.e., RyR Ca sensitivity). In Fig. 3, we increased \(K_{rel}\) by 20%; this produces 1:1 alternans of systolic release. The plot in Fig. 3B shows why alternans has occurred; there is a greatly increased steepness of the relationship between Ca\(^{2+}\) release and diastolic SR Ca\(^{2+}\) content compared with the control. Thus for a small reduction of Ca\(^{2+}\) in the SR at end diastole, there is a very large reduction in systolic release. However, in this case, the increased steepness in this plot does not rely on wave propagation, as there is no spatial dimension in the model.

In our studies of raising \(K_{rel}\), we have concentrated on a value of 1.2 times control, as this produces an alternans ratio of 1:1, i.e., for each large transient, there is a small transient. However, it is clear from Fig. 3C that other patterns of alternans are possible. In all cases, the cell is maintaining Ca flux balance, i.e., the amount of Ca that enters the cell must also leave. However, it is clear that the number of stimulation cycles required to make this balance can vary. How altering \(K_{rel}\) changes the pattern of alternans is not yet clear and warrants further studies.

Fragmentation of the release profile seen in cells when tetracaine or acid pH is applied can be reproduced in the full version of the model (75 release units in contact by diffusion between neighboring element’s cytoplasmic spaces) by applying a random pattern of increase of \(K_{rel}\) across the array of release units. Although L-type channel opening is uniform across the array, Ca\(^{2+}\) wave propagation was initiated at a limited number of release sites (Fig. 4A). If a region of the cell is not stimulated to release Ca by an L-type channel opening, it may then be able to support wave propagation. If Ca release takes place (triggered either way), on the next stimulus the SR in this region is depleted of Ca\(^{2+}\) (Fig. 4B) and so does not respond to either stimulus or arrival of a wave. This reliance on

Fig. 6. Ca\(^{2+}\) content and efflux during Ca\(^{2+}\) alternans produced by small depolarization pulses with 40 out of 75 elements blocked. A: [Ca\(^{2+}\)] in cytoplasmic space. Biphasic Ca release is obvious with a small initial release evoked by stimulus (small arrow) followed by a big release (large arrow). B: Ca\(^{2+}\) content in the SR. C: Na/Ca exchanger current (\(i_{NCX}\)). D: \(i_{Ca,L}\).

Fig. 7. A: propagation of cytoplasmic Ca\(^{2+}\) wave produced by localized CICR in response to \(i_{Ca,L}\) trigger (with the same condition as in Fig. 6) and spatial diffusion of Ca\(^{2+}\). Solid bars: elements with fully active \(i_{Ca,L}\) channels. B: SR Ca\(^{2+}\) transients for some local elements 32 and 56. C: spatial distribution of SR Ca\(^{2+}\) content at end diastole.
SR Ca does not, however, reflect a luminal effect (24), as no “sensor” of SR Ca content inside the SR has been built into the model, although it may reflect a change in the feedback of the CICR mechanism. Released Ca feeding back to activate more CICR will be more evident if SR Ca content is high.

Application of increased $K_{rel}$ in a random pattern across the cell requires explanation. Conditions throughout the cell are unlikely to be entirely uniform in experiments as they are in the model. Although other possibilities may exist, in real cells, fragmentation of the release profile might occur through small, local variations of SR Ca$^{2+}$ due to, e.g., spontaneous sparks and/or local differences in $K_{rel}$. In each case, a spatially inhomogeneous response of the SR to L-type current would be expected to result. The mechanism we propose for depletion and refilling of the SR would then maintain such inhomogeneity. This is confirmed in Fig. 4A, at the point where the dashed line (marking raised $K_{rel}$) becomes solid, the random changes of $K_{rel}$ cease, i.e., $K_{rel}$ is raised by 20% uniformly across the array. Despite this, the fragmented release profile is maintained.

As in real cells, at the whole cell level, the degree of alternans in the model is rather small compared to local alternans (Fig. 4C). This results from the lack of synchrony of release, i.e., regions alternating to a lesser extent or out of phase partially balance each other. This is just as we saw in experiments on rat ventricular myocytes in the presence of tetracaine and acidic pH (9).

We can now ask why it is that, in real cells, alternans is not uniform, but is produced by propagation of miniwaves? It is clear from Fig. 3 that uniform alternans is possible (75 release units together with a uniform increase of $K_{rel}$ would also produce uniform alternans); however, if we compare the SR Ca values produced by the model required for uniform alternans with those when miniwaves produce alternans, we see that alternans in a single unit requires higher SR Ca levels (1.81 vs. 1.56 mM). Thus there are two probable factors that combine to prevent the appearance of uniform alternans in the cell: conditions are unlikely ever to be sufficiently uniform to allow them to develop, and, in any case, alternans due to miniwaves will develop at a lower SR Ca, thereby preventing SR Ca rising high enough.

Small depolarizations and alternans. When alternans are produced by reducing the number of L-type Ca channels that respond to depolarization, rather than the number of RyR that respond to L-type current, the degree of alternans at the whole cell level is greater in both cells and the model (Fig. 5). In this case, a few elements with activated L-type channels trigger SR Ca release. This released Ca diffuses to neighboring sites, where it activates CICR. This two-stage process leads to the biphasic Ca$^{2+}$ release in Fig. 6, averaged over the whole cell. The time taken for the second phase to rise is dictated by the propagation velocity of the wave of CICR.

In the model, the number of release sites responding to small depolarization pulse is reduced by the imposition of a block of L-type channel current locally in some of elements (see Limitations of the model), which are randomly chosen from the 75 release elements in the array, giving a different pattern of L-type channel activation with each stimulus. The different pattern of L-type channel activation on each stimulus results in different initiation sites for Ca release (not shown). However, the pattern of L-type channel opening is not the only influence on where RyR openings result, as many L-type channel openings do not produce SR release (Fig. 7). Where release triggered by L-type Ca channels is absent or small, SR Ca content is low at the time of stimulation, e.g., site 56. The model has no luminal site that could confer extra sensitivity of the release unit at high Ca content. Thus it appears that Ca released can feed back to stimulate further release, if SR content is high.

The importance of SR Ca content. As we have reported before, during alternans, large releases deplete the SR due to greater activation of Ca$^{2+}$ efflux pathways. This can be seen in Fig. 6, where the large NCX current depletes the SR of Ca. By the time of the next stimulus, the junctional SR has not completely refilled. This small depletion of the SR means no wave propagation is possible, and a small release results. As little efflux is activated by this small release, the SR is able to refill back to the level able to support wave propagation, and a large release results once again on the next cycle. A similar explanation of alternans can be used on a local scale to explain the simulations of the effects of tetracaine or acid pH. The large releases cause either global (Fig. 6B) or local (Fig. 4B) SR depletion, depending on how inhibition of RyR opening is applied. This depletion leads to reduced release of Ca and prevents wave propagation. Obviously, for local depletions of SR Ca to be able to influence the amount of Ca released, it requires that the depletion persist long enough. The intercoupling between neighboring SR release sites allows for local depletion to persist sufficiently long to maintain alternans.

The actual shape of the relationship between systolic [Ca] and frequency is interesting: when alternans first appears, the ratio is large, but, as frequency increases, the alternans ratio declines until systolic [Ca] is once again uniform from beat to beat. Associated with these changes is a fall of SR Ca content, which suggests that alternans is only possible over a narrow range of SR Ca content. Perhaps as SR Ca content falls, the steepness of the relationship between SR Ca release and diastolic SR Ca content falls too low to provide sufficient depletion of the SR in any one cycle. Obviously, real cells exhibit alternans when frequency is raised. In the past, our laboratory has produced models of alternans that rely on a very steep relationship between SR Ca$^{2+}$ release and SR Ca$^{2+}$ content (12). Such a model produces alternans (e.g., Figs. 2 and 3 in this study); however, we now consider any approach that does not include a spatial element incomplete, as it cannot produce behavior that involves propagation of waves. It would be interesting in future simulations to impose some heterogeneity on the model (perhaps by application of a resting frequency of sparks to produce small variations of SR Ca content) to determine whether this will cause the Ca release profile to fragment at higher frequencies and allow miniwaves of Ca release and alternans to appear.

A recent report has shown (29) that, although present in the majority of cells studied, systolic Ca alternans can result without apparent alternans of SR Ca content. The simulations presented here, however, are intended to address the situation where the release of Ca from the SR is compromised, either by inhibition of CICR or limitation of L-type Ca current. It is entirely possible that the mechanism put forward here of fragmentation of the Ca release profile, followed by propagation of waves, is not the only one capable of leading to systolic Ca alternans. It would be interesting in the future to model the
conditions required for such alternans, independent of SR Ca content.

Limitations of the model. The present model has several limitations. In the present study, the SR Ca release was modeled by quasi-steady-state approximation of RyR gating kinetics; however, this seems reasonable considering the rapid activation (0.07–0.27 ms) (42) and extremely slow inactivation of the RyR gate (43) compared with the L-type Ca channel and the low stimulus frequency (1 Hz). In simulations, the mechanism of reversible SR uptake was not incorporated in the model. However, simulations with an alternative reversible SR uptake equation (33) (see Eq. A18.1 in Appendix) show similar results. We have made no attempt here to model electrophysiological consequences of systolic Ca2+ alternans, although it is clear from our previous work and here (Fig. 6) that currents generated as a result of the changes in Ca2+ release would be expected to alter action potential shape and/or duration. The model includes no influence of SR Ca content on the behavior of the Ca release channel (25); this is thought to play a role in control of Ca release, but should, if present, accentuate the effects of local differences of SR Ca content on Ca release. Importantly, the model uses whole cell behavior of L-type current in each of the elements rather than the behavior of single unitary L-type channels. As a result, the changes of voltage used in this paper reduce current flow through the L-type channel of each release unit rather than increase unitary channel current. Finally, the model is a one-dimensional simulation of a cell and, therefore, lacks the three-dimensional complexities of a whole cell, such as t-tubule networks (39) and spatial distribution of L-type current (4). Each of these limitations needs to be addressed in future studies.

Despite these limitations, the model demonstrates that propagation of waves of CICR following fragmentation of the systolic Ca release profile is able to produce systolic Ca alternans. In addition, the model highlights the importance of interactions between local SR Ca content, L-type Ca channel activity, and sensitivity to Ca of the Ca release channel of the SR in sustaining this behavior.

APPENDIX

Glossary

F Faraday constant
I Ionic current
R Universal gas constant
SR Sarcoplasmic reticulum
t Time
T Absolute temperature
V Membrane potential (in mV)

Ionic channel currents.

ICa.L L-type Ca2+ channel current
ICa,p Sarcolemmal Ca2+ pump current
INaCa Na+/Ca2+ exchanger (NCX) current

Cell geometry.

Vcell Cell volume
Vi Myoplasmic volume available for Ca2+ diffusion
Vrel Volume of junctional SR (Ca2+ release store)

Vsub Subspace volume
Vup Volume of network SR (Ca2+ uptake store)

Ionic concentrations.

[Ca2+]i Myoplasmic Ca2+ concentration
[Ca2+]o Extracellular Ca2+ concentration
[Ca2+]rel Ca2+ concentration in junctional SR
[Ca2+]sub Subspace Ca2+ concentration
[Ca2+]up Ca2+ concentration in network SR
[Mg2+]i Intracellular Mg2+ concentration
[Na+]i Intracellular Na+ concentration
[Na+]o Extracellular Na+ concentration

Equilibrium (reversal) potentials.

ECa.L Apparent reversal potential of ICa.L

Sarcolemmal ionic currents.

dL Activation gating variable for ICa.L
dNaCa Steady-state dL
INaCa Denominator constant for INaCa
fL Inactivation gating variable for ICa.L
fNaCa Steady-state fL
gCa,L Maximum ICa.L conductance
kNaCa Scaling factor for INaCa

γNaCa Position of Eyring rate theory energy barrier controlling voltage dependence of INaCa
αdL Opening rate constant of dL
αfL Opening rate constant of fL
βdL Closing rate constant of dL
βfL Closing rate constant of fL
τdL Time constant of dL
τfL Time constant of fL

Ca2+ diffusion flux.

JCa,sub diffusion flux from subspace to myoplasm
JCa,up diffusion flux between neighboring myoplasm
JCa,up,rel diffusion flux between neighboring SR networks
τCa,rel diffusion time constant for Ca2+ diffusion from subspace to myoplasm
τCa,up diffusion time constant for Ca2+ diffusion between neighboring SR networks

SR function.

Jrel Ca2+ release flux from junctional SR to subspace
Ju Ca2+ transfer flux from network SR to junctional SR
Ju,pump Ca2+ uptake flux from myoplasm to network SR
Krel Half-maximal [Ca2+]sub for Ca2+ release from junctional SR
Kpump Half-maximal [Ca2+]rel for Ca2+ uptake by Ca2+ pump in network SR
Prate Rate constant for Ca2+ release from junctional SR
Prelease Rate constant for Ca2+ uptake by Ca2+ pump in network SR
τw Time constant for Ca2+ transfer from network SR to junctional SR

Ca2+ buffering.

[CQ]tot Total calsequestrin concentration
[CM]tot Total calmodulin concentration
kCa,Ca Ca2+ dissociation constant for calmodulin
kCa,Ca,Ca2+ dissociation constant for calsequestrin
kCa,Ca,Mg Ca2+ dissociation constant for troponin-Ca complex
kCa,Mg dissociation constant for troponin-Mg complex
kMg,Mg dissociation constant for troponin-Mg complex

Intracellular Ca\(^{2+}\) diffusion flux. Ca\(^{2+}\) diffusion flux between neighboring elements. For each coupled element array, indexed by \(k\) (\(k\) varies between 1 and 75):

\[
J_{\text{Ca,} k_\text{diff}} = \frac{[\text{Ca}^{2+}]_{\text{sub}} - [\text{Ca}^{2+}]_{\text{up}}}{\tau_{\text{Ca,} k_\text{diff}}} \quad (A14)
\]

Ca\(^{2+}\) handling by SR:

\[
J_{\text{Ca,} k_\text{up}} = P_{\text{Ca,} k_\text{up}} \cdot \frac{[\text{Ca}^{2+}]_{\text{sub}}}{[\text{Ca}^{2+}]_{\text{sub}} + K_{\text{Ca,} k_\text{up}}} \quad (A17)
\]

\[
J_{\text{Ca,} k_\text{up}} = 12.0 \cdot \frac{[\text{Ca}^{2+}]_{\text{sub}}^{1.787} - 0.286 [\text{Ca}^{2+}]_{\text{sub}}^{1.787}}{1 + [\text{Ca}^{2+}]_{\text{sub}}^{1.787} + [\text{Ca}^{2+}]_{\text{sub}}^{1.787}} \quad (A18.1)
\]

Table 2. Model parameters and values

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See Glossary for definition of terms.
Model constants.

See Table 2 for model parameters and values.

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GRANTS

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