Published in final edited form as:
J Cardiovasc Electrophysiol. 2006 May ; 17(Suppl 1): S64–S70.

Restitution of Ca^{2+} Release and Vulnerability to Arrhythmias

ERIC A. SOBIE, PH.D.[*], **LONG-SHENG SONG, M.D.**[†], and **W.J. LEDERER, PH.D.**[†]
[*] *From the Division of Pediatric Cardiology, New York University School of Medicine, New York, New York, and*

[†] *Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland, USA*

Abstract

Ca^{2+} Release Restitution. New information has recently been obtained along two essentially parallel lines of research: investigations into the fundamental mechanisms of Ca^{2+}-induced Ca^{2+} release (CICR) in heart cells, and analyses of the factors that control the development of unstable rhythms such as repolariza-tion alternans. These lines of research are starting to converge such that we can begin to understand unstable and potentially arrhythmogenic cardiac dynamics in terms of the underlying mechanisms governing not only membrane depolarization and repolarization but also the complex bidirectional interactions between electrical and Ca^{2+} signaling in heart cells. In this brief review, we discuss the progress that has recently been made in understanding the factors that control the beat-to-beat regulation of cardiac Ca^{2+} release and attempt to place these results within a larger context. In particular, we discuss factors that may contribute to unstable Ca^{2+} release and speculate about how instability in CICR may contribute to the development of arrhythmias under pathological conditions.

Keywords

excitation-contraction coupling; alternans; calcium-induced calcium release; calcium transient; action potential duration; fibrillation; computer modeling

Interactions Between Ca^{2+} and Electrical Signaling in Heart Cells

To ensure efficient pumping of blood, the heart employs complex bidirectional interactions between electrical and chemical signaling systems, the latter primarily mediated through changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Each time the heart beats, depolarization of the cell membrane leads to a large, transient increase in $[Ca^{2+}]_i$ in every cell, and contraction results from Ca^{2+} ions binding to myofilaments. Over the past few decades, numerous experimental studies have provided significant insight into this phenomenon of excitation-contraction coupling, and, specifically, how Ca^{2+} entry through the cell membrane triggers the release of a larger amount of Ca^{2+} from sarcoplasmic reticular (SR) stores, a process known as Ca^{2+}-induced Ca^{2+} release (CICR). A major conceptual breakthrough occurred with the discovery that CICR occurs via the recruitment of a number of elementary units of Ca^{2+} release, Ca^{2+} sparks,[1–4] each of which is triggered by increases in $[Ca^{2+}]_i$ in the immediate vicinity of the SR release channels responsible for these events. Advances in understanding CICR and regulation of Ca^{2+} sparks have been reviewed recently by several groups.[5–7]

Address for correspondence: W.J. Lederer, Ph.D., Medical Biotechnology Center, 725 W. Lombard Street, Baltimore, MD 21201, USA. Fax: (410) 510-1545; E-mail: lederer@umbi.umd.edu.

This work was supported by grants from the National Institutes of Health, the Whitaker Foundation, and the American Heart Association.

Ca^{2+} current flowing through L-type Ca^{2+} channels provides the primary trigger for Ca^{2+} release in heart cells,[8–11] although under certain conditions sarcolemmal Ca^{2+} flux through T-type Ca^{2+} channels[12] or via "reverse mode" operation of the Na^+-Ca^{2+} exchanger (NCX) may also contribute.[13] Since both the open probability and the single-channel current of L-type channels depend strongly on voltage, changes in the action potential shape affect excitation-contraction coupling and can modulate CICR in subtle and sometimes surprising ways.[14,15] At the same time, the $[Ca^{2+}]_i$-dependence displayed by several ionic currents results in modulation of the action potential shape by changes in cellular Ca^{2+} transients. Potential sites for this regulation include Ca^{2+}-dependent inactivation of the L-type Ca^{2+} current,[16] Ca^{2+}-dependent K^+ and Cl^- currents,[17,18] and NCX, which, in "forward mode," imports three Na^+ ions for every Ca^{2+} ion expelled and therefore contributes inward, depolarizing current.[19] A larger Ca^{2+} transient on a given beat would be expected to simultaneously lead to increased Ca^{2+}-inactivation of L-type Ca^{2+} channels, which would tend to shorten the action potential, and increased Ca^{2+} extrusion via NCX, which, by supplying inward current, would tend to lengthen the action potential. The Ca^{2+} and electrical signaling systems in heart cells therefore influence one another in complicated, intricate ways. Under certain circumstances, it is also possible that mechanical movement of heart myocytes affects membrane potential more directly through stretch-sensitive channels, a process known generally as mechano-electric feedback.[20]

To relate cardiac excitation-contraction coupling to more general questions of beat-to-beat heart electrical rhythms, one of the key questions is: "Once CICR has been triggered, what determines the size of the Ca^{2+} transient at the next beat?" This deceptively simple question is not as easy to answer as it might seem. After Ca^{2+} is released from the SR, several time, voltage, and/or concentration-dependent processes may play a role in the recovery or restitution of the Ca^{2+} transient. These include recovery of L-type Ca^{2+} channels from both voltage and Ca^{2+}-dependent inactivation, refilling of SR Ca^{2+} stores, and recovery of the SR release channels, known as ryanodine receptors (RyRs), from whatever mechanism terminated release. Early studies that investigated recovery of Ca^{2+} transient amplitude, or restitution, had to distinguish between different hypotheses using indirect methods and inferences.[21,22] More recently, technical advances have allowed for more precise experimental control of Ca^{2+} release triggers and for the visualization of local release events. A handful of new studies have examined the recovery of Ca^{2+} release at the local level and have provided important new information.

Local Recovery of CICR from Refractoriness

A few recent studies have been able to gain insight into the mechanisms underlying the local recovery of Ca^{2+} release in ventricular myocytes. For instance, Sobie et al. applied low doses of the RyR agonist ryanodine to rat cells loaded with the Ca^{2+} indicator fluo-3 and observed repeated activations of individual Ca^{2+} spark sites.[23] Spontaneous Ca^{2+} sparks occur stochastically in resting ventricular cells at a low rate of approximately 100 per cell/sec.[2] This translates to roughly 1 spark/sec when images are acquired with a confocal microscope operating in "line scan" mode, and the scan line spans the length of the cell. Exposure to 50 nM ryanodine caused sequences of Ca^{2+} sparks to originate from a limited number of sites within the cell, as shown in Figure 1A, and spark pairs originating from these sites were analyzed to examine how the delay between sparks influenced the amplitude of the second spark and the probability that a second spark would arise. Representative Ca^{2+} spark pairs taken from the data presented in this study are shown in Figure 1B. The plots show that the amplitude of the second spark, relative to the first, increases with an increase of the delay between sparks. Analysis of numerous Ca^{2+} spark pairs demonstrated that the recovery of Ca^{2+} spark amplitude could be well fit by a single exponential with a time constant of roughly 90 msec, whereas the probability of Ca^{2+} spark triggering followed a more sigmoidal time

course that lagged behind the recovery of Ca^{2+} spark amplitude. The two recovery functions obtained in that study[23] are plotted together in Figure 1C.

How should the delay between the recoveries of Ca^{2+} spark amplitude and triggering probability be interpreted? Several possibilities exist but are difficult to distinguish, and the current work by us and other groups is aimed at discriminating between various hypotheses. A key factor in the recovery of Ca^{2+} spark amplitude is the refilling of local SR Ca^{2+} stores, which become significantly depleted during Ca^{2+} release, as direct measurements of free $[Ca^{2+}]_{SR}$ have recently shown.[24,25] If Ca^{2+} spark amplitude recovery, to a first approximation, reflects this refilling, the slower recovery of spark triggering probability may indicate that this is controlled by a different mechanism, such as recovery of RyRs from inactivation after dissipation of high local $[Ca^{2+}]_i$. An alternative possibility is that the local refilling of SR stores completely controls Ca^{2+} spark restitution, but the delay occurs because the triggering probability depends on $[Ca^{2+}]_{SR}$ raised to a power greater than 1. Several factors could contribute to this. One is that, in the experiments presented by Sobie et al., the trigger for Ca^{2+} release is a spontaneous opening of a ryanodine-bound channel within the repetitively firing RyR cluster. The amplitude of this trigger increases as the local SR refills, and could increase the probability that a spontaneous RyR opening would activate neighboring RyRs via CICR. Moreover, since planar lipid bilayer studies of RyR gating indicate a sigmoidal dependence of open probability on cytosolic $[Ca^{2+}]$,[26] this would translate to a nonlinear increase in triggering probability as $[Ca^{2+}]_{SR}$ refills. In addition, the open probability of RyRs depends on $[Ca^{2+}]$ in the SR lumen, with RyRs more sensitive to triggering by cytosolic Ca^{2+} when $[Ca^{2+}]_{SR}$ is high.[27,28] If dynamic changes in $[Ca^{2+}]_{SR}$ during and after sparks modulate RyR gating, as has been suggested,[29,30] this factor would add an additional nonlinearity and further contribute to the lag between the two recovery functions. A final possible factor could be that after local $[Ca^{2+}]_{SR}$ returns to its control value, a slow conformational change must occur before the RyRs in the cluster recover their sensitivity to activation by cytosolic Ca^{2+}. This could happen if the lumenal dependence of RyR gating depends not on free $[Ca^{2+}]_{SR}$ but instead on interactions of Ca^{2+} with calsequestrin, junction and/or triadin on the lumenal side of the SR membrane. Specifically, it has recently been hypothesized that the higher open probability of RyRs at high $[Ca^{2+}]_{SR}$ is due to the dissociation of calsequestrin, which can inhibit RyRs in its Ca^{2+}-free form.[31] Although the kinetics of these protein-protein interactions have not been well characterized, it is easy to envision a scheme in binding and dissociation reactions such as those proposed contribute to a delay.

Restitution of Cellular Ca^{2+} Transients

No matter which mechanism accounts for the recoveries of Ca^{2+} spark amplitude and triggering probability measured recently by Sobie et al.,[23] the results obtained in this and other recent studies can be helpful for understanding how Ca^{2+} release at the cellular level will recover with time. To a first approximation, the amplitude of a cellular Ca^{2+} transient will be proportional to the product of Ca^{2+} spark amplitude and the probability of Ca^{2+} spark triggering. This probability, in turn, may in general depend on both the recovery from refractoriness of CICR and recovery of the trigger for Ca^{2+} release. Figure 2 displays Ca^{2+} transient recovery functions assessed in four recent studies[23,32–34] on a single set of axes. By comparing the plots and noting the differences in experimental conditions and protocols, insight into the relative importance of different factors may be gained. To avoid potential problems caused by differences between species, in particular expression of the SR/ER Ca^{2+} ATPase (SERCA) responsible for SR refilling after Ca^{2+} release, we have only plotted results obtained in small rodents (rats and mice). These species display robust SERCA function, high SR load even after long periods of quiescence, and Ca^{2+} transients that rely almost entirely on Ca^{2+} release from the SR.[5]

Several points of interest can be noted on the composite plot. One is that the recovery of Ca^{2+} transient amplitude assessed by Szentesi et al.[34] (cyan line) is slightly slower than the estimate of Sobie et al.[23] (black line). For the latter study we have plotted the product of the Ca^{2+} spark amplitude and triggering probability recoveries, a quantity that should be roughly proportional to the Ca^{2+} transient amplitude recovery. Szentesi et al. loaded mouse ventricular myocytes with the light-sensitive Ca^{2+} buffer DM-nitrophen, then delivered brief flashes of UV light to liberate Ca^{2+} from the buffer and trigger Ca^{2+} release.[34] With this technique repeatable triggers for Ca^{2+} release could be given at any interval. Thus, both the Sobie et al. and Szentesi et al. experiments investigated restitution of Ca^{2+} release without any complications due to partial or complete refractoriness of the trigger. The key difference between the two studies is that Sobie et al. measured local restitution of Ca^{2+} sparks whereas Szentesi et al. triggered cell-wide Ca^{2+} transients. The somewhat slower recovery observed in the latter study may therefore reflect slower SR refilling after triggering of global Ca^{2+} transients versus individual Ca^{2+} sparks. This could happen if local refilling after isolated Ca^{2+} sparks depend on both SERCA activity and diffusion of Ca^{2+} from neighboring regions of network SR, which would be predicted based on cellular ultrastructure.

The red plot in Figure 2 is from the study of Cheng et al.,[32] who recorded Ca^{2+} transients induced by electrical field stimuli after spontaneous Ca^{2+} waves in Ca^{2+} overloaded cells and plotted, for various subcellular locations, the local Ca^{2+} transient amplitude versus the delay since the wave had passed. The somewhat slower recovery seen by these investigators may reflect refractoriness in the trigger for Ca^{2+} release. In other words, the Ca^{2+} wave could have caused Ca^{2+}-dependent inactivation of L-type Ca^{2+} channels, leading to partial refractoriness of these channels at the time of the field stimulus. Finally, the slowest Ca^{2+} transient amplitude recovery was observed by Sham et al.[33] (green line), who delivered paired voltage-clamp depolarizations and recorded the resulting "Ca^{2+} spikes"[35] in rat ventricular myocytes. The extremely slow and unphysiological (for rats) recovery recorded in this study could have resulted from the addition of a high concentration (4 mM) of exogenous Ca^{2+} buffer (EGTA) to the cytosol to allow for the recording of "spikes." This result therefore supports the hypothesis that SR refilling plays a key role in Ca^{2+} transient restitution. In summary, this comparison of several recent studies suggests that (1) refilling of global SR Ca^{2+} content may proceed more slowly after cell-wide transients than local refilling after isolated Ca^{2+} sparks, (2) in healthy cells the recoveries of both Ca^{2+} release and L-type Ca^{2+} current may contribute to Ca^{2+} transient restitution, and (3) recovery of $[Ca^{2+}]_{SR}$ may become an especially important factor when refilling is slowed. Additional experiments, of course, must be performed under carefully controlled conditions to confirm or refute such ideas.

Restitution of Ca^{2+} Transients and Unstable Rhythms

As progress in understanding the molecular mechanisms underlying local and global of Ca^{2+} release recovery continues to be made, one of the key questions will be: what factors determine whether Ca^{2+} release is stable from beat to beat, and under what conditions are regular fluctuations, or Ca^{2+} transient alternans, produced? The answer to this question should provide insight not just into the physiological pathways that control calcium homeostasis in heart cells but also into the factors that predispose the heart to arrhythmias in certain disease states.

Regular, beat-to-beat alternations in the strength of contraction or the characteristics of the ECG, referred to as mechanical and electrical alternans, respectively, have long been associated with lethal arrhythmias and sudden cardiac death.[36,37] More recently, clinical studies have demonstrated that small alternans in the T-wave amplitude, invisible to the naked eye but detectable with signal processing techniques, can identify patients with an increased risk sudden cardiac death in diverse populations.[38–41] Additionally, experiments in an animal model have shown that action potential duration (APD) alternans occur in cells concurrently

with T-wave alternans and that spatially discordant APD alternans can immediately precede and facilitate the development of reentrant arrhythmias.[42] These observations have been given a theoretical grounding by analyses of cellular dynamics based on the APD restitution curve. This literature, which has a long history,[43] predicts that restitution properties which allow for the development of alternans in individual cells will encourage the degeneration of ventricular tachycardia into fibrillation in tissue.[44–46] In particular, a region in the restitution curve with a slope greater than unity is thought to be required for stable APD alternans. This prediction has been supported by experimental studies showing that pharmacological agents that "flatten" the APD restitution curve (decrease its slope) can prevent the development of fibrillation in animal models.[47,48]

Analyses based on the APD restitution curve are complicated; however, by the fact that this cellular property is not a static function but instead can vary dynamically as circumstances change. Altered "conditions" that can modify the APD restitution curve can be as simple as a change in the pacing rate,[49] and a few recent studies have highlighted the difficulty of defining a "true" cellular APD restitution curve at a particular instant in time.[50,51] Recent studies have also obtained some counterintuitive results, such as alternans developing at locations or under conditions that would not be expected based on static restitution analyses or vice versa.[52–54] For instance, APD alternans can display "hysteresis" such that the threshold pacing rate is higher when the pacing rate is progressively increased than when it is decreased.[55] Several of these investigations have pointed to the potential importance of Ca^{2+} cycling in the development of APD alternans. Mechanical and Ca^{2+} transient amplitude alternans have long been known to occur simultaneously with APD alternans;[56–58] however, it has not been clear whether APD alternans leads to Ca^{2+} transient alternans or vice versa. Some of the new, unexpected results recently obtained suggest that Ca^{2+} transient alternans may in fact develop before and cause APD alternans.[52,53,55] This hypothesis has been supported by the observation that Ca^{2+} transient alternans can be produced in isolated cells even when voltage-clamp techniques are used to fix the beat-to-beat voltage waveform.[59–61] Overall, these findings have pointed to the importance of examining the beat-to-beat dynamics of cardiac Ca^{2+} cycling. In particular, there is at present a relative lack of straightforward quantitative predictions regarding which conditions promote and which inhibit Ca^{2+} transient alternans, at least compared with the extensive analyses of APD restitution. Despite the limitations of these analyses that have been noted,[62,63] their relative simplicity leads to a satisfying intuitive understanding and provides a useful framework for considering questions of stability. Analogous analyses of Ca^{2+} dynamics are just beginning to be developed.

Here, we present results from a simple computer model to show how particular nonlinearities in restitution of Ca^{2+} release may contribute to the development of Ca^{2+} transient alternans. These nonlinear relationships have been identified experimentally, but quantitative information is still somewhat lacking, so the model presented should be considered phenomenological and ad hoc. Results from simulations performed with this model are shown in Figure 3.

In the model, a fraction of the SR Ca^{2+} content is released into the cytosol at the instant each "beat" occurs, after which $[Ca^{2+}]_{SR}$ follows an exponential time course as it increases from its minimum and approaches a new steady state. The fraction released at a given beat is specified by a term P_{TRIG}, which varies between 0 and 1 and also increases as the interval since the previous beat increases. In some simulations this factor depends nonlinearly on the SR Ca^{2+} content;[64–66] in others it follows an independent time course. An additional important regulatory factor is the balance between the amount of Ca^{2+} entering the cell versus the amount exiting during each cardiac cycle. The influence of this factor is explored in the model through the parameter $[\overline{Ca^{2+}}]_{SR}$, the steady-state value toward which $[Ca^{2+}]_{SR}$ relaxes during the interval between beats. In some simulations, this is fixed, implying that the Ca^{2+} entry and

Ca^{2+} extrusion are exactly in balance. In others, $[\overline{Ca^{2+}}]_{SR}$ is inversely related to the amount of Ca^{2+} released on the previous beat, $\Delta[Ca^{2+}]_i$. This reflects the observation that the cell tends to accumulate Ca^{2+} when little SR Ca^{2+} is released, for instance following depletion of $[Ca^{2+}]_{SR}$. [67,68] and tends to extrude more Ca^{2+} than the amount entering when Ca^{2+} transients are especially large.[66] This property of the cell has been referred to as "high feedback gain."[60]

Figure 3 shows how these nonlinear elements in Ca^{2+} release restitution may interact with changes in pacing rate to promote the development of alternans. Panel A displays simulations performed with a nominal pacing cycle length of 1 second. The top graph (i), which plots $\Delta[Ca^{2+}]_i$ for the last 5 beats in a sequence of 40, shows that the beat-to-beat Ca^{2+} transient amplitude is extremely stable whether $[\overline{Ca^{2+}}]_{SR}$ is constant (black symbols) or depends on the preceding value of $\Delta[Ca^{2+}]_i$ (green symbols). Panels (ii) and (iii) in Figure 3A plot the recovery functions corresponding to these two conditions. These graphs show how $[Ca^{2+}]_{SR}$ (solid lines) and $\Delta[Ca^{2+}]_i$ (dashed lines) evolve as the time since the beat increases for the next-to-last (blue) and last (red) beats in the sequence simulated. These curves are virtually identical from one beat to the next, reflecting the stability observed in the amplitude plot at the top.

When the pacing cycle length is reduced to 500 msec, however, different behavior is observed. When $[\overline{Ca^{2+}}]_{SR}$ is fixed from one beat to the next, implying that Ca^{2+} entry and Ca^{2+} extrusion are exactly balanced, the beat-to-beat Ca^{2+} transient amplitude is stable (black symbols at top and middle curves). However, when $[\overline{Ca^{2+}}]_{SR}$ depends on $\Delta[Ca^{2+}]_i$, alternans are produced. This is consistent with the hypothesis of Diaz et al. that increased Ca^{2+} extrusion after large Ca^{2+} transients (high feedback gain) is a factor that promotes instability.[60] The bottom traces in Figure 3B show that, under these conditions, Ca^{2+} release restitution can no longer be described by a single function. After relatively large beats, $[Ca^{2+}]_{SR}$ increases toward a smaller value before the next beat (blue traces). This leads to a smaller Ca^{2+} transient, which in turn results in $[Ca^{2+}]_{SR}$ increasing toward a larger value before the subsequent beat (red traces). This plot also shows that small differences in the SR Ca^{2+} content from one beat to the next can become amplified by the nonlinear relationship between the SR Ca^{2+} content and the fraction released (see figure legend for details). Indeed, additional simulations (not shown) confirmed that alternans could be abolished if the dependence of P_{TRIG} on $[Ca^{2+}]_{SR}$ were replaced with an independent time-dependent function that did not depend on $[Ca^{2+}]_{SR}$. Since an infinite number of potential functions could have been chosen in the model, this does not prove that nonlinear gain is critical, but it does suggest that this steep relationship may promote the development of unstable beat-to-beat Ca^{2+} release. The simulations presented with this phenomenological and ad hoc model are simply meant to be illustrative and to suggest that future, more mechanistic analyses may lead to insight into which factors control the stability of Ca^{2+} release. A useful analysis of this type has recently been performed by Shiferaw et al., [69] but many more such studies will need to be performed in the future to determine which potentially unstable features of Ca^{2+} cycling are particular to individual mathematical models and which represent general characteristics of cardiac cellular physiology.

The simulations displayed in Figure 3, by calculating individual values of $[Ca^{2+}]_{SR}$ and $\Delta[Ca^{2+}]_i$ on each beat, implicitly assume that conditions within the cell are identical. However, since CICR release represents the recruitment of individually triggered Ca^{2+} sparks, as mentioned above, this may not always be the case. Experiments have shown that certain pharmacological interventions can promote "subcellular Ca^{2+} transient alternans," in which different regions of the cell may alternate out-of-phase with one another.[70] Such subcellular heterogeneities may become especially important in disease states such as heart failure. Structural and/or functional alterations to the EC coupling machinery in HF may promote conditions in which SR Ca^{2+} content and/or CICR efficiency is high in certain locations, due

to high local SR load or the local subcellular geometry, but low in others. Such local heterogeneities would be consistent in principle with the decreased synchrony of Ca^{2+} release that has been observed in cells isolated from failing hearts.[71]

The future challenge will be to combine quantitative examinations of Ca^{2+} restitution dynamics with analyses of APD to gain a richer understanding of how the electrical and chemical signaling systems of heart cells influence one another. As features that are critical to the development of unstable beat-to-beat rhythms such as alternans are recognized, new targets for therapies may be identified. As mechanistic insight continues to be gained, we may be able to find molecular targets that can be safely modified to prevent rhythm disturbances and reduce the risk of sudden cardiac death in patients while still maintaining adequate heart function.

References

1. Cannell MB, Cheng H, Lederer WJ. The control of calcium release in heart muscle. Science 1995;268:1045–1049. [PubMed: 7754384]
2. Cheng H, Lederer WJ, Cannell MB. Calcium sparks: Elementary events underlying excitation-contraction coupling in heart muscle. Science 1993;262:740–744. [PubMed: 8235594]
3. Lipp P, Niggli E. Modulation of Ca^{2+} release in cultured neonatal rat cardiac myocytes. Insight from subcellular release patterns revealed by confocal microscopy. Circ Res 1994;74:979–990. [PubMed: 8156645]
4. Lopez-Lopez JR, Shacklock PS, Balke CW, Wier WG. Local, stochastic release of Ca^{2+} in voltage-clamped rat heart cells: Visualization with confocal microscopy. J Physiol (Lond) 1994;480:21–29. [PubMed: 7853223]
5. Bers DM. Cardiac excitation-contraction coupling. Nature 2002;415:198–205. [PubMed: 11805843]
6. Guatimosim S, Dilly K, Santana LF, Jafri MS, Sobie EA, Lederer WJ. Local Ca^{2+} signaling and EC coupling in heart: Ca^{2+} sparks and the regulation of the $[Ca^{2+}]i$ transient. J Mol Cell Cardiol 2002;34:941–950. [PubMed: 12234764]
7. Wier WG, Balke CW. Ca^{2+} release mechanisms, Ca^{2+} sparks, and local control of excitation-contraction coupling in normal heart muscle. Circ Res 1999;85:770–776. [PubMed: 10532944]
8. Beuckelmann DJ, Wier WG. Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. J Physiol (Lond) 1988;405:233–255. [PubMed: 2475607]
9. Cannell MB, Berlin JR, Lederer WJ. Effect of membrane potential changes on the calcium transient in single rat cardiac muscle cells. Science 1987;238:1419–1423. [PubMed: 2446391]
10. Cleemann L, Morad M. Role of Ca^{2+} channel in cardiac excitation-contraction coupling in the rat: Evidence from Ca^{2+} transients and contraction. J Physiol (Lond) 1991;432:283–312. [PubMed: 1653321]
11. London B, Krueger JW. Contraction in voltage-clamped, internally perfused single heart cells. J Gen Physiol 1986;88:475–505. [PubMed: 2431095]
12. Sipido KR, Carmeliet E, Van de WF. T-type Ca^{2+} current as a trigger for Ca^{2+} release from the sarcoplasmic reticulum in guinea-pig ventricular myocytes. J Physiol (Lond) 1998;508:439–451. [PubMed: 9508808]
13. Litwin SE, Li J, Bridge JH. Na-Ca exchange and the trigger for sarcoplasmic reticulum Ca release: Studies in adult rabbit ventricular myocytes. Biophys J 1998;75:359–371. [PubMed: 9649393]
14. Bassani RA, Altamirano J, Puglisi JL, Bers DM. Action potential duration determines sarcoplasmic reticulum Ca^{2+} reloading in mammalian ventricular myocytes. J Physiol 2004;559:593–609. [PubMed: 15243136]
15. Sah R, Ramirez RJ, Kaprielian R, Backx PH. Alterations in action potential profile enhance excitation-contraction coupling in rat cardiac myocytes. J Physiol 2001;533:201–214. [PubMed: 11351028]
16. Yue DT, Backx PH, Imredy JP. Calcium-sensitive inactivation in the gating of single calcium channels. Science 1990;250:1735–1738. [PubMed: 2176745]
17. Hume JR, Duan D, Collier ML, Yamazaki J, Horowitz B. Anion transport in heart. Physiol Rev 2000;80:31–81. [PubMed: 10617765]

18. Tohse N. Calcium-sensitive delayed rectifier potassium current in guinea pig ventricular cells. Am J Physiol 1990;258:H1200–H1207. [PubMed: 2331008]
19. Bridge JH, Smolley JR, Spitzer KW. The relationship between charge movements associated with ICa and INa-Ca in cardiac myocytes. Science 1990;248:376–378. [PubMed: 2158147]
20. Kohl P, Hunter P, Noble D. Stretch-induced changes in heart rate and rhythm: Clinical observations, experiments and mathematical models. Prog Biophys Mol Biol 1999;71:91–138. [PubMed: 10070213]
21. Vornanen M, Shepherd N. Restitution of contractility in single ventricular myocytes of guinea pig heart. Cardiovasc Res 1997;33:611–622. [PubMed: 9093531]
22. Wier WG, Yue DT. Intracellular calcium transients underlying the short-term force-interval relationship in ferret ventricular myocardium. J Physiol (Lond) 1986;376:507–530. [PubMed: 2432238]
23. Sobie EA, Song LS, Lederer WJ: Local recovery of Ca^{2+} release in rat ventricular myocytes. J Physiol (Lond) 2005.
24. Brochet DX, Yang D, Di Maio A, Lederer WJ, Franzini-Armstrong C, Cheng H. Ca^{2+} blinks: Rapid nanoscopic store calcium signaling. Proc Natl Acad Sci USA 2005;102:3099–3104. [PubMed: 15710901]
25. Shannon TR, Guo T, Bers DM: Ca^{2+} scraps: Local depletions of free [Ca2+] in cardiac sarcoplasmic reticulum during contractions leave substantial Ca^{2+} reserve. Circ Res 2003.
26. Zucchi R, Ronca-Testoni S. The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: Modulation by endogenous effectors, drugs and disease states. Pharmacol Rev 1997;49:1–51. [PubMed: 9085308]
27. Ching LL, Williams AJ, Sitsapesan R. Evidence for Ca^{2+} activation and inactivation sites on the luminal side of the cardiac ryanodine receptor complex. Circ Res 2000;87:201–206. [PubMed: 10926870]
28. Gyorke I, Gyorke S. Regulation of the cardiac ryanodine receptor channel by luminal Ca^{2+} involves luminal Ca^{2+} sensing sites. Biophys J 1998;75:2801–2810. [PubMed: 9826602]
29. Sobie EA, Dilly KW, Dos Santos CJ, Lederer WJ, Jafri MS. Termination of cardiac Ca^{2+} sparks: An investigative mathematical model of calcium-induced calcium release. Biophys J 2002;83:59–78. [PubMed: 12080100]
30. Terentyev D, Viatchenko-Karpinski S, Valdivia HH, Escobar AL, Gyorke S. Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. Circ Res 2002;91:414–420. [PubMed: 12215490]
31. Gyorke I, Hester N, Jones LR, Gyorke S. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. Biophys J 2004;86:2121–2128. [PubMed: 15041652]
32. Cheng H, Lederer MR, Lederer WJ, Cannell MB. Calcium sparks and $[Ca^{2+}]$i waves in cardiac myocytes. Am J Physiol 1996;270:C148–C159. [PubMed: 8772440]
33. Sham JS, Song LS, Chen Y, Deng LH, Stern MD, Lakatta EG, Cheng H. Termination of Ca^{2+} release by a local inactivation of ryanodine receptors in cardiac myocytes. Proc Natl Acad Sci USA 1998;95:15096–15101. [PubMed: 9844021]
34. Szentesi P, Pignier C, Egger M, Kranias EG, Niggli E. Sarcoplasmic reticulum Ca^{2+} refilling controls recovery from Ca^{2+}-induced Ca^{2+} release refractoriness in heart muscle. Circ Res 2004;95:807–813. [PubMed: 15388639]
35. Song LS, Sham JS, Stern MD, Lakatta EG, Cheng H. Direct measurement of SR release flux by tracking 'Ca^{2+} spikes' in rat cardiac myocytes. J Physiol (Lond) 1998;512:677–691. [PubMed: 9769413]
36. Windle JD. The incidence and prognostic value of the pulsus alternans in myocardial and arterial disease. Q J Med 1913;6:462.
37. Lewis T. Notes up alternation of the heart. Q J Med 1910;4:141–144.
38. Hohnloser SH, Klingenheben T, Li YG, Zabel M, Peetermans J, Cohen RJ. T wave alternans as a predictor of recurrent ventricular tachyarrhythmias in ICD recipients: Prospective comparison with conventional risk markers. J Cardiovasc Electrophysiol 1998;9:1258–1268. [PubMed: 9869525]

39. Ikeda T, Saito H, Tanno K, Shimizu H, Watanabe J, Ohnishi Y, Kasamaki Y, Ozawa Y. T-wave alternans as a predictor for sudden cardiac death after myocardial infarction. Am J Cardiol 2002;89:79–82. [PubMed: 11779531]
40. Platt SB, Vijgen JM, Albrecht P, Van Hare GF, Carlson MD, Rosenbaum DS. Occult T wave alternans in long QT syndrome. J Cardiovasc Electrophysiol 1996;7:144–148. [PubMed: 8853024]
41. Rosenbaum DS, Jackson LE, Smith JM, Garan H, Ruskin JN, Cohen RJ. Electrical alternans and vulnerability to ventricular arrhythmias. N Engl J Med 1994;330:235–241. [PubMed: 8272084]
42. Pastore JM, Girouard SD, Laurita KR, Akar FG, Rosenbaum DS. Mechanism linking T-wave alternans to the genesis of cardiac fibrillation. Circulation 1999;99:1385–1394. [PubMed: 10077525]
43. Nolasco JB, Dahlen RW. A graphic method for the study of alternation in cardiac action potentials. J Appl Physiol 1968;25:191–196. [PubMed: 5666097]
44. Courtemanche M, Glass L, Keener JP. Instabilities of a propagating pulse in a ring of excitable media. Phys Rev Lett 1993;70:2182–2185. [PubMed: 10053491]
45. Karma A. Electrical alternans and spiral wave breakup in cardiac tissue. Chaos 1994;4:461–472. [PubMed: 12780121]
46. Qu Z, Weiss JN, Garfinkel A. Spatiotemporal chaos in a simulated ring of cardiac cells. Phys Rev Lett 1997;78:1387–1390.
47. Garfinkel A, Kim YH, Voroshilovsky O, Qu Z, Kil JR, Lee MH, Karagueuzian HS, Weiss JN, Chen PS. Preventing ventricular fibrillation by flattening cardiac restitution. Proc Natl Acad Sci USA 2000;97:6061–6066. [PubMed: 10811880]
48. Riccio ML, Koller ML, Gilmour RF Jr. Electrical restitution and spatiotemporal organization during ventricular fibrillation. Circ Res 1999;84:955–963. [PubMed: 10222343]
49. Boyett MR, Jewell BR. Analysis of the effects of changes in rate and rhythm upon electrical activity in the heart. Prog Biophys Mol Biol 1980;36:1–52. [PubMed: 7001542]
50. Koller ML, Riccio ML, Gilmour RF Jr. Dynamic restitution of action potential duration during electrical alternans and ventricular fibrillation. Am J Physiol 1998;275:H1635–H1642. [PubMed: 9815071]
51. Kalb SS, Dobrovolny HM, Tolkacheva EG, Idriss SF, Krassowska W, Gauthier DJ. The restitution portrait: A new method for investigating rate-dependent restitution. J Cardiovasc Electrophysiol 2004;15:698–709. [PubMed: 15175067]
52. Goldhaber JI, Xie LH, Duong T, Motter C, Khuu K, Weiss JN. Action potential duration restitution and alternans in rabbit ventricular myocytes: The key role of intracellular calcium cycling. Circ Res 2005;96:459–466. [PubMed: 15662034]
53. Pruvot EJ, Katra RP, Rosenbaum DS, Laurita KR. Role of calcium cycling versus restitution in the mechanism of repolarization alternans. Circ Res 2004;94:1083–1090. [PubMed: 15016735]
54. Banville I, Chattipakorn N, Gray RA. Restitution dynamics during pacing and arrhythmias in isolated pig hearts. J Cardiovasc Electrophysiol 2004;15:455–463. [PubMed: 15089996]
55. Walker ML, Wan X, Kirsch GE, Rosenbaum DS. Hysteresis effect implicates calcium cycling as a mechanism of repolarization alternans. Circulation 2003;108:2704–2709. [PubMed: 14581412]
56. Kihara Y, Morgan JP. Abnormal Cai2+ handling is the primary cause of mechanical alternans: Study in ferret ventricular muscles. Am J Physiol 1991;261:H1746–H1755. [PubMed: 1750531]
57. Lab MJ, Lee JA. Changes in intracellular calcium during mechanical alternans in isolated ferret ventricular muscle. Circ Res 1990;66:585–595. [PubMed: 2306800]
58. Spear JF, Moore EN. A comparison of alternation in myocardial action potentials and contractility. Am J Physiol 1971;220:1708–1716. [PubMed: 5087820]
59. Chudin E, Goldhaber J, Garfinkel A, Weiss J, Kogan B. Intracellular Ca^{2+} dynamics and the stability of ventricular tachycardia. Biophys J 1999;77:2930–2941. [PubMed: 10585917]
60. Diaz ME, O'Neill SC, Eisner DA. Sarcoplasmic reticulum calcium content fluctuation is the key to cardiac alternans. Circ Res 2004;94:650–656. [PubMed: 14752033]
61. Huser J, Wang YG, Sheehan KA, Cifuentes F, Lipsius SL, Blatter LA. Functional coupling between glycolysis and excitation-contraction coupling underlies alternans in cat heart cells. J Physiol 2000;524:795–806. [PubMed: 10790159]

62. Franz MR. The electrical restitution curve revisited: Steep or flat slope—which is better? J Cardiovasc Electrophysiol 2003;14:S140–S147. [PubMed: 14760916]

63. Ideker RE, Rogers JM, Gray RA. Steepness of the restitution curve: A slippery slope? J Cardiovasc Electrophysiol 2002;13:1173–1175. [PubMed: 12475112]

64. Bassani JWM, Yuan W, Bers DM. Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. Am J Physiol 1995;268:C1313–C1319. [PubMed: 7762626]

65. Shannon TR, Ginsburg KS, Bers DM. Potentiation of fractional sarcoplasmic reticulum calcium release by total and free intra-sarcoplasmic reticulum calcium concentration. Biophys J 2000;78:334–343. [PubMed: 10620297]

66. Trafford AW, Diaz ME, Sibbring GC, Eisner DA. Modulation of CICR has no maintained effect on systolic Ca2+: Simultaneous measurements of sarcoplasmic reticulum and sarcolemmal Ca2+ fluxes in rat ventricular myocytes. J Physiol 2000;522:259–270. [PubMed: 10639102]

67. Spencer CI, Berlin JR. Control of sarcoplasmic reticulum calcium release during calcium loading in isolated rat ventricular myocytes. J Physiol 1995;488:267–279. [PubMed: 8568669]

68. Trafford AW, Diaz ME, Negretti N, Eisner DA. Enhanced Ca2+ current and decreased Ca2+ efflux restore sarcoplasmic reticulum Ca2+ content after depletion. Circ Res 1997;81:477–484. [PubMed: 9314828]

69. Shiferaw Y, Watanabe MA, Garfinkel A, Weiss JN, Karma A. Model of intracellular calcium cycling in ventricular myocytes. Biophys J 2003;85:3666–3686. [PubMed: 14645059]

70. Diaz ME, Eisner DA, O'Neill SC. Depressed ryanodine receptor activity increases variability and duration of the systolic Ca2+ transient in rat ventricular myocytes. Circ Res 2002;91:585–593. [PubMed: 12364386]

71. Litwin SE, Zhang D, Bridge JH. Dyssynchronous Ca^{2+} sparks in myocytes from infarcted hearts. Circ Res 2000;87:1040–1047. [PubMed: 11090550]

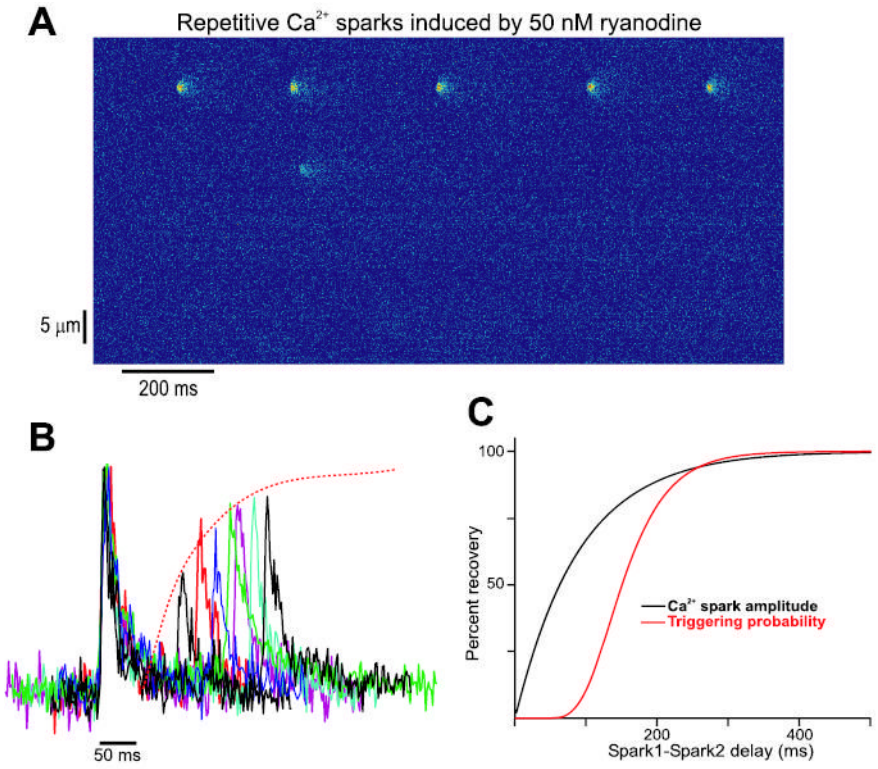

Figure 1.

Restitution of Ca^{2+} sparks in rat myocytes. (A) Addition of 50 nM ryanodine to a quiescent rat ventricular myocyte loaded with the Ca^{2+}-sensitive dye fluo-3 causes a limited number of Ca^{2+} spark sites to display repetitive activity. Spark pairs derived from repeating sites such as the one near the top of the line scan image displayed can be analyzed to probe the time course of Ca^{2+} spark restitution. (B) Example of Ca^{2+} spark pairs recorded in rat ventricular myocytes after application of 50 nM ryanodine. Individual amplitudes of the initial Ca^{2+} sparks within each pair have been normalized so that spark pairs could be overlaid. Initial Ca^{2+} sparks display very similar time courses. The amplitude of the second spark in each pair, relative to the first, increases with an increase in the delay between sparks. (C) Ca^{2+} spark amplitude and triggering probability recovery functions derived from the analysis of spark pairs. Several potential regulatory factors, discussed in the text, could plausibly account for roughly 90 msec delay (measured at 50% recovery) between the two plots. The results shown have been replotted from the original data discussed in a recent study,[23] with the permission of the authors.

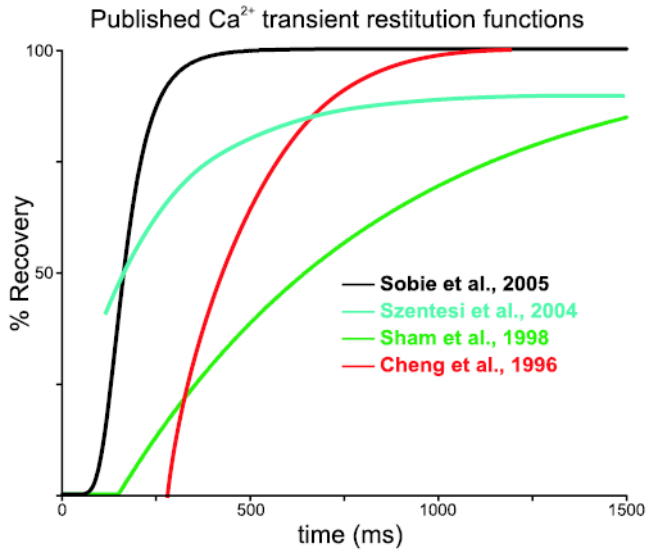

Figure 2.

Comparison of Ca^{2+} transient restitution functions measured in different studies. Ca^{2+} transient recovery functions obtained in four recent studies displayed on a single set of axes to allow for easier comparisons. As explained in the text, differences in the experimental conditions and/or protocols used to assess restitution may account for the quantitative differences observed.

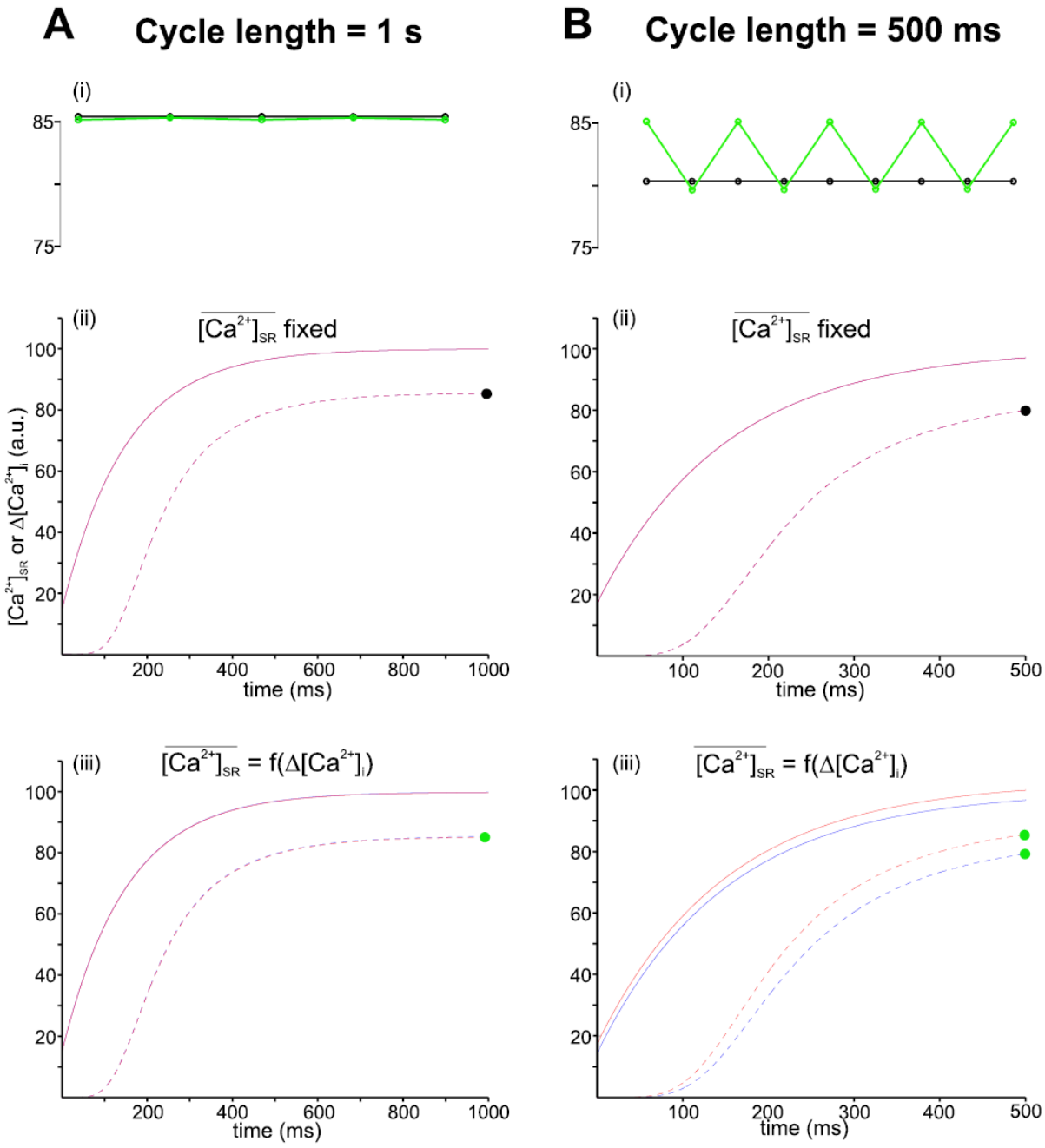

Figure 3.

Factors that may contribute to Ca^{2+} *transient alternans. Simulations were performed with a simple, phenomenological model to explore the characteristics of* Ca^{2+} *release restitution that may contribute to rate-dependent* Ca^{2+} *transient alternans.* Ca^{2+} *transient amplitude is stable from one beat to the next at a pacing rate of* 1 Hz (A), *but* Ca^{2+} *transient alternans may develop, depending on model choices, at a pacing rate of* 2 Hz (B). *The top plots (i) display the quantity of* Ca^{2+} *released at each beat* ($\Delta[Ca^{2+}]_i$) *for the last* 5 (at 1Hz) *or last* 9 (at 2 Hz) *beats in a sequence of* 40 *or* 80, *respectively. The middle (ii) and bottom (iii) plots show how the* SR Ca^{2+} *content* ($[Ca^{2+}]_{SR}$) *and* $\Delta[Ca^{2+}]_i$ *recover with time between beats during the next-to-last (blue) and last (red) beats in each sequence. The points on the* Ca^{2+} *transient recovery curves*

in (ii) and (iii) corresponding to the amplitude plots in (i) are marked with black and green dots as appropriate. The key features of the model that lead to alternans are a nonlinear dependence of $\Delta[Ca^{2+}]_i$ *on* $\Delta [Ca^{2+}]_{SR}$ *and the dependence of the value toward which* $[Ca^{2+}]_{SR}$ *increases between beats* ($[\overline{Ca^{2+}}_{SR}]$) *on the quantity just released. Specifically, in the simulations indicated with the green symbols at the top and plotted on the bottom graphs,* $\Delta[Ca^{2+}]_i = P_{TRIG} * [Ca^{2+}]_{SR}$ *and* $P_{TRIG} = [Ca^{2+}]^n_{SR} / ([Ca^{2+}]^n_{SR} + K_m{}^n)$ *where the exponent n is set at 8 and the SR load that produced* 50% *release,* K_m *is equal to* 80. *Also in these simulations,* $[\overline{Ca^{2+}}_{SR} = -0.52 * (\Delta[Ca^{2+}]_i - 85) + 100$. *In the simulations represented with black symbols at the top and displayed in the middle plots,* $[\overline{Ca^{2+}}_{SR}$ *does not depend on* $\Delta[Ca^{2+}]_i$ *and is equal to 100 after all beats. In additional simulations not shown,* P_{TRIG} *was assumed to not depend on* $[Ca^{2+}]_{SR}$ *but instead to drop to zero immediately after each release and then exponentially rise toward a maximum value of 0.8 with a time constant of* 200 msec. *Either of these changes to the model abolished alternans at a pacing rate of 2 Hz, confirming that these nonlinear elements are responsible for the instability.*