Published in final edited form as:

J Mol Cell Cardiol. 2013 May ; 58: 53–58. doi:10.1016/j.yjmcc.2013.01.011.

Pernicious Attrition and Inter-RyR2 CICR Current Control in Cardiac Muscle

Dirk Gillespie and Michael Fill

Rush University Medical Center, Department of Molecular Biophysics & Physiology, Section of Cellular Signaling, 1750 West Harrison Street, Chicago, IL 60612

Abstract

In cardiac muscle cells, ryanodine receptor (RyR) mediated Ca^{2+} release from the sarcoplasmic reticulum (SR) drives the contractile apparatus. Spontaneous bouts of inter-RyR Ca^{2+} induced Ca^{2+} release (CICR) generate an elemental unit of SR Ca^{2+} release called a spark. Sparks are localized events that terminate soon after they begin. The local control of sparks is not clearly understood. In this article, we review the potential regulatory role that the changing single RyR Ca^{2+} current may play. Moreover, we aggregate RyR data into a working scheme of inter-RyR CICR current control of sparks and a potential inter-RyR CICR termination mechanism that we call pernicious attrition.

In cardiac muscle, the action potential activates dihydropyridine receptor (DHPR) Ca^{2+} channels across the surface membrane and transverse tubule membrane. Each DHPR mediates a small Ca^{2+} influx that activates underlying cardiac ryanodine receptor (RyR2) Ca^{2+} release channels on the sarcoplasmic reticulum (SR). Each open RyR2 mediates a relatively large Ca^{2+} flux into the cytosol. This DHPR-RyR2 Ca^{2+} -induced Ca^{2+} release (CICR) initiates cardiac excitation-contraction coupling (ECC). RyR2s are clustered at discrete SR release sites and just some are associated with DHPRs. Non-DHPR linked RyR2s are activated via inter-RyR2 CICR. The result is the large cytosolic Ca^{2+} transient that drives the contractile apparatus.

During diastole, RyR2 channels are predominantly closed. There is, however, always some probability that a RyR2 will spontaneously open. When one does, it mediates a Ca^{2+} flux into the cytosol and this Ca^{2+} can trigger inter-RyR2 CICR at that release site, generating a Ca^{2+} spark [1]. If not, the Ca^{2+} released represents non-spark RyR-mediated SR Ca^{2+} leak [2,3]. The cytosolic Ca^{2+} signal generated by a typical spark is usually insufficient to activate RyR2s at neighboring release sites and thus sparks are local spatially-confined events. Abnormally large (or frequent sparks) can activate RyR2s at neighboring release sites, generating propagating SR Ca^{2+} waves that travel across the cells during diastole [4].

Sparks are thought to be normal events in cardiac muscle cells that contribute to the normal SR Ca^{2+} leak and consequently govern overall SR Ca^{2+} load [5]. Spontaneous Ca^{2+} waves are abnormal events. During a wave, some of the released Ca^{2+} will be extruded from the

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Corresponding Author: Michael Fill, Ph.D., Ph#: 312-942-6434, mfill@rush.edu.

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Disclosures: None Declared

cell via Na-Ca²⁺ exchange (NCX) activity. Since NCX function is electrogenic, this may depolarize a cell during diastole sufficiently to trigger an action potential and possibly initiate a life-threatening arrhythmia. Despite decades of effort, the mechanisms that control the inter-RyR2 CICR underlying sparks and waves remain poorly understood. For example, the local negative control that counters the inherent positive feedback CICR is unknown. Also, we know that sparks are critically controlled by local intra-SR (luminal) Ca²⁺ levels but exactly how is unclear. Here, we describe the role of the single RyR2 Ca²⁺ current in CICR local control and its possible contribution to the luminal Ca²⁺ sensitivity and termination of sparks.

This review briefly describes how the luminal Ca²⁺ sensitivity of local SR Ca²⁺ release may rise in mammalian cardiac muscle. It describes the changes in single RyR2 Ca²⁺ current amplitude that naturally occur during a spark as well as the local cytosolic Ca²⁺ signal generated by a single RyR2 opening event. The potential role of this signal in modulating local RyR2 activity is discussed and we coin the term pernicious attrition to describe the failure of local inter-RyR2 CICR as the signal becomes smaller. An attempt was made to present this growing complex body of information in a readable easily-understood way. It was impractical to describe and include all the quality studies that comprise this field but we hope this effort helps general interest readers understand local CICR control in heart better.

Luminal Ca²⁺ Sensitivity

Spark frequency increase dramatically as SR Ca²⁺ load rises [6] and sparks terminate when the local SR load (intra-SR free Ca²⁺ level) falls to a critical level [7-9], the termination threshold. It is thought that single RyR2 channels somehow “sense” changes in local SR load. This intra-SR Ca²⁺ sensing may arise from Ca²⁺ binding to the luminal side of the RyR2 protein itself or to an intra-SR protein that is closely associated with the RyR2 [10]. The most popular RyR2 luminal Ca²⁺ sensing mechanism involves the calsequestrin (CASQ), triadin (TD) and junctin (JN) proteins [11-14]. Indeed, there are naturally occurring RyR2, CASQ and TD mutations [15-19] linked to catecholaminergic polymorphic ventricular tachycardia (CPVT), a form of wave-evoked arrhythmia associated with abnormal RyR2 luminal Ca²⁺ sensitivity. It is commonly believed that intra-SR Ca²⁺ sensing mechanisms entirely explained the luminal Ca²⁺ sensitivity of SR Ca²⁺ sparks. There is, however, another way that local SR Ca²⁺ load may control local Ca²⁺ release.

The local SR Ca²⁺ load defines the magnitude of the trans-SR Ca²⁺ driving force. Thus, local load determines the single RyR2 Ca²⁺ current amplitude. The single RyR2 Ca²⁺ current provides the local Ca²⁺ signal that drives the inter-RyR2 CICR underlying the spark. As SR load rises, this local Ca²⁺ signal will become larger because the current is bigger. The larger local Ca²⁺ signal intuitively will increase the likelihood of inter-RyR2 CICR (i.e. sparks). As load falls, the local Ca²⁺ signal will become smaller and the likelihood that inter-RyR2 CICR starts (or continues) becomes less. The possible contribution of single RyR2 Ca²⁺ current in CICR local control is an embedded element, typically not emphasized, in several modeling studies [20-24]. Recently, Sato & Bers [24] have even described current control of sparks with impressive quantitative detail and current control seems to be gaining in popularity as a potentially significant load-dependent process that helps govern sparks and waves [8,9,25,26]. Until recently, this current control scenario has been experimentally difficult to verify because single RyR2 Ca²⁺ current and local SR load are difficult to differentially manipulate in cells. We recently overcame this obstacle and experimentally showed that single RyR2 Ca²⁺ current amplitude does indeed substantially contribute to spark local control [27]. Thus, RyR2 luminal Ca²⁺ controls of sparks and waves is very likely due to a composite of processes that include intra-SR Ca²⁺ sensing mechanisms and single RyR2 current control of inter-RyR2 CICR.

The Single RyR2 Ca²⁺ Current

Single RyR2 function has been defined by incorporating the channel into planar lipid bilayers[10]. The RyR2 is a poorly selective Ca²⁺ channel [28] and multiple RyR2-permeable cations (K⁺, Mg²⁺ and Ca²⁺) are present in cells. There are normally no trans SR K⁺ or Mg²⁺ gradients and the high SR K⁺ permeability holds the SR at 0 mV [29], the K⁺ equilibrium potential. When the RyR2 opens, all RyR2-permeable ions enter the poorly selective pore. There is a net SR Ca²⁺ efflux (at 0 mV) because there is a trans-SR Ca²⁺ gradient. Without K and Mg present, the single RyR2 Ca²⁺ current (at 0 mV) can be as large as ~4 pA when super-physiological luminal Ca²⁺ levels (e.g. 10-50 mM) are present [30]. In cell-like salt solutions (i.e. 120 mM K, 1 mM free Mg and <100 μ M free Ca²⁺ in the cytosol; 120 mM K, 1 mM free Mg and 1 mM free Ca²⁺ in the SR lumen), single RyR2 channel Ca²⁺ current amplitude at 0 mV is ~0.35 pA [27,31-33]. Since robust SR countercurrent assures that the SR V_m never strays far from 0 mV [29,33,34], the amplitude of the single RyR2 channel Ca²⁺ current is likely near 0.35 pA in cells if local intra-SR free Ca²⁺ is 1 mM.

Measurements suggest the local free Ca²⁺ level in the SR falls roughly 50% (to ~0.5 mM) during a spark [7,35,36]. Mathematical modeling, however, suggests it may fall even further (90%) depending on the speed of release termination and rate of Ca²⁺ transfer between regions inside the SR [8,9]. Our extensive single RyR2 permeation works [33,34,37-41] indicate that single RyR2 Ca²⁺ current amplitude (at 0 mV) will vary almost proportionally with local SR Ca²⁺ load. Thus, single RyR2 Ca²⁺ current (at 0 mV) may be 0.35 pA when a spark begins but would fall to 0.175 pA if local load decreased by 50% (or to 0.035 pA if it decreased by 90%) when the spark terminates.

The Local Cytosolic Ca²⁺ Signal Generated by a Single RyR2 Opening

The local cytosolic Ca²⁺ signal generated around a Ca²⁺ point source, like an open RyR2 conducting a 0.35 pA Ca²⁺ current, can be calculated using standard diffusion theory [42-44]. Figure 1 plots the steady state local Ca²⁺ concentration around a 0.35 pA point source as a function of distance (thick solid curve). For the RyR, these steady state concentrations are likely established in <10 μ s [45,46] and are sustained as long as the current is sustained. The elevation in local cytosolic Ca²⁺ generated by a 0.175 and 0.035 pA current are also shown (thin solid curve). The dashed curve is the elevation in local cytosolic Ca²⁺ level with the 0.35 pA current in the presence of 1 mM cytosolic BAPTA, a fast-binding Ca²⁺ buffer. To help interpret these curves, 3 adjacent RyR2s are depicted at the bottom of Figure 1. The adjacent RyR2s shown roughly reflect the known physical dimensions of the RyR2 [47,48] (central pore, cytosolic domain ~28 nm wide) as well as the known spacing between RyR2s at a release site [49] (center-to-center distance ~30 nm). The center of one RyR2 (Fig. 1, bottom left) is aligned with 0 nm on the plot and thus this RyR2 can be envisioned as the Ca²⁺ point source that is generating the various Ca²⁺ plots above.

In cell-like salt solutions, the measured RyR2 cytosolic Ca²⁺ activation EC₅₀ is ~10 μ M [27,30]. Figure 1 shows that the physiological RyR2 Ca²⁺ current of 0.35 pA will elevate the local cytosolic Ca²⁺ to >20 μ M around the open RyR2 and to >10 μ M across half of the immediately adjacent RyR2. The super-EC₅₀ Ca²⁺ levels over an adjacent closed RyR2 would intuitively trigger inter-RyR2 CICR and initiate a spark. When the single RyR2 Ca²⁺ current is 0.175 pA (not 0.35 pA), there is sub-EC₅₀ Ca²⁺ levels over the adjacent RyR2 and thus inter-RyR2 CICR would be less likely to occur. Note that these predictions are based on well-established experimental measurements (RyR2 Ca²⁺ EC₅₀, RyR2 current amplitude and RyR2 physical size/spacing in cells) as well as simple diffusion theory. How accurately this describes local RyR2 Ca²⁺ cross talk in living cells remains to be determined.

If single RyR2 Ca^{2+} current is large enough to act upon the Ca^{2+} regulatory sites of a neighboring RyR2, then should also have some action on the Ca^{2+} regulatory sites of RyR2 actually mediating the current. After all, the Ca^{2+} regulatory sites of the RyR2 mediating the current are much closer to the open pore. We will define this prospect as auto-RyR2 Ca^{2+} feed through. Auto-RyR2 Ca^{2+} feed through is when the Ca^{2+} flux through an open RyR2 feeds back and acts on the cytosolic regulatory sites of the same RyR2 conducting the flux. This is not inter-RyR2 CICR where the Ca^{2+} flux through an open RyR2 acts on the cytosolic regulatory sites of a nearby neighboring RyR2 channel. Several studies have reported auto-RyR2 Ca^{2+} feed through [43,44,50,51]. However, these studies usually done with super-physiological RyR2 Ca^{2+} fluxes (>2.5 pA) and/or enhanced RyR2 cytosolic Ca^{2+} sensitivity. The cytosolic Ca^{2+} sensitivity is typically enhanced using caffeine or ATP (without cytosolic Mg^{2+} present). In cell-like salt solutions, the physiological Ca^{2+} flux is considerably smaller and cytosolic Ca^{2+} sensitivity is normally not pharmacologically potentiated in cells. In cell-like salt solutions, experiments show that there is very little (if any) auto-RyR2 Ca^{2+} feed through activation in cells [44], despite popular belief.

While experiments show there is little (if any) auto-RyR2 feed through, the explanation is not totally clear. Figure 1 illustrates that the cytosolic local free Ca^{2+} around an open RyR2 mediating even a small Ca^{2+} current is above that RyR2's cytosolic Ca^{2+} EC_{50} (~ 10 μM). Also, the cytosolic Ca^{2+} EC_{50} of Cs^+ conducting (no Ca^{2+} flux) and Ca^{2+} conducting (>4 pA of Ca^{2+} flux) RyR2's are the same (~ 2 μM) [44]. These observations imply that a RyR2's cytosolic Ca^{2+} activation sites are somehow insensitive to the Ca^{2+} fluxing through that same channel's open pore. In other words, single RyR2's are immune to auto-RyR2 Ca^{2+} feed through activation [44]. We have speculated that this immunity may arise because Ca^{2+} is already occupying the cytosolic activation site(s) of the open RyR2 [44] and thus the fluxed Ca^{2+} has little effect. Note that cytosolic Ca^{2+} activation sites on neighboring RyR2s would not be occupied. Thus, the Ca^{2+} fluxing through an open RyR2 can act on the cytosolic sites of neighboring RyR2's and drive inter-RyR2 CICR. The current control discussed in this review applies to inter-RyR2 CICR, not auto-RyR2 feed through activation.

Possible Role of Inter-RyR2 Current Control of Sparks

Logically, the likelihood of inter-RyR2 CICR occurring at a SR Ca^{2+} release site will critically depend on the magnitude of the local cytosolic Ca^{2+} signal generated by the opening of an individual RyR2 channel [20-25,27]. The magnitude of this signal will depend on both the amplitude of the single RyR2 Ca^{2+} current and the duration of the opening event. It is clear that local SR Ca^{2+} load modulates single RyR2 open times (duration and frequency) by acting on intra-SR RyR2 Ca^{2+} regulatory mechanisms [11-14] perhaps in part by modulating the RyR2's cytosolic Ca^{2+} and/or ATP sensitivity [11,13,52]. Local SR Ca^{2+} load also determines single RyR2 current amplitude because it sets the SR Ca^{2+} driving force. These two actions likely work in concert to govern the likelihood of inter-RyR2 CICR (sparks) and generate the load sensitivity of SR Ca^{2+} release.

During diastole in cardiac cells, there is always a low but finite probability that a RyR2 will open spontaneously. When one does, the likelihood that it triggers inter-RyR2 CICR (i.e. a spark) depends on the magnitude of the local cytosolic Ca^{2+} signal it generates. Since RyR2 open times are exponentially distributed, there are many more short than long spontaneous openings. We have reported that only the longest single RyR2 openings are likely to drive inter-RyR2 CICR and evoke sparks [30]. This suggests spark frequency is normally low during diastole primarily because spontaneous long duration RyR2 openings are relatively rare.

When local SR load rises above normal levels, each spontaneous single RyR2 opening will carry a larger than normal Ca^{2+} flux and thus generate a larger than normal local Ca^{2+} signal. Consequently, shorter openings (and there are exponentially more of them) will be sufficient to drive inter-RyR2 CICR (sparks). If so, then spark frequency will increase nearly exponentially as local load rises. Moreover, higher intra-SR Ca^{2+} also shifts (via intra-SR RyR2 regulatory mechanisms) the distribution of RyR2 open times to longer values [11,13,53], so spark frequency will sky rocket (super-exponentially increase) as local SR Ca^{2+} load increases. The sparks generated will be bigger because the larger local Ca^{2+} signals (generate by each individual opening) will recruit more RyR2s per spark. The resulting frequent large sparks are more likely to trigger arrhythmogenic Ca^{2+} waves. On the other hand, lower SR Ca^{2+} load simultaneously decreases single RyR2 current amplitude (due to smaller Ca^{2+} driving force) and shortens single RyR2 open time (via intra-SR Ca^{2+} regulator mechanisms). The possible ramifications of the latter warrants further consideration.

As local load falls during a spark, the cytosolic Ca^{2+} signal generated by the open RyR2's will intuitively become at some point too small to drive (or sustain) inter-RyR2 CICR. At this point, RyR2's that spontaneously close will no longer be re-activated by inter-RyR2 CICR. Eventually, all RyR2's will close and local release will terminate. In this scheme, sparks terminate because RyR2's inherently/spontaneously close and fail to re-open. They fail to re-open because the cytosolic Ca^{2+} signal generated by individual RyR2 openings is no longer sufficient to drive inter-RyR2 CICR. We call this current-dependent termination mechanism pernicious attrition. Pernicious attrition is not stochastic attrition. Stochastic attrition specifically refers to the probability that all open RyR2 will spontaneously close at the same moment. During pernicious attrition, RyR2 closing is asynchronous. We call it pernicious because the smaller RyR2 Ca^{2+} current devastates inter-RyR2 CICR in a way that is not easily seen or noticed. This is an interesting possibility because termination is commonly attributed solely to processes that directly turn-off RyR2's. Pernicious attrition contributes to termination by limiting a signal that turns-on RyR2s (i.e. the local Ca^{2+} trigger signal that drives inter-RyR2 CICR). We have presented a preliminary report describing this mechanism [26]. Subsequently Laver et al [25] described similar process that they refer to as "induction decay". Much more work is clearly required to fully understand the potential contribution of these mechanisms to spark local control.

Single RyR2 Current and Spark/Wave Control

Single inter-RyR2 current control likely contributes to the local control of sparks and waves in cardiac muscle cells. The key word here is contributes. For example, the presence of current control does not mean that calsequestrin (CASQ) dependent RyR2 luminal Ca^{2+} regulation is not important. Current control very likely works in synergy with CASQ-dependent RyR2 regulation during overall spark local control. Also, the existence of pernicious attrition does not mean that other potential CICR termination mechanisms are not significant. Current control likely operates in concert with these other local control phenomena to terminate sparks.

Some possible contributions of current control during various spark/wave phenomena are outlined in Table 1. Current control likely contributes to the luminal Ca^{2+} sensitivity of sparks. It may help explain why sparks terminate when SR Ca^{2+} falls to a set termination threshold and why that threshold changes when caffeine is present. Current control also explains the existence of non-spark SR Ca^{2+} leak as recently described by Williams et al. [23] and Sato & Bers [24]. It also may help explain how drugs that shorten RyR2 open time end up limiting SR Ca^{2+} waves. Indeed, few proposed local control process in our

experience integrate this smoothly and broadly with our present understanding of CICR local control.

What is the experimental evidence supporting the existence of inter-RyR2 CICR current control? In cells, single RyR2 Ca^{2+} current and SR load inherently vary in parallel. This makes it very difficult to distinguish their individual regulatory contributions. We recently devised a way to differentially manipulate single RyR2 Ca^{2+} current and SR Ca^{2+} load in permeabilized cardiomyocytes [27]. This method involves replacing cytosolic K with large RyR2-permeable organic cations that enter the RyR2 pore and consequently limits Ca^{2+} flux through the pore. Single channel studies confirmed that this experimental manipulation does not alter other aspects of RyR2 function (i.e. its gating). Spark frequency followed the change in single RyR2 Ca^{2+} current (frequency decreased), not the change in load. Sparks terminated earlier even though SR Ca^{2+} load was higher, suggesting local CICR termination is tightly linked to decreasing single RyR2 current. We also reported that high SR Ca^{2+} loads did not evoke Ca^{2+} waves when the large RyR2-permeable organic cation was present (i.e. when the single RyR2 Ca^{2+} current was limited). At the high SR loads, subsequent removal of the large organic cation immediately resulted in waves [27]. This suggests waves (like sparks) are not solely governed by intra-SR Ca^{2+} acting at intra-SR RyR2 Ca^{2+} regulatory sites. Another process, one linked to single RyR2 current amplitude also plays a substantial role in local spark/wave control [27]. That other process is likely current control.

There is also evidence supporting current control that does not involve the use of non-physiological large organic cations. In SR-load controlled permeabilized cardiomyocytes, Bovo et al. [54] recently reported that spark frequency decreases when a small amount of cytosolic BAPTA, instead of EGTA, is present. Since load was controlled, the change in spark frequency was not due to a change in SR Ca^{2+} load. They reasoned that BAPTA, a fast Ca^{2+} buffer, had likely limited cytosolic Ca^{2+} diffusion around the open RyR2 pore, in effect reducing the local cytosolic Ca^{2+} signal generated by each open RyR2. The reduced signal would be less likely to activate neighboring RyR2's (i.e. driving inter-RyR2 CICR). This does not prove current control but it is consistent with it. Also, flecainide [55] and carvedilol [56] both reduce single RyR2 mean open time, which will reduce the size the cytosolic Ca^{2+} signal generated by an individual RyR2 channel opening. Current control would explain why these drugs ultimately reduce SR Ca^{2+} wave frequency. Thus, it seems clear that inter-RyR2 CICR current control plays at least some role in regulating SR Ca^{2+} sparks.

One caveat, when considering the possibility of inter-RyR2 CICR current control, is the existence of RyR2 coupled gating. In our hands [27], individual RyR2s incorporated into planar bilayers have a mean open time of 8.5 ms when activated by 10 μM cytosolic Ca^{2+} (1 mM Ca^{2+} luminal). Since the average spark time-to-peak (TTP) in analogous solutions is ~20 ms, we suggest that than 96% of measured single RyR2 openings are shorter than spark TTP [27]. This implies individual RyR2s may open and close many times during a spark. However, there is evidence that gating of neighboring RyR2s may be allosterically coupled by the FK-506 binding protein (FKBP) [57]. This allosteric coupling is proposed to functionally couple channels so that neighboring RyR2s open and close together (i.e. in a lock-step fashion). Lock-step closing of multiple channels would represent a robust means to terminate local CICR. Consequently, allosteric coupled gating has been included in various theoretical models that describe CICR local control [21,58,59]. Does such allosteric coupling exist in cells? This is a difficult question to answer. Allosterically coupled RyR2 gating has been observed in vitro in a few single channel studies [57,60]. However, functional coordination of neighboring RyR2 channels by inter-RyR2 CICR has also been reported in some in vitro studies [27,44,61]. In our view, the almost incomprehensible free energy that would be required to allosterically couple the gating of huge macromolecules

like RyR2 channels strongly suggests that it is unlikely to occur in cells. Further, simultaneous sparks and blink measurements indicate that sparks peak (i.e. release termination begins) before the underlying SR Ca^{2+} release ends [7]. This implies RyR2 closing during spark termination is not synchronous. This is consistent with the relatively rounded peaks of cardiac sparks (i.e. synchronous lock-step closing of all active RyR2s would intuitively generate sharp spark peaks). Of course, these observations might be explained if small subgroups of allosterically coupled RyR2s were closing asynchronously [62]. The more prudent explanation, however, is that individual RyR2s are simply closing asynchronously during spark termination. If so, then RyR2 function during a spark is interdependent because neighboring channels interact through inter-RyR2 CICR but each active channel gates individually (i.e. not in an allosterically coupled fashion).

In summary, experiment and modeling both indicate that the single RyR2 Ca^{2+} current amplitude plays a substantial regulatory role in the local control of inter-RyR CICR at SR Ca^{2+} release sites in cardiac muscle. Local inter-RyR2 CICR current control is load dependent because the trans-SR Ca^{2+} driving force is determined by the local SR Ca^{2+} load. We suggest that inter-RyR2 CICR current control works in synergy with other well-established RyR luminal Ca^{2+} regulatory mechanisms to generate the load sensitivity of cardiac SR Ca^{2+} release. We have also discussed a process we call pernicious attrition that describes the point where the single RyR2 Ca^{2+} current (load) becomes insufficient to support the continuation of local inter-RyR2 CICR.

Acknowledgments

This effort was supported by National Institutes of Health grant R01-HL057832 and R01-AR054098 (M.F.). We would like to thank Dr. Demetrio J. Santiago for help preparing this manuscript.

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Highlights

1. Single RyR2 current is a key determinant of local inter-RyR2 CICR control.
2. This local current control of CICR works in synergy with other RyR2 regulatory mechanisms.
3. Pernicious attrition may contribute to the termination of local CICR events.

FIGURE 1.

Local Ca^{2+} diffusion around one open RyR2 channel. Local free Ca^{2+} concentration is plotted as a function of distance from the center of a Ca^{2+} point source of 0.35, 0.175 or 0.035 pA. Local Ca^{2+} concentrations were calculated as specified elsewhere [44]. Three RyR channels (one open, two closed) are illustrated at bottom to provide interpretive context. The Ca^{2+} activation EC_{50} of single the cardiac RyR channel in cell-like salt solution is $\sim 10 \mu\text{M}$ [30] and super- EC_{50} Ca^{2+} concentrations are indicated by the shaded area on the plot. The dashed line represents local Ca^{2+} levels when there is a 0.35 pA Ca^{2+} current with 1 mM cytosolic BAPTA present.

Table 1

Finding	Current Control Contribution
Intra-SR RyR2 Ca²⁺ Regulatory Sites <i>Gyorke et al. (2004)</i> <i>Jiang et al. (2004)</i>	These sites modulate single RyR2 open time (OT). Higher load increases OT while lower loads decrease it. Both single RyR2 current amplitude (defined by trans-SR Ca driving force) and RyR2 OT (in part defined by intra-SR Ca sites) determine the size [*] of local cytosolic Ca ²⁺ signal generated by a single RyR2 opening. This in turn determines the likelihood of inter-RyR2 CICR.
SR Load Controls Spark Frequency <i>Lukyanenko et al. (2001)</i> <i>Zima et al. (2010)</i>	Load influences both single RyR2 current amplitude (via Ca driving force) and RyR2 OT (via intra-SR Ca sites). This dual action renders inter-RyR2 CICR exquisitely sensitive to even small changes in local SR Ca load.
Sparks terminate when SR Ca²⁺ falls to a termination threshold <i>Rice et al (1999), Hinch (2004), Zima et al (2008),</i> <i>Ramay et al (2011) & Sato and Bers (2011)</i>	As local SR load falls, single RyR2 current amplitude gets smaller and RyR2 OTs shorter. At some point (threshold), RyR2's spontaneously that close will no longer be re-activated by local CICR and will remain closed. Soon all RyR2's will be closed and local spark terminated. We call this process pernicious attrition.
Sparks Peak Before Underlying Local SR Ca²⁺ Release Ends <i>Zima et al (2008)</i>	This implies RyR2s close asynchronously during spark termination and is consistent with current control and pernicious attrition (see above).
Calsequestrin KO Causes CPVT but SR Release Still Load Dependent <i>Knollmann et al. (2006)</i>	Calsequestrin participates in intra-SR RyR2 Ca ²⁺ regulation and its absence alters this regulation leading to larger more frequent sparks. Load still defines single RyR2 current amplitude so release/sparks are still load dependent.
RyR2, CASQ or TD Mutations Can Promote Ca²⁺ Waves Causing CPVT <i>Priori et al. (2002) & Roux-Buisson (2012) Terentyev et al. (2008)</i>	The RyR2-TD-CASQ complex participates in intra-SR RyR2 Ca ²⁺ regulation and alterations in this complex result in larger more frequent waves. They likely lengthen RyR2 OT, increasing likelihood of inter-RyR2 CICR at any set load.
Caffeine Promotes Sparks/Waves <i>Traford et al. (1995)</i> <i>Porta et al. (2011)</i>	Caffeine makes single RyR2 more Ca ²⁺ sensitive. Consequently, smaller single RyR2 Ca ²⁺ currents can drive inter-RyR2 CICR, increasing spark/wave frequency.
Caffeine Reduces SR Ca²⁺ Spark Termination Threshold <i>Domeier et al. (2010)</i>	Since caffeine heightens RyR2 Ca ²⁺ sensitivity, local load must fall further before the cytosolic signal driving inter-RyR2 CICR fails (i.e. pernicious attrition starts).
Non-spark SR Ca²⁺ Leak Exists <i>Zima et al. (2010)</i> <i>Santiago et al. (2010)</i>	During diastole, single RyR2 spontaneously open at a very low frequency. If the cytosolic Ca ²⁺ signal generated by an opening fails to trigger inter-RyR2 CICR, then Ca ²⁺ released during that opening represents non-spark SR Ca ²⁺ leak.
Ryanodol & Imperatoxin Evoke Trains of Sparks at SR Release Sites <i>Terentyev et al. (2002)</i> <i>Ramos-Franco et al. (2010)</i>	These drugs likely evoke sustained sub-conductance openings of 1 or 2 RyR2's at a release site, generating a prolonged elevation in the local cytosolic Ca ²⁺ level. This makes RyR2 more likely to open and/or respond to smaller than normal local Ca ²⁺ trigger stimuli (generated by local single RyR2 openings).
Carvedilol & Flecainide Reduce RyR2 Open Time, Stopping CPVT <i>Watanabe et al. (2009)</i> <i>Zhou et al. (2011)</i>	The drug-evoked reduction in RyR2 OT decreases the size [*] of the cytosolic Ca ²⁺ signal generated by an individual RyR2 opening. This reduces the likelihood of inter-RyR2 CICR and limiting the initiation of sparks/waves that trigger CPVT.

^{*} size refers to the combination amplitude, duration and spatial width of the local Ca²⁺ trigger signal.