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Quenching the spark: Termination of CICR in the sub-microscopic space of the dyad.

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Summary. Many mechanisms have been proposed for the termination of regenerative calcium-induced calcium release (CICR). Robust termination of CICR by ‘induction decay’ is made possible by significant local depletion of the sarcoplasmic reticulum (albeit underestimated in imaging experiments) decreasing RyR (re)activation.

Introduction

Cardiac excitation-contraction (E-C) coupling is a transduction cascade that results in muscle contraction and relaxation. In ventricular myocytes, the arrival of an action potential activates sarcolemmal L-type Ca^{2+} channels (LCCs) and the subsequent inward Ca^{2+} current (I_{Ca}), in turn, activates the Ca^{2+} release unit (CRU), which incorporates several ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) membrane. Activation of a CRU causes more Ca^{2+} to be released into the local cytoplasm in a process called Ca^{2+} -induced Ca^{2+} release (CICR) (Fabiato, 1983), and is observed as a Ca^{2+} spark (Cannell et al., 1994). The spatio-temporal summation of these elementary events forms the Ca^{2+} transient that enables cross-bridge cycling. The rise in cytosolic Ca^{2+} is short-lived, as removal mechanisms such as the Na^+ - Ca^{2+} exchange (NCX) and SR Ca^{2+} ATPase (SERCA) restore Ca^{2+} back to resting conditions -once SR Ca^{2+} release stops by one or more mechanism(s) whose relative contributions remain unclear (Stern and Cheng, 2004; Hinch, 2004).

CICR as an amplifier needs local control

CICR amplifies a small trigger Ca^{2+} flux by about an order of magnitude by inducing Ca^{2+} release from the SR. While the trigger is provided (mostly) by L-type Ca^{2+} channel gating during the action potential, the SR Ca^{2+} release is mediated by Ca^{2+} -dependent gating of RyRs that are the SR Ca^{2+} release channels. As soon as it became possible to measure Ca^{2+} levels inside voltage-clamped cardiac cells it became apparent that regenerative CICR never escaped tight control by the timing and amplitude of the trigger Ca^{2+} influx, although the RyRs should be regeneratively activated by their own Ca^{2+} release (as both sources feed the adjacent cytoplasm or the “common pool”) (Cannell et al., 1987). Stern’s mathematical analysis showed that common pool CICR models should operate in an “all or none” fashion at realistic flux amplification levels (Stern, 1992) which was clearly at odds with the graded SR Ca^{2+} release seen in numerous single cell voltage clamp experiments (e.g. (Cannell et al., 1987; Barceñas-Ruiz and Wier, 1987). The solution to this problem was provided by “local control” (Stern, 1992; Cannell et al., 1995; Stern et al., 1999) wherein small groups of RyRs and L-type Ca^{2+} channels form an autonomous calcium release unit (CRU) in the micro-anatomical dyad

structure (Franzini-Armstrong et al., 1998). The physical separation of CRUs by ~700 nm prevents cell-wide regenerative behavior and gives rise to microscopic packets of Ca^{2+} release which were first detected in the form of “ Ca^{2+} sparks” (Cheng et al., 1993). Thus activation of one or a small number of RyRs within a CRU leads rapid recruitment of the adjacent RyRs within that CRU to produce a Ca^{2+} spark, but RyRs in adjacent CRUs are not normally activated because of the diffusion and buffering of Ca^{2+} outside the source dyad. Graded cell-wide Ca^{2+} release is then provided by the time- and trigger-dependent recruitment of Ca^{2+} sparks whose amplitude depends on SR Ca^{2+} levels (Cannell et al., 1995; Soeller and Cannell, 2004).

However, the regenerative problem inherent in CICR was not solved at the scale of the CRU by the discovery of Ca^{2+} sparks. Once a CRU is activated, a Ca^{2+} spark should still progress independently of the trigger due to regenerative CICR within the dyad junction itself (Cannell et al., 1995). Put another way, the dyad space should (essentially) recapitulate the original common pool problem. Mathematical analysis has shown that most proposed mechanisms can contribute to stability (Hinch, 2004) but, the question is: which is most important or key?

To date, the mechanism(s) responsible for the control of SR release termination remain unclear, although evidence for several mechanisms that may contribute to RyR closure have been obtained: (1) Time-dependent inactivation and/or “adaptation” of the RyR channel (Györke and Fill, 1993; Zahradnikova and Zahradnik, 1996; Velez et al., 1997). (2) Stochastic attrition, which describes the probabilistic event that all (n) RyR channels within a CRU close at the same time to allow the local Ca^{2+} to dissipate and thus terminate regenerative CICR (Stern, 1992). (3) Allosteric coupling between RyRs so that spontaneous closure of one RyR promotes closure of the others (Stern, 1992; Marx et al., 2001; Sobie et al., 2002). (4) SR Ca^{2+} -dependent RyR gating changes due to the presence of a RyR luminal Ca^{2+} sensor either on the RyR itself (Gyorke and Györke, 1998; Ching et al., 2000) or via an accessory protein such as calsequestrin (CSQ, (Qin et al., 2008)). (5) ‘Induction decay’ (Laver et al., 2013) or ‘pernicious attrition’ (Gillespie and Fill, 2013) wherein a decreasing RyR release flux leads to local cytoplasmic Ca^{2+} levels becoming insufficient to maintain CICR. All of these mechanisms with the exception of 5 have been discussed in previous focused reviews, e.g. (Fill and Copello, 2002; Stern and Cheng, 2004; Cannell and Kong, 2012), so this perspective will not

exhaustively examine their literature except to raise problems in their sufficiency for CICR termination.

SR lumen control and SR Ca²⁺ depletion

Ca²⁺ in the lumen of the SR is highly-buffered and CSQ appears to be able to explain most of the measured buffering power (Shannon and Bers, 1997). In addition to this important role, CSQ may also directly modulate RyR gating –an idea supported by the Ca²⁺ handling abnormalities associated with CSQ mutants and CSQ expression changes (see (Knollmann et al., 2006; Terentyev et al., 2011)). In addition, histidine-rich Ca²⁺ binding proteins (HRC) are also present in the SR and may modulate SERCA Ca²⁺ uptake as well as RyR gating (for review see (Arvanitis et al., 2011)). The amount of HRC present in the SR is uncertain but seems capable of supplanting Ca²⁺ binding in CSQ knockout mice (Murphy et al., 2011). However, most (if not all) Ca²⁺ transport/balance models have focused on CSQ as the principal SR Ca²⁺ buffer and not included HRC. Finally, it should be noted that SERCA also buffers Ca²⁺ in the lumen of the SR and this buffer can modify Ca²⁺ cycling (Higgins et al., 2006).

Eventual termination of CICR would be assured if the SR ran out of buffered Ca²⁺ (Fig. 1A), however measurements of SR content using caffeine as a probe of releasable Ca²⁺ suggested that less than 50% of the SR Ca²⁺ content was released in a single twitch (Bassani et al., 1993). Thus extensive SR wide Ca²⁺ depletion is unlikely to explain release termination. Since CICR is a local control phenomenon in the dyad (Stern et al., 1999; Cannell et al., 1995), attention has turned naturally to evaluating Ca²⁺ levels in the jSR. Measurements with low affinity Ca²⁺ indicators trapped within the SR also showed that jSR Ca²⁺ depletion was far from complete (Shannon et al., 2003; Brochet et al., 2005) and it was suggested that depletion by itself could not explain CICR termination (Sobie et al., 2002). However, a more moderate depletion could (possibly) be augmented by SR luminal control of RyR gating (Fig. 1B).

RyR gating appears to be sensitive to the level of Ca²⁺ in the SR lumen (Sitsapesan and Williams, 1994; Lukyanenko et al., 1996; Gyorke and Györke, 1998). Varying luminal Ca²⁺ over the likely physiological range (0.5 -2 mM) alters RyR open probability (P_o) approximately 2-fold

(Gyorke and Györke, 1998) and, although weaker than cytoplasmic regulation, could be important in adjusting CICR gain. A large part of RyR luminal Ca^{2+} sensitivity may be related to CSQ binding as, when CSQ is stripped from the RyR complex, RyR luminal Ca^{2+} sensitivity is reduced by a factor of ~ 2 , as is the maximum P_o (Qin et al., 2008) (see also (Ching et al., 2000)). Such a moderate change in RyR gating would not seem capable of terminating SR Ca^{2+} release without augmentation by additional mechanisms. Our modeling suggests that such luminal control is only a weak modifier of Ca^{2+} release during Ca^{2+} sparks (Cannell et al., 2013) and produces effects that are hard to distinguish from modifiers of the cytoplasmic Ca^{2+} sensitivity of RyRs.

RyR inactivation/adaptation

Time-dependent inactivation and/or “adaptation” (Györke and Fill, 1993) may be seen under some conditions and many models of CICR include RyR inactivation to achieve stability. However, adaptation appears to be too slow (in the order of ~ 100 ms see (Valdivia et al., 1995)) to be responsible for Ca^{2+} spark termination and, on the ~ 30 ms time scale of the Ca^{2+} spark, significant adaptation/inactivation is not seen (Zahradnikova et al., 1999). Furthermore, direct evidence against adaptation as a primary termination mechanism was provided by local Ca^{2+} release measurements (Sham et al., 1998). However, this does not mean that adaptation-type mechanisms are incapable of adding some modulation to other CICR termination (and activation) processes. In connection with this point, it has been suggested that resting Ca^{2+} spark rate can increase slowly during rest with Ca^{2+} influx blocked and no change in SR Ca^{2+} load (Sato et al., 1997) –a phenomenon that would be compatible some weak time dependent process. In addition, RyRs may undergo modal gating behavior with a slow transition between a high availability mode and other states (Zahradnikova and Zahradnik, 1995). Again, while such gating changes could contribute to longer term changes in RyR responses, the rate of mode shifting appears to be too slow for this process to play a major role in Ca^{2+} spark termination.

Stochastic attrition

Stochastic attrition (Fig. 1C) also appears to be too slow to explain normal Ca^{2+} spark termination for typical RyR open times, open probabilities and likely number of RyR in a CRU (see (Cannell and Kong, 2012; Stern and Cheng, 2004)). However, recent super-resolution data is indicating that the number of RyRs in each junctional cluster may be lower than originally inferred from junctional area and the assumption of tight RyR packing within circular clusters (Baddeley et al., 2009; Hou et al., 2014). Previous EM and confocal imaging studies suggested that up to several hundred RyRs might form a functional CRU in each junction (Franzini-Armstrong et al., 1999; Soeller et al., 2007), but the organization of the RyRs in the CRU is highly variable and occupy an average area that would correspond to 40-60 RyRs per CRU if tightly packed (Hou et al., 2014). Since RyRs may not be tight packed (see below), the number of RyRs inferred from junctional image area should probably be reduced by about 30-50% to give ~30-40 RyRs in each functional CRU. From this we can calculate that the maximum release flux would be ~7 pA (from a single channel current of ~0.4 pA (Gillespie and Fill, 2008) and peak P_O of 0.5 (Cannell et al., 2013)), which is close to that estimated from Ca^{2+} spark model fitting (Soeller and Cannell, 2002). However, with an open time of ~2 ms, the time constant of stochastic attrition would still be too long for attrition to play a key role unless P_O is reduced to <0.1 (Stern and Cheng, 2004), which seems unlikely for junctional Ca^{2+} levels >10 μM being associated with a release flux as low as a 0.2 pA (Soeller and Cannell, 1997).

A key defining feature of the stochastic attrition mechanism is the near simultaneous closure of all currently open RyRs in the CRU to allow local cytoplasmic Ca^{2+} to decline to a level that does not reopen them. Stochastic attrition should be associated with a rather abrupt cessation of release flux but our detailed release flux calculations suggested a rather smooth decrease in release flux during the Ca^{2+} spark (Soeller and Cannell, 2002)(see also (Kong et al., 2013)), although this is not a very strong argument against stochastic attrition in the face of uncertainties due to noise and microscope blurring (see below).

For CICR to stop fatefully under stochastic attrition, local Ca^{2+} levels in the dyad must decline to a level that prevents any RyRs from reopening. It takes local Ca^{2+} about 5 ms to decrease to near average cytoplasmic levels after SR release stops (Soeller and Cannell, 1997). If this is much shorter than the number of RyRs divided by their mean closed time, CICR would not be able to

reignite the Ca^{2+} spark and release would be terminated. Therefore, while stochastic attrition might be an initiating event for termination of CICR, full termination still requires that RyR closed time times the number of RyRs in a cluster be >5 ms. For a cluster of ~ 35 RyRs, this would imply a RyR closed time of $>\sim 165$ ms and this is seen at cytoplasmic $\text{Ca}^{2+} < 4\text{-}40$ μM (depending on species) (Cannell et al., 2013). Such levels are likely to be achieved within ~ 5 ms of CRU closure, so stochastic attrition by itself could terminate CICR, although it is unclear that stochastic attrition would happen quickly enough.

Timescale-based arguments against stochastic attrition being the mechanism for Ca^{2+} spark termination do not apply when the availability of RyRs is reduced with tetracaine as long lasting Ca^{2+} sparks can occur (Zima et al., 2008a). This is associated with an apparently steady level of SR Ca^{2+} and so SR release termination cannot be due to changes in luminal Ca^{2+} (or a luminal SR Ca^{2+} sensing site) in these conditions (Zima et al., 2008b). However, the termination of such long lasting release events lasting ~ 300 ms (or more) would, we suggest, be compatible with the stochastic attrition mechanism.

Allosteric coupling

The equation for the time constant for stochastic attrition (Stern and Cheng, 2004) depends on the assumption of independent RyR gating, but it has been suggested that RyR gating might not be independent ('X' in Fig. 1C). When RyRs are reconstituted in bilayers, RyRs can show coupled gating (Marx et al., 2001) and RyRs are closely packed in the junctional space (Franzini-Armstrong et al., 1999) suggesting the possibility of allosteric interactions between RyRs. Such allosteric coupling could produce positive cooperativity which would cause a CRU to behave as if there were fewer RyRs in the cluster (Stern, 1992; Sobie et al., 2002; Stern and Cheng, 2004) (and in the limit of very strong coupling causes the cluster to gate as one). Although a viable mechanism to produce reliable Ca^{2+} sparks and spark termination (Stern and Cheng, 2004; Groff and Smith, 2008), how possible *physical* interaction (as distinct from effects mediated by changes in Ca^{2+}) might occur is unclear. FK506 binding protein (FKBP) was initially identified as a protein modifier of *RyR1* interactions (Marx et al., 1998) but its possible role in coupled RyR gating is controversial with conflicting

evidence for roles in determining Ca^{2+} spark frequency and properties (see (Guo et al., 2010) for references). A protein that acts as a linker between RyR tetramers might be expected to have 1:1 or 1:2 stoichiometry with RyR but <20% of RyR have FKBP12.6 (the isoform that appears to modify RyR gating) bound although RyR binds nearly all the FKBP12.6 in the cell (Guo et al., 2010). Allosteric interactions require RyR to be very closely apposed, if not actually touching. Recent high resolution tomographic data suggests that RyRs in cardiac dyads do not exhibit a regular geometric organization with only ~50% actually touching each other (Asghari et al., 2014). This result, while compatible with the low fraction of RyR actually having FKBP bound, would place an important limit on the extent to which RyR allosteric interactions (and consequent increase in likelihood of stochastic attrition) can help eventual Ca^{2+} spark termination by attrition (Hinch, 2004). This conclusion is supported by experiments in FKBP12.6 null mice which show only modest increases in spontaneous Ca^{2+} spark frequency and duration (Xin et al., 2002). Recently, high resolution electron micrographs of purified RyRs appear to show that there may be some preferred regions of RyR interaction that can cause them to form dimers (Cabra et al., 2016), but this interaction is likely weak as most RyRs did not dimerize or form higher number assemblies. We suggest that, if coupled gating via physical interactions occurs, it is neither central to the spark termination problem nor a major modifier of Ca^{2+} release during Ca^{2+} sparks.

Induction decay

None of the above mechanisms, in isolation, appears to be capable of providing a sufficient explanation for Ca^{2+} spark termination (Stern and Cheng, 2004). However, most prior models for CICR did not include realistic geometry for the RyRs in the dyad nor an accurate description for RyR gating under physiological conditions. These shortcomings were addressed in a new ‘induction decay’ model (Fig. 1D) which included a simplified RyR gating model (based on actual RyR gating measured in planar lipid bilayers) as well as dyad geometry (Laver et al., 2013). The mechanism of Ca^{2+} spark termination that appeared as an emergent property of the model was called ‘induction decay’ because it reflected the gradual loss of the regenerative capacity (or gain) within Ca^{2+} induced Ca^{2+} release (CICR). In the model, a gradual decline in local Ca^{2+} due to a decreasing open RyR Ca^{2+}

flux resulted in an increase in the closed time of adjacent RyRs so it became increasingly unlikely for CICR to continue (as also shown in the mathematical analysis of (Hinch, 2004)). The decline in RyR release flux was entirely due to the local Ca^{2+} depletion in the jSR which refilled, once release was finished, from the network SR. Importantly, the model also explained the time course of Ca^{2+} spark restitution described by Sobie and co-workers (Sobie et al., 2005) without additional free parameters. That a decreasing RyR flux could affect SR release was shown directly in cotemporaneous experiments using RyR permeation blockers (Guo et al., 2012). While these observations were subsequently incorporated into a termination mechanism called ‘pernicious attrition’ (Gillespie and Fill, 2013), the idea of induction decay is central to both the computational and conceptual models. The strength of the computer model (Cannell et al., 2013) resides in its ability to show that the measured Ca^{2+} -dependence of RyR closed times is sufficient to terminate CICR, as well as reproduce other effects such as Ca^{2+} spark refractoriness. However, it remains unclear whether any SR load-dependent RyR gating effects might also be present to modulate induction decay (see above). The coupling of jSR load to the ability to support CICR via the dyad cytoplasmic space provides an effective “use-dependence” that was observed by Sham et al. in “ Ca^{2+} spike” recordings that give a measure of local release fluxes (Sham et al., 1998).

While various alternative models can be tuned to control CICR under a fixed set of conditions (Stern, 1992), the induction decay model produced similar Ca^{2+} sparks with variable numbers of RyRs, RyR organization and RyR Ca^{2+} sensitivity and this remarkable property was due to the extent of local jSR depletion associated with the CRU. The relative insensitivity to the number of RyRs in the dyad in the induction decay model is unlike models that rely on simple attrition schemes and would be an advantage for variable RyR expression in dyads. Similarly, a significant increase in RyR sensitivity (as seen in sheep RyRs) does not prevent Ca^{2+} spark termination because the jSR simply depleted to a lower level (Cannell et al., 2013) –a feature reminiscent of the behavior of CICR as seen in the presence of RyR gating modifiers (Eisner et al., 2000). A more recent study (Walker et al., 2014) using the geometry and RyR gating used in the original induction decay model showed that it could also mimic the SR Ca^{2+} leak-load relationship as seen in intact cells (Zima et al., 2010). In the induction decay model, such effects are mediated by cytoplasmic dependence of RyR opening rate

and consequent support of CICR (initiated by a spontaneous RyR opening) rather than a luminal $[Ca^{2+}]$ effect *per se*. It is important to note that in the induction decay model, the number of open RyRs gradually decreases, unlike the abrupt simultaneous closure required for stochastic attrition. Of course, once the number of open RyRs becomes small enough, stochastic attrition may finish the induction decay process (see (Hinch, 2004) for an analysis), but simultaneous closure of multiple RyRs is not needed and does not usually occur.

Perhaps unexpectedly, our induction decay simulations also showed that the standard deviation of Ca^{2+} spark durations ($\sim 10\%$ of the mean see Table 1 in (Cannell et al., 2013)) was smaller than might be expected for a purely stochastic closing process. A part of this behavior can be explained by RyR gating being supplied with an effective memory of the prior RyR gating pattern due to the coupling of prior RyR openings to the level of Ca^{2+} in the jSR that, in turn, affects RyR gating (primarily via the dyad space). This behavior also creates a type of “allosteric coupling”, although not mediated by direct RyR contact but rather via Ca^{2+} -dependent crosstalk.

SR depletion as a local control problem/non-uniform depletion

Local depletion of the jSR is required for induction decay and the depth of depletion (to $\sim 10\%$ of the original level) is much larger than suggested by previous studies. Using caffeine to probe the total SR Ca^{2+} content, it has been estimated that the SR releases 17%-53% of its content (Bassani et al., 1993; Delbridge et al., 1996; Diaz et al., 1997). A similar estimate ($\sim 50\%$) is provided by Ca^{2+} imaging with SR loaded Fluo 5N (e.g. (Shannon et al., 2003; Picht et al., 2011; Zima et al., 2010)). Such moderate depletion might seem to be a problem for the induction decay mechanism. However, we suggest the local jSR is more deeply depleted than the latter imaging studies suggest. Ca^{2+} sparks that were repeatedly activated from the same site showed that Ca^{2+} spark amplitude decreased with decreasing interval between activations (Δt) and at t short Δt (~ 50 ms) Ca^{2+} spark amplitude was only $\sim 10\%$ of the initial Ca^{2+} spark amplitude (Sobie et al., 2005) suggesting that local SR Ca^{2+} may be similarly reduced. Importantly, the restitution of Ca^{2+} spark amplitude in the latter study was reproduced by the induction decay model, further strengthening the idea of significant local SR depletion, although this would not rule out a lesser depletion augmented by some other form of luminal control.

Re-analysis of SR Ca²⁺ depletion signals

To further examine the possibility deeper local jSR depletion than suggested by fluorescence measurements, we carried out a detailed Ca²⁺ spark model fitting exercise (Kong et al., 2013), similar to an earlier analysis of Ca²⁺ spark flux by a reconstruction method (Soeller and Cannell, 2002). We constructed a spherical reaction-diffusion model centred on a single dyad. Cellular structures and associated Ca²⁺ buffers were homogeneously distributed over each model compartment (see Fig. 1B in (Kong et al., 2013)) and the calculated fluorescence signals at all model spatiotemporal coordinates were then convolved with a microscope PSF to simulate experimental Ca²⁺ spark recordings. Importantly, the model confocal PSF was not assumed to be diffraction-limited, but matched to that observed in live cell experiments. The jSR was given a volume and buffering power consistent with other models and high quality Ca²⁺ spark records were fitted by modifying a release flux basis function. This model accurately fitted Ca²⁺ spark data (Fig 2A) both temporally and spatially (Fig 2A right) and produced a reasonable “Ca²⁺ blink” depletion signal (Fig. 2B), although the actual level of SR Ca²⁺ depletion was lower than the fluorescence record (F/F₀) might suggest. This difference arises from the blurring of the Fluo-5N signal, which is more spatially restricted compared to that of a Ca²⁺ spark and its non-linear response to Ca²⁺ (Kong et al., 2013). The time to minimum of the induction decay model blink signal (~25 ms) is very similar to that reported in the original work of Brochet et al. (24 ms) (Brochet et al., 2005) (see also (Terentyev et al., 2008)), although another study in skinned cells suggested longer times to peak of the Ca²⁺ spark and blink (~60 ms) (Zima et al., 2008b). The time to nadir depends on the degree of jSR connectivity, jSR buffering as well as RyR cytoplasmic Ca²⁺ sensitivity, so some variability among experiments should be expected. Nevertheless, one consistently observed property is that the time to nadir of a Ca²⁺ blink is 1.5-3 times longer than the time to peak of the associated Ca²⁺ spark and this is reproduced in the computational model (see Fig.2).

From the deduced release flux and jSR depletion, the apparent RyR gating time-course was derived (nP₀ Fig. 2C). Two features of this analysis were notable: 1) the release flux appeared to decay monotonically, while 2) the jSR permeability declined more slowly. The experimentally

constrained model flux was very similar to the average (stochastic) induction decay model results (Fig 2D). The time-course of RyR gating differs between the models. The decline of RyR permeability is sensitive to the Ca^{2+} -dependence of RyR closed time, as shown by the more Ca^{2+} -sensitive sheep RyRs (dotted line, Fig 2D). Despite these changes in gating time-course, these models suggest that the decay of release flux is driven mainly by the local Ca^{2+} gradient across the jSR membrane rather than the time course of RyR gating *per se*. The calculated jSR depletion levels are consistent with the depletion required for induction decay.

Conclusion

The induction decay mechanism provides a self-sufficient explanation for CICR termination. In this mechanism, a decline in jSR Ca^{2+} during a Ca^{2+} spark is transduced via the steep cytoplasmic Ca^{2+} -dependence of RyR gating, and this rapidly increases RyR closed time until CICR cannot be maintained. The other mechanisms described here may be able to modulate induction decay, although further studies will be needed to establish their relative contributions. We suggest that additional modification(s) of the gain and sensitivity of CICR may be necessary because of the criticality of CICR for cardiac function and therefore the need for more than one point of control.

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Figure Legends

Figure 1. Possible CICR termination mechanisms. (A) SR Ca²⁺ depletion. Reduction in SR Ca²⁺ levels will reduce release flux regardless of RyR gating (P_o). However, fateful termination by this mechanism alone is problematic because the jSR lumen is continually

refilled from the rest of the SR. (B) SR luminal control of RyR gating may be modulated either by a direct on the RyR itself (red), and/or via an accessory protein such as CSQ (green). However, the extent to which these mechanisms could reduce RyR P_O sufficiently to terminate release is unclear. (C) Stochastic attrition. If all RyRs close simultaneously, then the release flux is terminated. However, it is unlikely that this will occur within the time scale of a Ca^{2+} spark. Stochastic attrition could be accelerated by coupled gating between RyRs, either by direct contact, or by a protein linker, 'X'. (D) Induction decay. After CRU activation, jSR Ca^{2+} levels decline which results in a decreasing release flux. The local cytoplasmic Ca^{2+} is proportional to the release flux and this is transduced via the steep Ca^{2+} -dependence of the RyR closed time. As the closed time becomes longer, it becomes less and less likely for an RyR to re-open to provide the flux and local Ca^{2+} levels required to continue CICR.

Figure 2. Estimating jSR Ca^{2+} depletion and RyR gating time-course from flux reconstructions. (A) A 3D model of Ca^{2+} reactions, diffusion and microscope blurring generates a Ca^{2+} spark record, which is fitted to experimental data by varying a basis function for the jSR permeability time-course (Kong et al., 2013). (B) Although not fitted, the model can also simulate the corresponding SR Ca^{2+} depletion signals which are similar to those recorded experimentally (see (Zima et al., 2008b)). (C) From the calculated release flux (red) and the jSR Ca^{2+} levels, the jSR permeability time-course can be derived (blue). (D) Release flux (red) and permeability (blue) changes from the induction decay model of Cannell et al (2013). Note the concordance of flux estimates by both models, although the permeability time-course is dependent on the species-dependent RyR Ca^{2+} sensitivity.