

New and Notable

Extinguishing the Sparks

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The mechanical contraction of cardiac muscle is triggered by membrane depolarization through a process known as calcium-induced calcium-release (CICR). The structural basis of CICR is exquisite in its detail. At thousands of distinct locations throughout the myocyte, the junctional sarcoplasmic reticulum (JSR) membrane approaches to within ~12 nm of the t-tubule membrane to form structures known as dyads. Dyads consist of a handful of L-type calcium (Ca^{2+}) channels (LCCs) in the t-tubule membrane, a larger number of ryanodine-sensitive Ca^{2+} -gated Ca^{2+} -release channels (RyR2s) in the closely apposed JSR membrane, regulatory proteins that modify LCC and RyR2 function, and the fluid volume that separates them. Membrane depolarization leads to openings of LCCs, flux of Ca^{2+} into the dyadic space, Ca^{2+} binding to and opening of RyR2s, and release of Ca^{2+} from the JSR into the dyadic space. This Ca^{2+} then diffuses out of the dyad and binds to the mechanical machinery of the cell. The sum of these Ca^{2+} release events drives contraction of heart muscle.

Despite its apparent simplicity, one of the major challenges in cardiac electrophysiology has been to achieve a quantitative understanding of this process via a combination of experimental and modeling approaches. Early computational models of the cardiac myo-

cyte did not incorporate localized dyadic JSR Ca^{2+} release. Instead, the Ca^{2+} trigger flux through all LCCs and the Ca^{2+} release flux through all RyR2s were assumed to be directed into a single compartment with volume equal to that of all dyads within the cell. However, these so-called common pool models failed to reconstruct two key properties of CICR (1). The first is that release is a smoothly varying, graded function of Ca^{2+} trigger flux through LCCs. The second is that at the cardiac action potential plateau, the ratio of RyR2 release flux to LCC trigger flux (gain) is large. In one of the greatest achievements in cardiac mathematical and computational modeling to date, Stern (2) showed that common pool models cannot simultaneously describe both high gain and graded release.

The reason is that once Ca^{2+} release through RyR2s is initiated, increased subspace Ca^{2+} concentration promotes further RyR2 opening and regenerative Ca^{2+} release that is no longer under sarcolemmal control. Building from what was known about the structure of the dyad, Stern (2) went on to pose the landmark theory of “local control” of CICR. This theory posits that because RyR2s are arranged near one another in the dyad, opening of one or a few RyR2s and the resulting spread of Ca^{2+} within a dyad could trigger openings of other RyR2s within the same, but not different dyads. Graded release results from the fact that elementary dyadic Ca^{2+} release events could be recruited by LCCs in a voltage-dependent manner, reflecting the graded dependence of both LCC open probability and unitary current on membrane potential. This theory was strengthened upon the experimental discovery of “ Ca^{2+} sparks” (3), which are the elementary stereotypical Ca^{2+} release events that underlie local control of CICR. Sparks are now known to be produced not only in response to LCC-mediated trigger Ca^{2+} , but also occur spontaneously due to the random opening of RyR2s

in a resting cell in the absence of any Ca^{2+} trigger flux.

At this point, local-control theory offered a quantitative, mechanistic interpretation of the JSR Ca^{2+} release process. However, there was still no clear understanding of how the positive feedback loop leading to locally regenerative Ca^{2+} release was broken to terminate release. This was the beginning of what has become a more than one-decade-long debate about the mechanisms of release termination. Stern and others have proposed a number of different hypotheses.

The first is known as stochastic attrition (2). In this hypothesis, release termination results from the near-simultaneous random closing of a sufficient number of RyR2s so that dyad Ca^{2+} concentration is reduced to below the regenerative threshold. The likelihood of this occurring, in the absence of other contributing mechanisms, becomes small as the number of RyR2s in a dyad increases. Therefore, stochastic attrition is no longer considered to be a dominant mechanism of release termination.

The second hypothesis is that RyR2s undergo some form of Ca^{2+} -dependent inactivation (4). However, the observed timescale of inactivation is too slow to underlie the observed rate of release termination.

The third is referred to as “allosteric coupling” (5). Allosteric coupling is a phenomenon by which opening and closing of adjacent RyR2s is correlated, presumably as a result of mechanical linkage via the FKBP12.6 binding protein. This process can contribute to release termination because the closing of one RyR2 increases the likelihood that adjacent RyR2s will close. Whether or not RyR2s gate in a coupled manner is a subject of debate. Coupled gating has only been observed in a few studies. It has also been argued that the interaction energy required for coupled gating of the huge

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RyR2 macromolecule is so large as to be unlikely (6).

The fourth is regulation of RyR2 gating via a luminal JSR Ca^{2+} sensing mechanism (7,8). It has been shown that higher JSR Ca^{2+} levels lead to an increased RyR2 open probability and mean open time. Therefore, as local JSR Ca^{2+} becomes depleted upon release, RyR2 open probability and mean open time both decrease, contributing to release termination.

The fifth hypothesis is that reduction of RyR2 release flux due to a decrease of the trans-JSR Ca^{2+} concentration gradient produced by local depletion of JSR Ca^{2+} plays a critical role in release termination (9). A decrease in unitary RyR2 Ca^{2+} flux, which requires that the JSR refill rate be sufficiently slow for depletion to occur, would be expected to weaken CICR among RyR2s, and has recently received renewed attention as a key termination mechanism.

Given this plethora of possible mechanisms, it is no wonder that debate over the mechanism of Ca^{2+} release termination has been underway for so long. Modeling has played an increasingly important role in this debate. Models based firmly on experimental data provide a means by which the relative importance of each release termination mechanism can be explored. Many RyR2 models have been formulated with an intrinsic inactivation mechanism to reconstruct the correct time course of JSR Ca^{2+} release termination (10). Recent approaches, however, have recognized that no such process has been demonstrated experimentally with sufficiently rapid kinetics to underlie the termination mechanism. Recent models presented by Williams et al. (11) and Sato and Bers (12) use elegantly simple formulations of RyR2 gating in which intrinsic inactivation is absent to demonstrate the mechanistic basis for nonspark-mediated JSR Ca^{2+} leak. These studies have elucidated how single RyR2 openings fail to trigger sufficient CICR to produce a Ca^{2+} spark as the

JSR becomes depleted. In both models, the JSR Ca^{2+} depletion-dependent reduction of RyR2 open probability arises due to a combination of mechanisms including reduced unitary RyR2 current, JSR Ca^{2+} -dependent regulation of RyR2 gating, and in the case of Williams et al. (11), allosteric RyR2 interactions. The demonstrated loss of fidelity of inter-RyR2 CICR in these studies serves not only to hinder spark generation, but also as a mechanism of spark termination.

In this issue, Cannell et al. (13) introduce, what is to our knowledge, a novel model of CICR, and use it to examine the process of spark termination. The model is novel in that it incorporates spatial properties and electro-diffusion of Ca^{2+} within the dyad, as well as refilling of local JSR by way of Ca^{2+} diffusion from the tortuous network sarcoplasmic reticulum to the JSR. Armed with this model and new experimental data, they examine the robustness of induction decay as a mechanism for termination of JSR Ca^{2+} release. Induction decay (14) refers to the chain of events in which gradual JSR Ca^{2+} depletion leads to declining RyR2 unitary flux, which reduces the dyadic Ca^{2+} concentration in the vicinity of open RyR2s and their closed neighbors, and hence reduces the open probability (via an increase in mean RyR2 closed time) of these neighboring RyR2s, interrupting the positive feedback of local regenerative release. Induction decay is the manifestation of the decline in trans-SR Ca^{2+} concentration gradient that occurs with JSR depletion (described above). To test the hypothesized role of induction decay in release termination, they experimentally characterized single-channel properties of rat and sheep RyR2s in the presence of physiological levels of Mg^{2+} and ATP.

The data were used to develop simple empirical two-state (open and closed) models of RyR2 gating, in which the opening and closing rates depend only on dyadic Ca^{2+} concentration. A novel feature of this formu-

lation is the absence of any intrinsic release termination mechanism (e.g., inactivation, allosteric coupling, and regulation by JSR Ca^{2+}) other than stochastic attrition (the existence of which is impossible to eliminate due to the inherent stochastic gating of RyR2s). Stochastic simulation of these RyR2s within a detailed structural three-dimensional model of the cardiac dyad revealed that nanoscopic gradients of cardiac dyad Ca^{2+} regulate CICR and the mechanism of induction decay is sufficient, in the context of limited JSR volume and Ca^{2+} buffering, to terminate Ca^{2+} release on the timescale of a spark in the absence of other mechanisms, even with the relatively heightened Ca^{2+} sensitivity of sheep RyR2s.

Furthermore, the incorporation of regulation by luminal JSR Ca^{2+} into the model had little effect on Ca^{2+} sparks and blinks, suggesting it may have less of a role than induction decay in the termination of JSR Ca^{2+} release. One caveat, however, is that Cannell et al. (13) incorporated this luminal regulation by altering the RyR2 opening rate to match their experimental data that show dependence of RyR2 open probability on luminal JSR Ca^{2+} (their Fig. 2). Because it is known that JSR Ca^{2+} influences RyR2 open time (8), it may be that incorporation of luminal RyR2 regulation via alteration of the closing rate (i.e., mean open time) would reveal a more prominent role of JSR Ca^{2+} regulation. Despite this, these simulations demonstrate that the spatio-temporal evolution of dyadic Ca^{2+} plays a very important role in regulation of CICR that cannot be captured in models that assume each dyad is a single homogeneous compartment. Such models necessarily have relied more heavily on the other mechanisms of release termination described above.

The work of Cannell et al. (13) serves as an example of how a model can be used to isolate and study coupled biological processes in a way that is challenging to accomplish experimentally. The model demonstrates

the sufficiency of induction decay to terminate inter-RyR2 CICR by reconstructing this phenomenon in the absence of all other possible mechanisms. In cells, the ability to experimentally distinguish the role of unitary RyR2 current from luminal Ca^{2+} regulation is hampered by the fact that these vary together with changes in JSR Ca^{2+} level. Recently, however, Guo et al. (15) devised a clever way to manipulate unitary RyR2 current independently of JSR Ca^{2+} concentration using large RyR2-permeable organic cations. Their results led to a similar conclusion that spark local control follows unitary RyR2 current amplitude, and this group has referred to the process by which the decay of local Ca^{2+} concentration in the vicinity of an open RyR2 leads to failure of local inter-RyR2 CICR as “pernicious attrition” (6). Manipulation of the unitary RyR2 current in the model to mimic the experimental protocol yielded model reconstructions of spark properties that were a good match to the experimental results.

The work of Cannell et al. (13) is particularly notable in that it uses an experimentally based structurally detailed model of JSR Ca^{2+} release to demonstrate the sufficiency of induction decay as a mechanism of release termination in the absence of

other mechanisms, a finding that is appealing in terms of its simplicity. Although these findings do not prove that other mechanisms are insignificant, and no doubt the debate will continue, they make a case for placing induction decay at the top of the list. In addition, the fact that modeling spatial gradients of Ca^{2+} within the dyad is of high importance raises significant challenges as to how first-principles models of CICR may be formulated at the level of the cell.

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