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The Ca^{2+} leak paradox and “rogue ryanodine receptors”: SR Ca^{2+} efflux theory and practice

Eric A. Sobie^{a,b}, Silvia Guatimosim^{a,c}, Leticia Gómez-Viquez^a, Long-Sheng Song^a, Hali Hartmann^a, M. Saleet Jafri^d, and W.J. Lederer^{a,e,*}

^a Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard Street, Baltimore, MD 21201, USA

^b Department of Pediatrics, NY University School of Medicine, New York, NY, USA

^c Department of Physiology and Biophysics, Federal University of Minas Gerais, Brazil

^d School of Computational Sciences, George Mason University

^e Department of Physiology, University of Maryland Baltimore

Abstract

Ca^{2+} efflux from the sarcoplasmic reticulum (SR) is routed primarily through SR Ca^{2+} release channels (ryanodine receptors, RyRs). When clusters of RyRs are activated by trigger Ca^{2+} influx through L-type Ca^{2+} channels (dihydropyridine receptors, DHPR), Ca^{2+} sparks are observed. Close spatial coupling between DHPRs and RyR clusters and the relative insensitivity of RyRs to be triggered by Ca^{2+} together ensure the stability of this positive-feedback system of Ca^{2+} amplification. Despite evidence from single channel RyR gating experiments that phosphorylation of RyRs by protein kinase A (PKA) or calcium-calmodulin dependent protein kinase II (CAMK II) causes an increase in the sensitivity of the RyR to be triggered by $[\text{Ca}^{2+}]_i$ there is little clear evidence to date showing an increase in Ca^{2+} spark rate. Indeed, there is some evidence that the SR Ca^{2+} content may be decreased in hyperadrenergic disease states. The question is whether or not these observations are compatible with each other and with the development of arrhythmogenic extrasystoles that can occur under these conditions. Furthermore, the appearance of an increase in the SR Ca^{2+} “leak” under these conditions is perplexing. These and related complexities are analyzed and discussed in this report. Using simple mathematical modeling discussed in the context of recent experimental findings, a possible resolution to this paradox is proposed. The resolution depends upon two features of SR function that have not been confirmed directly but are broadly consistent with several lines of indirect evidence: (1) the existence of unclustered or “rogue” RyRs that may respond differently to local $[\text{Ca}^{2+}]_i$ in diastole and during the $[\text{Ca}^{2+}]_i$ transient; and (2) a decrease in cooperative or coupled gating between clustered RyRs in response to physiologic phosphorylation or hyperphosphorylation of RyRs in disease states such as heart failure. Taken together, these two features may provide a framework that allows for an improved understanding of cardiac Ca^{2+} signaling.

1. Introduction

The amount of Ca^{2+} within the sarcoplasmic reticulum (SR) plays a central role in cardiac Ca^{2+} signaling in ventricles. During normal excitation–contraction coupling in adult hearts, this Ca^{2+} store contributes 70–90% (depending on species) of the $[\text{Ca}^{2+}]$ transient that leads to the initiation of contraction. Far from being a passive repository of excess Ca^{2+} ions, however, the Ca^{2+} content within the SR ($[\text{Ca}^{2+}]_{\text{SR}}$) serves as a critical regulator of the release

* Corresponding author. Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard Street, Baltimore, MD 21201, USA. Tel.: +1 410 706 8182; fax: +1 410 510 1545. E-mail address: lederer@umbi.umd.edu (W.J. Lederer).

process itself. Dynamic decreases in local $[Ca^{2+}]_{SR}$ are thought to aid in terminating the elementary units of release, Ca^{2+} sparks (Sobie et al., 2002; Terentyev et al., 2002), and, when SR Ca^{2+} content is excessive, regenerative release of Ca^{2+} is thought to produce arrhythmias (Lederer and Tsien, 1976; Kass et al., 1978; Berlin et al., 1989; Cheng et al., 1996). Here we discuss the balance of influx of Ca^{2+} into the SR and its release through Ca^{2+} sparks and Ca^{2+} “leak”. Ca^{2+} “leak” is broadly defined as loss of Ca^{2+} from the SR under resting or quiescent conditions. This includes SR Ca^{2+} efflux visible experimentally as Ca^{2+} sparks, but may also, we will argue below, include the silent loss of Ca^{2+} from the SR. At any moment the Ca^{2+} content of the SR depends on the balance between Ca^{2+} leak and Ca^{2+} uptake as well as the amount previously present.

Since dynamic regulation of the SR Ca^{2+} content occurs during physiological perturbations in heart function and in diverse cardiac diseases (Gomez et al., 1997; Pogwizd et al., 2001), a thoughtful analysis of the regulatory principles of SR Ca^{2+} balance can lead to an improved understanding of heart function. Recent findings have led to an increased appreciation of the complexity of the regulatory elements as the molecular details that underlie the regulation unfold (Cheng et al., 1993; Gomez et al., 1997; Marx et al., 2000; Pogwizd et al., 2001; Trafford et al., 2001; Diaz et al., 2004) and the relevant governing rules are debated (Marks, 2003; Bers et al., 2003). The SR Ca^{2+} influx is primarily due to the SR/endoplasmic reticulum (ER) Ca^{2+} -ATPase (SERCA), a protein found widely in both SR and ER. SR Ca^{2+} efflux occurs principally through Ca^{2+} release channels in the SR/ER membrane. In muscle the ryanodine receptor (RyR) is the primary Ca^{2+} release channel but inositol trisphosphate receptors (IP3Rs) are also present. The role(s), if any, of IP3Rs in ventricular myocytes remain elusive.

The RyRs are largely clustered in near-crystalline arrays and exhibit interactive group behavior. Gating experiments performed in planar lipid bilayers suggest that these channels tend to open and close together and that this coupling is mediated by a regulatory protein known as FKBP 12.6 (Marx et al., 2001). Computer modeling studies that have incorporated cooperative or allosteric interactions between RyRs have suggested that such inter-channel interactions may provide important regulation of Ca^{2+} release (Stern et al., 1999; Sobie et al., 2002). There is growing evidence that RyRs are distributed both in clusters at the junctions between the SR and the transverse tubules (TT) (Franzini-Armstrong et al., 1998, 1999) and also as individual RyRs associated with other structures (Brookes et al., 2004; Pare et al., 2005). In addition to the clustered RyRs that are found at the jSR, we are also interested in the unknown fraction of non-junctional RyRs. Non-clustered RyRs in the SR membrane have not been visualized directly, but their existence is suggested by the finding that under certain conditions SR Ca^{2+} release may occur via a spatially uniform, non-spark mechanism (Lipp et al., 2002). In this discussion, we refer to isolated, non-junctional RyRs as “rogue RyRs” and the jSR RyRs as “clustered” or “junctional” RyRs. RyRs in “corbular” SR are also clustered as in the jSR but are distinguished from the latter in that they are not apposed to a triggering L-type Ca^{2+} channel (dihydropyridine receptor, DHPR). The function of these RyRs may also have important regulatory implications but will not be discussed further here. The distributed nature of the SR within a cell suggests that this organelle is highly interconnected such that Ca^{2+} influx or efflux in one region will, in principle, affect the Ca^{2+} content of the remaining SR, albeit with a time-delay.

Here we examine how the spatial distribution of SR Ca^{2+} release channels and changes in RyR gating may contribute to the regulation of Ca^{2+} content. This presentation includes new, relatively simple mathematical models relevant to Ca^{2+} signaling in ventricular myocytes and a novel analysis of information that we and others have published. This work seeks to develop testable hypotheses and practical insight regarding Ca^{2+} signaling in heart cells that broadens our understanding of specific features for cardiovascular disease and molecular medicine.

2. The paradox

Our analysis is motivated largely by the difficulty of reconciling disparate and sometimes controversial experimental results related to the pathophysiology of heart failure (HF). HF is generally acknowledged to be a disease state in which normal beta-adrenergic signaling is disrupted. Even though circulating levels of catecholamines are constitutively higher than in healthy persons, beta-adrenergic receptors appear to be down-regulated, and the robust responsiveness to agents such as isoproterenol seen in healthy heart cells is severely attenuated (Marks, 2001; Chien et al., 2003). Biochemical studies have indicated that this abnormal, sustained “hyperadrenergic” state leads to excess phosphorylation of RyRs (Marx et al., 2000), although this result remains controversial (Jiang et al., 2002). Complementary planar lipid bilayer studies of RyR gating have predicted that this hyperphosphorylation will lead to increased RyR open probability. Additional key results are that RyR phosphorylation causes dissociation of FKBP12.6 from the RyR macromolecular complex (Marx et al., 2000) and that in HF RyRs display decreased binding to this regulatory protein (Ono et al., 2000). Together, one might expect that these effects would lead to increased Ca^{2+} leak from the SR and an increased rate of Ca^{2+} sparks in quiescent ventricular myocytes isolated from failing hearts. However, studies of Ca^{2+} sparks and EC coupling in HF have failed to detect an increased Ca^{2+} spark rate (Gomez et al., 1997). One significant complicating factor, however, is that other changes in Ca^{2+} cycling seen in HF, namely decreased activity of SERCA and increased activity of Na^{+} - Ca^{2+} exchange (NCX), would lead to decreased SR load. This effect, by causing a decrease in spark rate independent of effects on RyR phosphorylation, could have hidden the putative increase in Ca^{2+} spark rate. However, Li et al. did not observe an increase in Ca^{2+} spark rate upon protein kinase A (PKA) phosphorylation even when the SR load was made the same as in control conditions (Li et al., 2002). The picture has been further clouded by the decrease in SR Ca^{2+} leak seen in cultured adult myocytes upon over-expression of FKBP12.6 (Prestle et al., 2001), the relatively robust survival of mice lacking FKBP12.6 (Xin et al., 2002; Wehrens et al., 2003), and more recent results demonstrating the overall rate of Ca^{2+} leak from the SR is in fact increased at a given SR load after beta-adrenergic stimulation (Lindegger and Niggli, 2005) and in myocytes isolated from failing hearts (Shannon et al., 2003). This has forced a re-examination of the question of whether all diastolic SR leak can be accounted for by the resting rate of Ca^{2+} sparks. Since it is clear that a significant percentage of the SR Ca^{2+} efflux can be accounted for by measuring Ca^{2+} sparks (Bers, 2001), this question has seemed to be of only secondary importance, but the confusing results mentioned above suggest that this question should be considered anew.

This discussion of diastolic Ca^{2+} leak in healthy versus diseased heart cells may appear to be a minor technical issue of secondary importance, but it is intimately related to a much broader paradox regarding the pathophysiology of this disease state. Changes in Ca^{2+} cycling that act to reduce SR Ca^{2+} load would be expected to increase the stability of Ca^{2+} release, but patients with HF suffer from arrhythmias that are thought to be related to improper Ca^{2+} cycling and are generally associated with increased rather than decreased SR Ca^{2+} load, so-called “ Ca^{2+} overload” arrhythmias. One important factor, demonstrated elegantly by Pogwizd et al. (Pogwizd et al., 2001), may be that reduced background K^{+} current (I_{K1}) and increased NCX current can act synergistically such that a spontaneous cell-wide release of Ca^{2+} will lead to a larger membrane depolarization, and thus be potentially more arrhythmogenic, than an equivalent Ca^{2+} release in a healthy cell. Nonetheless, this does not answer the question of what led to the spontaneous Ca^{2+} release in the first place. The high rate of mortality due to arrhythmia suggests that regenerative cellular Ca^{2+} release in the form of propagating Ca^{2+} waves is far more common in HF, but the mechanisms underlying this, and potential links to diastolic SR Ca^{2+} leak, remain murky.

In this paper, we explore new hypotheses that may account for some of these apparent contradictions. One novel suggestion is the idea that “rogue” RyR2s may be responsible for SR Ca^{2+} leak that cannot be observed directly. Other seeming contradictions may result from changes that can be attributed to the increased non-uniformity of Ca^{2+} spark activation and SR Ca release.

3. Results and discussion

3.1. Nano-scale and micro-scale Ca^{2+} signaling

Fig. 1 displays simulation results of the spatial spread of Ca^{2+} within and between RyR clusters to illustrate some of the pertinent “boundary conditions.” The quantitative $[\text{Ca}^{2+}]$ gradients produced in cells depend on many details that remain uncertain, including fluxes through DHPR and RyR channels, the spatial distribution of Ca^{2+} buffers, and possible cellular diffusion barriers, but simulations such as those shown are nonetheless valuable to place constraints on thinking. Fig. 1A shows “nano-scale” Ca^{2+} signaling, the predicted spread of local $[\text{Ca}^{2+}]_i$ within the subspace between the TT membrane that contains the DHPR and the jSR membrane that contains the RyR cluster. When the DHPR is open, a gradient of $[\text{Ca}^{2+}]_i$ centered around the channel exists within the subspace, and $[\text{Ca}^{2+}]$ is estimated to be elevated above $10 \mu\text{M}$ within $\pm 60 \text{ nm}$ of the channel mouth. This $[\text{Ca}^{2+}]$ elevation ought to be sufficient to activate RyRs in the jSR and trigger a locally regenerative Ca^{2+} spark from the cluster. The compressed geometry of the fuzzy space facilitates this communication through Ca^{2+} by restricting diffusion. Since single channel RyR current has a similar amplitude under physiological conditions (Kettlun et al., 2003), this result also implies that spontaneous openings of RyR channels situated within clusters can, with some finite probability, activate neighboring channels via Ca^{2+} -induced Ca^{2+} release (CICR).

Once a spark is triggered the nature of slightly more macroscopic $[\text{Ca}^{2+}]_i$ signaling is shown in Figs. 1B and C. The spatial separation between RyR clusters and the steep gradients observed in three-dimensional diffusion ensure that neighboring RyR clusters only experience small increases in $[\text{Ca}^{2+}]$. At distances greater than $1 \mu\text{m}$ from the source, Ca^{2+} does not increase much above the resting level of 100 nM . However, $[\text{Ca}^{2+}]_i$ is elevated to 351 nM at a distance 500 nm from the center, an issue that could potentially be important if RyR clusters are relatively tightly packed.

3.2. Effect of RyR coupling on $[\text{Ca}^{2+}]_i$ sensitivity

As mentioned, planar lipid bilayer gating experiments suggest that RyRs can exhibit cooperativity or coupling, a feature consistent in principle with the tightly packed arrangement of clustered RyRs in cells. Computer modeling studies that have incorporated this feature have demonstrated that coupling can result in important functional consequences (Stern et al., 1999), including a role in Ca^{2+} spark termination (Sobie et al., 2002). Inter-RyR interactions that can lead to coupled behavior and results from a stochastic, Monte-Carlo computer model that included such interactions are shown in Fig. 2. Fig. 2A schematically illustrates the state transitions of an RyR energetically coupled to two neighbors, following a strategy used in studies of bacterial chemotaxis (Duke and Bray, 1999; Duke et al., 2001) and similar in spirit to numerous previous studies of allostery in various biological systems (see Changeux and Edelstein (1998), Bray and Duke (2004) for review). The diagram in Fig. 2A refers to the potential transitions of the middle channel in each group of three. If both neighbors are in the same state as the middle channel, this state will be energetically stabilized by a variable coupling energy E_J , and transitions to the opposite state will be unlikely. Sample results from a stochastic model incorporating these inter-RyR interactions are shown in Fig. 2B. Eight RyRs arranged in a ring open and close independently when coupling is absent (top trace) but tend to open and close together when coupling is strong ($E_J = 0.8kT$; bottom trace). An important

consequence of cooperativity between RyRs is that the steady-state open probability of the group will have a much steeper dependence on $[Ca^{2+}]_i$ than an isolated channel. When $[Ca^{2+}]_i$ is below the concentration at which the open probability of a single channel is 50%, the largely closed RyRs will discourage transitions to the open state and tend to keep the remaining channels closed. When the population opens it will tend to open abruptly (i.e. with a steeper $[Ca^{2+}]_i$ dependence). Fig. 2C compares results of the Monte-Carlo simulations under conditions of strong coupling (filled circles, dashed line) with the prediction of P_O versus $[Ca^{2+}]_i$ for an isolated channel (solid black line). Although the dashed curve was generated with stochastic simulations, a closed-form solution for a relatively simple system such as this can be derived (see Rice et al., 2003). Two other ways to consider this behavior are shown in Fig. 3, which schematically compares the open probability of a coupled cluster with that of an uncoupled channel. Coupling can lead to an increase or a decrease in P_O , depending on the baseline P_O , and, hence, the cytosolic $[Ca^{2+}]_i$. Two important implications of this result are as follows. First, this may explain, at least in part, why Li et al. (Li et al., 2002), who performed experiments in skinned cardiac myocytes at very low ambient $[Ca^{2+}]_i$ (10 and 50 nM), failed to detect an increase in Ca^{2+} spark frequency after PKA phosphorylation of RYRs. Second, these predictions are consistent in principle with the planar lipid bilayer results of Valdivia et al. (Valdivia et al., 1995), who observed that PKA phosphorylation of isolated RyRs caused an increase in the peak channel P_O after rapid jumps in $[Ca^{2+}]$ but little increase at 100 nM.

3.3. Effects of coupling on diastolic Ca^{2+} sparks

To examine how changes in coupling might affect the resting Ca^{2+} spark rate, we generated simulated line-scan images with a simplified but nonetheless probabilistic model of Ca^{2+} spark triggering, as shown in Fig. 4. Simulated Ca^{2+} spark line-scan images produced by a previously published computer model (Sobie et al., 2002) were added to a simulated line-scan where random variables indicated they had occurred. Details of this model are provided in the figure legend. Line-scan images as might be obtained with strong coupling and with no coupling are compared in Figs. 4A and B, respectively. Identical numbers of spontaneous Ca^{2+} sparks seem to occur under the two conditions. The detailed views in Fig. 4C of the two “sparks” indicated by the white boxes, (right) however, shows an important but subtle model prediction. Because weakly coupled RyR clusters display an increased sensitivity to triggering by small increases in $[Ca^{2+}]_i$ above the diastolic level, there is an increased probability that one Ca^{2+} spark will trigger a second spark from a neighboring RyR cluster. The probability is not high enough to produce any cell-wide instability and thus is not readily observed, but uncoupling, as modeled here, leads to an increased occurrence of “double sparks” such as that shown, events which are virtually never observed in control conditions. Without unusual care, the change in the Ca^{2+} spark appearance might not be observed. If, however, some additional factor, such as a transient increase in SR load, were to increase the sensitivity of RyRs, then instability could occur and arrhythmias would become prominent. This combination of factors may account for the increased prevalence of delayed afterdepolarizations following exercise in FKBP 12.6 knockout mice seen by Wehrens et al. (Wehrens et al., 2003).

3.4. Effect of PKA phosphorylation on RyR2 opening

Fig. 5A compares the relative P_O of RyRs when activated by $[Ca^{2+}]_i$ under steady-state conditions in the presence and absence of PKA phosphorylation. The separate experiments are presented in Wehrens et al. (2003), Fig. 4B and re-analyzed to compare the effects of PKA phosphorylation. These are steady-state experiments but are largely consistent with the findings of others who also explored the effects of transient increases in $[Ca^{2+}]_i$ (Valdivia et al., 1995). The relative effect of PKA on P_O is shown in Fig. 5B, revealing that PKA phosphorylation of RyR increases P_O at low to moderate $[Ca^{2+}]_i$ levels but tends to decrease the relative P_O at higher levels of $[Ca^{2+}]_i$. A similar overall result is found if one compares a control RyR2s with those containing a mutation that is known to be associated with

catecholaminergic polymorphic ventricular tachycardias (CPVT) in human patients (R2474S). This mutation causes a similar but much larger change in the curve describing the relative increase (or decrease) in P_O following PKA phosphorylation of the mutant RyR2.

3.5. Role of “leak” versus the triggering of extrasystoles and of arrhythmias

RyRs in the SR membrane that are not part of clusters, or “rogue RyRs,” may provide a pathway through which SR Ca^{2+} leak may occur in a largely silent or invisible mode. Fig. 6 displays the results of simulations of the steady-state SR load that explore this possibility. These simulations compute the equilibrium $[\text{Ca}^{2+}]_{\text{SR}}$ in a resting myocyte assuming that the SERCA pump uptake rate depends linearly on diastolic $[\text{Ca}^{2+}]_i$ and that Ca^{2+} can exit the SR through either clustered or rogue RyRs. The model assumes that, in control conditions, rogue channels are twice as likely to open as RyRs in clusters (solid black line). This could theoretically occur because small elevations in local $[\text{Ca}^{2+}]$, such as those occurring when Ca^{2+} sparks arise spontaneously in the general vicinity, would be more likely to activate the uncoupled rogue channels (see Fig. 2C).

When there are no rogue RyRs, the model system suggests that the SR Ca^{2+} level does not change even when the RyRs are phosphorylated by PKA. This finding is consistent with all of the experimental results to date. If, however, there are rogue RyRs, the Ca^{2+} content of the SR declines to a new steady state that depends on the fraction of RyRs that are rogue (see solid black curve). This occurs because there may be brief openings of the rogue RyRs that do not occur with the jSR RyRs. Assuming that one examines cells at a given rogue fraction and then phosphorylates the RyRs via PKA, the question is: What happens to SR Ca^{2+} content?

The model predicts that there will be a further decrease in SR Ca^{2+} when PKA phosphorylation occurs but that this further decrease is due entirely to the rogue RyRs (dashed black line). There are important assumptions that appear to be consistent with many experimental findings. But the one element that has not yet been properly documented is the relative number of rogue RyRs.

Paradox possibly resolved—In this short article, we have discussed the mechanisms that regulate steady-state SR Ca^{2+} content in resting ventricular myocytes and how these processes may be altered physiologically and in disease states. In so doing, we have proposed new hypotheses for how Ca^{2+} “leak” from the SR may be increased without an obvious increase in the Ca^{2+} spark rate. One possibility is that rogue RyRs can operate almost invisibly to produce a fraction of the overall Ca^{2+} leak. Another important factor may be coupling between clustered RyRs, the disruption of which may destabilize the Ca^{2+} release system. Together these ideas, to the extent that they are validated by new experimental data, may help to resolve apparent paradoxes and provide insight into the cellular regulation of Ca^{2+} cycling in healthy and diseased hearts.

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Fig. 1.

Nano-scale and micro-scale Ca^{2+} signaling in heart cells. (A) Ca^{2+} signaling within the restricted “fuzzy” space between the sarcolemmal and SR membranes containing a functional release unit (diagram). The plot shows a prediction of $[Ca^{2+}]$ versus distance from the center of an L-type Ca^{2+} channel (gray) assumed to conduct 0.5 pA for an open time of 0.5 ms. This brief opening produces a local elevation in $[Ca^{2+}]$ sufficient to trigger Ca^{2+} release from RyRs (black) located within ± 60 nm. The compressed geometry of the fuzzy space facilitates this triggering. Since single channel RyR current has a similar amplitude under physiological conditions (Kettlun et al., 2003), this result also implies that spontaneous openings of RyR channels situated within clusters can, with some finite probability, activate neighboring channels via Ca^{2+} -induced Ca^{2+} release. (B) Ca^{2+} signaling between release sites. Because RyR clusters are spatially separated, Ca^{2+} release from one cluster does not usually trigger release from neighboring clusters. Release from a particular cluster therefore only occurs if Ca^{2+} enters the cell through an L-type Ca^{2+} channel situated nearby. (C) Calculation of $[Ca^{2+}]$ in the region surrounding a cluster at the end of a simulated Ca^{2+} spark (2 pA, 10 ms duration). This calculation was performed with a published model of cellular Ca^{2+} diffusion and buffering (Sobie et al., 2002), with recent findings of cellular buffering power incorporated (Vadakkadath et al., 2004). The ability of Ca^{2+} released from a cluster to potentially trigger

release from neighboring clusters depends on the spatial separation between the clusters and the sensitivity of the clusters to small increases in $[Ca^{2+}]$. At a distance of $\pm 1 \mu m$, Ca^{2+} does not increase above the resting level of 100 nM. However, at 500 nm from the source, $[Ca^{2+}]$ is elevated to 351 nM at the end of the assumed 10 ms period of release.

Fig. 2.

Model of coupling between cardiac RyRs. (A) Schematic of energetic interactions through which RyRs within a cluster are coupled, following the strategy outlined by Bray and coworkers (Duke and Bray, 1999; Duke et al., 2001). The difference in energy between the open and closed states of an isolated RyR (top) determines the channel's equilibrium open probability (P_O). For clarity, we display the open and closed states at the same energy ($P_O = 0.5$) in the figure, but in general this energy difference is $[Ca^{2+}]$ dependent. The energy of a channel within a cluster (bottom) also depends on the states of the channel's two nearest neighbors, as represented schematically for the middle channel in each group of three. Each neighboring channel in the same state reduces the channel's energy by E_J whereas each channel in the opposite state increases the energy by E_J . This inter-channel coupling makes certain transitions more or less likely, as indicated by the length and thickness of the arrows. (B) Example Monte-Carlo simulation of a group of eight RyRs, arranged in a ring, gating stochastically. In this example the equilibrium P_O of an isolated channel is 0.5, and the activation energy for each transition is assumed to be equal to $1kT$. For this example we selected simulations performed with eight RyRs so that individual transitions could be visualized and arranged the channels in a ring to preserve periodic boundary conditions (each channel is coupled to two neighbors), but the general behavior of the model does not depend on these specifics. Without coupling the trace of composite P_O versus time resembles white noise (top trace). However, when channels are strongly coupled ($E_J = 0.8$; bottom trace), channels tend to open and close together. (C) Strong energetic coupling between RyRs in a ring causes the steady-state P_O versus $[Ca^{2+}]$ curve to become very steep (filled circles, dashed line) compared with the relationship for an isolated channel (black, solid line). This "steepness effect" means that reducing coupling between RyRs could theoretically cause P_O to either increase or decrease, depending on $[Ca^{2+}]$.

Fig. 3.

Effect of coupling of RyRs on open probability. (A) An illustrative plot of P_O of RyRs under two conditions compared to P_O of the uncoupled RyR. This plot compares uncoupled (line of unity) to coupled RyR (see Fig. 2). (B) Normalized effect of coupling plotted versus $[\text{Ca}^{2+}]_i$. Coupling clearly decreases the relative P_O at low to moderate $[\text{Ca}^{2+}]_i$ when the P_O of the uncoupled receptors is low. However, the P_O of the coupled RyRs is relatively higher than that of uncoupled RyRs at high P_O .

Fig. 4.

Hypothetical effect of uncoupling of RyRs on apparent “leak.” (A) and (B) show simulated transverse line-scan images of spontaneous Ca²⁺ sparks in quiescent ventricular myocytes under conditions of strong and weak RyR coupling, respectively. In either case 50 RyR clusters, each containing 50 RyRs, are assumed to be spaced at 0.5 μm intervals along the scan line. The probability that an RyR in a cluster will open in a given interval was derived from the steady-state open probabilities shown in Fig. 2C and the assumption that the rate of spontaneous RyR opening at resting [Ca²⁺] (100 nM) was 10⁻⁴ s⁻¹. A Ca²⁺ spark occurring at a particular cluster was assumed to increase [Ca²⁺] at neighboring clusters to 351 nM (see Fig. 1C) for 20 ms, thereby increasing the probability that a spark could occur at the neighboring cluster during that interval. The relative increase in opening rate during this period was derived from the ratio of P_O at 350 nM [Ca²⁺] to P_O at 100 nM [Ca²⁺] in Fig. 2C. The apparent Ca²⁺ spark rate in either case is identical, 1 spark per line per second. (C) However, close inspection of the “spark events” indicated by the white boxes demonstrates that when coupling between RyRs is low, spontaneous Ca²⁺ sparks are more likely to trigger sparks from neighboring RyR clusters, due to the greater P_O at 351 nM [Ca²⁺] when RyRs are uncoupled. This may mean that uncoupling can produce conditions under which “double spark” events are more likely to occur. These may only be apparent as such when examined carefully.

Fig. 5.

Effect of PKA phosphorylation on the relative open probability (P_O) of single RyRs in the steady-state observed in planar lipid bilayers. (A) Normalized P_O plotted as a function of cytosolic $[Ca^{2+}]_i$ in the presence and absence of PKA phosphorylation of RyRs taken from wild-type (WT) channels expressed in HEK293 cells under steady-state conditions. Data are taken from Wehrens et al. (2003) and re-plotted. (B) Relative change in P_O following PKA phosphorylation of RyR2. At low $[Ca^{2+}]_i$, there was an increase in P_O while at moderate to high $[Ca^{2+}]_i$, there was a relative decrease in P_O . (C) Normalized P_O plotted as a function of cytosolic $[Ca^{2+}]_i$ in the presence and absence of PKA phosphorylation of RyRs taken from mutant RyR2 channels (R2474S) expressed in HEK293 cells under steady-state conditions. Again, data presented in Wehrens et al. (Wehrens et al., 2003) have been re-plotted. The R2474S mutation is associated with catecholaminergic polymorphic ventricular tachycardia (CPVT). (D) Relative change in P_O following PKA phosphorylation of mutant RyR2 channels. At low $[Ca^{2+}]_i$, there was an increase in P_O while at moderate to high $[Ca^{2+}]_i$, there was a relative decrease in P_O . These effects were larger than those of WT channels.

Fig. 6.

Hypothetical effect of “rogue” RyRs on leak and steady-state SR $[Ca^{2+}]$ load. Simulations were performed with a model of SR pump-leak balance to determine how “rogue” RyRs may affect steady-state SR load. In this simple model, SR leak through either RyR clusters or rogue channels is proportional to the current SR content, and re-uptake is proportional to the cytosolic $[Ca^{2+}]$. Ca^{2+} influx and efflux across the cell membrane were ignored, and constants were adjusted so that the steady-state SR load with no rogue channels present is 1 mM. In control, leak through rogue channels is assumed to be twice that through RyRs in clusters. Thus, steady-state SR load decreases as the fraction of rogue channels increases (solid black line). We assume that PKA activation or heart failure causes leak through rogue channels to increase to 3 times that through RyRs in clusters but does not change the rate of leak through clusters (dashed black line). For a non-zero percentage of rogue channels, this situation would manifest itself as an increase in SR leak, and a decrease in steady-state SR load, that may not be visible as an increased rate of Ca^{2+} sparks.