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Does the Goldilocks Principle apply to calcium release restitution in heart cells?

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In heart cells, membrane potential and intracellular calcium ($[Ca^{2+}]_i$) are closely linked. Each time the heart beats, membrane depolarization opens L-type Ca^{2+} channels in the cell membrane. Ca^{2+} entering through these channels triggers the release of a larger amount of Ca^{2+} via ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR) membrane, a process known as CICR, for Ca^{2+} -induced Ca^{2+} release. The increase and subsequent decrease in $[Ca^{2+}]_i$, the Ca^{2+} transient, is the signal that initiates contraction. Conversely, changes in $[Ca^{2+}]_i$ can alter membrane potential through Ca^{2+} -dependent ion channels, pumps, and transporters in the cell membrane. Not surprisingly, numerous proteins and pathways are involved in regulating Ca^{2+} transport in myocytes. When these regulatory systems become disrupted or unstable in disease, Ca^{2+} transients may be altered, and pathological changes in membrane potential such as arrhythmias can result. Understanding beat-to-beat regulation of CICR is therefore critical to gain insight into Ca^{2+} -dependent arrhythmias.

Ca^{2+} transient restitution is one of the fundamental cellular properties important in determining the beat-to-beat stability of CICR. In this context restitution refers to the following: if two Ca^{2+} transients are triggered in succession, how does the amplitude of the second relative to the first depend on the interval between triggers? Although restitution is straightforward to measure in heart cells, determining the underlying mechanisms is challenging because several time, voltage, and Ca^{2+} -dependent processes can contribute, including electrical refractoriness, recovery of L-type channels from inactivation, reloading of SR Ca^{2+} stores, and recovery of RyRs. To infer underlying mechanisms, investigators have used a variety of techniques aimed at separating the contributing processes, such as triggering Ca^{2+} release via photolytic “uncaging” of Ca^{2+} from light-sensitive compounds [1] and [2], and pharmacological manipulation of RyR gating [[3], [4], [5] and [6]]. In this issue of the JMCC, Kornyejev et al. address restitution mechanisms by comparing Ca^{2+} transients in genetically-modified versus wild-type mice [7]. This represents a novel approach to this question, and the results presented in this clever study provide new information that enhances our understanding of restitution. These data may also have interesting implications for how changes in myocyte Ca^{2+} regulation can either increase or decrease susceptibility to arrhythmias.

Disclosures

None.

1. Restitution is faster in the absence of calsequestrin

In their study [7], Kornyejev et al. utilized an established mouse with targeted deletion of type 2 calsequestrin (Casq2), the most abundant SR Ca^{2+} binding protein [8]. Previous studies with this mouse (Casq2^{-/-}) have shown that it exhibits a phenotype characteristic of catecholaminergic polymorphic ventricular tachycardia (CPVT), namely instability of CICR, a tendency towards spontaneous cell-wide Ca^{2+} waves, and a greater propensity for Ca^{2+} -dependent arrhythmias [8]. Besides reducing buffering in the SR, Casq2 knockout causes an increase in SR volume, a reduction in the SR accessory proteins junctin and triadin, and possibly a change in RyR gating (see discussion below). In the present study, the authors used pulsed local-field fluorescence microscopy [9] to record optically from a multicellular region in whole hearts. With this technique, they were able to optically measure (in separate experiments) $[\text{Ca}^{2+}]_i$, SR $[\text{Ca}^{2+}]$, and action potentials. The recovery of each process was assessed by delivering two stimuli at a variable interval and plotting the response to the second stimulus as a function of the interval. The authors found that recovery of both Ca^{2+} transients and SR $[\text{Ca}^{2+}]$ are considerably faster in the Casq2^{-/-} mice compared with the wild-type (Casq2^{+/+}) mice. Recovery of action potentials and L-type Ca^{2+} current, however, are normal in the Casq2^{-/-} mice. Together, these results therefore indicate that the difference between the groups results from faster recovery of the Ca^{2+} release process itself, rather than from recovery of the trigger. Thus, RyRs appear to recover from refractoriness more quickly in Casq2^{-/-} mice.

2. Restitution of release depends on changes in SR $[\text{Ca}^{2+}]$

When interpreted in the context of results obtained over the past several years, the data presented by Kornyejev et al. help us to understand the mechanisms underlying recovery of CICR in heart cells and, by extension, the mechanisms of release termination. For instance, if release terminated due to Ca^{2+} -dependent inactivation on the cytosolic side, and recovery from this process were the rate-limiting step in Ca^{2+} transient restitution, then knockout of an SR Ca^{2+} binding protein would have no effect. Thus, the markedly faster restitution observed in cells from Casq2^{-/-} mice implies that changes in SR $[\text{Ca}^{2+}]$ have a much greater effect on restitution than do changes in cytosolic $[\text{Ca}^{2+}]$. As recently reviewed [10], this result is consistent with several studies on Ca^{2+} spark termination and recovery over the past decade [2], [3], [4], [5], [6], [11] and [12] that have indicated the importance of dynamic changes in SR $[\text{Ca}^{2+}]$. Indeed, the study of Kornyejev et al. is just one of several recent results that argue against an important role for inactivation of RyRs by cytosolic Ca^{2+} (see Table 1). Together these data have important implications for mathematical models of CICR, since they suggest that models that omit Ca^{2+} -dependent inactivation of RyRs will produce more realistic results than those that continue to assume a prominent role for RyR inactivation in cardiac myocytes (see Table 1).

In the present study [7], measurements were made in intact hearts, and each recording reflected the activity of numerous cells. Work at the sub-cellular level has shown, however, that restitution of Ca^{2+} release from an individual cluster of RyRs can be decomposed into two components, a recovery of Ca^{2+} spark amplitude and a recovery of Ca^{2+} spark triggering probability [5] and [6]. To provide additional insight into the results obtained by Kornyejev et al., we performed simulations of Ca^{2+} release recovery in the absence of Casq2 using our mathematical model of the Ca^{2+} spark [6] and [11].

Fig. 1A illustrates schematically the previously described [6] protocol for simulating Ca^{2+} spark restitution in the model. We opened a single channel within a cluster of stochastically gating RyRs at time $t = 0$ to potentially trigger a Ca^{2+} spark, then opened a single RyR again after a specified interval. By running many trials at each interval and computing

statistics, we could assess how Ca^{2+} spark amplitude (Fig. 1B) and Ca^{2+} spark triggering probability (Fig. 1C) evolved as a function of the interval between programmed RyR openings (see [6] for details). In these simulations we assumed that Casq2 knockout completely eliminated Ca^{2+} buffering within the junctional SR but caused no additional changes. The model predicts that this change will dramatically accelerate the recovery rates of both Ca^{2+} spark amplitude and Ca^{2+} spark triggering probability.

Interestingly, in the model Casq2 knockout is also predicted to substantially decrease the probability that, in diastole, a spontaneous RyR opening triggers the remaining RyRs in the cluster (44.1% Casq2^{+/+} vs. 1.23% Casq2^{-/-}). This occurs because, without buffers in the junctional SR, local depletion of SR [Ca^{2+}] occurs more quickly when RyRs are open. This in turn makes the Ca^{2+} flux through each spontaneous RyR opening much less likely to trigger its neighbors because the total Ca^{2+} released during the opening is much smaller. Data obtained in the present study [7] and previously [8], however, suggest that Ca^{2+} spark triggering is normal in Casq2^{-/-} mice. The model prediction therefore suggests that additional factors besides changes in buffering must be considered to recapitulate the phenotype of the Casq2^{-/-} mouse.

3. Besides acting as a buffer, does Casq2 directly regulate the RyR?

It is intuitive that knockout of an SR Ca^{2+} buffer will accelerate the rate of recovery of SR [Ca^{2+}] after release, as the data obtained in the present study demonstrate. The simulation results in Fig. 1 show that this effect would be predicted to cause faster recovery of both Ca^{2+} spark amplitude and Ca^{2+} spark triggering probability. The question arises, then, of whether the reduction in SR buffering in Casq2^{-/-} mice is sufficient to explain the experimental results obtained in the present study and elsewhere.

Although no single result can be considered definitive, several pieces of evidence suggest that Casq2 knockout leads to changes in RyR gating in addition to altered Ca^{2+} buffering. First, in vitro studies have convincingly demonstrated that Casq2 can directly modulate RyR gating [13] and [14]. Second, data in the present study [7] showed that the degree of SR Ca^{2+} depletion during a second Ca^{2+} transient was greater in the Casq2^{-/-} mice when matched to the same relative level of SR [Ca^{2+}]. Third, the increased diastolic SR Ca^{2+} leak observed in Casq2^{-/-} mice and the propensity of these mice to develop Ca^{2+} -dependent arrhythmias [8] suggest changes in RyR gating. Finally, the simulation results shown in Fig. 1 also provide a hint that Casq2 knockout may alter RyR function. When SR Ca^{2+} buffering is reduced in the model and no other changes are made, the simulations predict a dramatic decrease in the frequency of spontaneous Ca^{2+} sparks. Reproducing the normal or slightly enhanced Ca^{2+} spark triggering in Casq2^{-/-} mice would therefore require increasing the RyR opening rate (i.e. increasing the sensitivity of RyRs to be activated by [Ca^{2+}]_i), suggesting that RyR gating is altered in the knockout mice.

Some caveats should be mentioned, however. One is that it is difficult to precisely quantify the levels of free SR [Ca^{2+}] in these mice. Relative changes in SR [Ca^{2+}] can be measured using an SR-targeted dye as in the present study, but calibration of these dyes is challenging, and absolute levels of SR [Ca^{2+}] cannot usually be determined. Previous work has documented a slight reduction in total SR [Ca^{2+}] in Casq2^{-/-} mice [8]; if we assume that roughly half of total SR [Ca^{2+}] is bound to Casq2 in WT mice [10], this suggests a significant elevation in free SR [Ca^{2+}] in the knockouts. An additional unresolved question concerns the precise role played by Casq2 in sensing changes in SR [Ca^{2+}]. RyR gating is known to be affected by SR [Ca^{2+}], and in vitro studies [13] and [14] suggest that Casq2 influences the ability of the RyR to respond to changes in SR [Ca^{2+}]. Casq2 knockout might

therefore be expected to influence the dependence of RyR gating on SR $[Ca^{2+}]$, although the quantitative effects of such an alteration remain unclear at present.

4. Is there an optimal speed of Ca^{2+} release restitution in heart cells?

Terentyev et al. proposed that faster CICR restitution due to decreased Casq2 may explain the increased risk of arrhythmia observed in patients with Casq2 mutations [4]. According to this idea, reduced levels of Casq2 would cause faster recovery of RyRs after a triggered Ca^{2+} transient, and this would increase the probability that pro-arrhythmic Ca^{2+} release in the form of regenerative Ca^{2+} waves could occur during diastole. Subsequent studies documenting Ca^{2+} waves and increased arrhythmia risk in mice with genetically-modified Casq2 [8], [15] and [16] were consistent with this hypothesis. The present study provides the important additional confirmation that Ca^{2+} transient restitution is indeed faster in the Casq^{-/-} mice. When considered along with additional recent findings [17] and [18], these studies provide compelling support for the hypothesis that unusually fast Ca^{2+} release restitution can increase arrhythmia risk.

Korneyev et al., however, obtained an additional interesting result. Upon rapid pacing, the authors found that Casq2^{-/-} mice were less susceptible than Casq2^{+/+} littermates to T-wave alternans, or beat-to-beat alternation in the amplitude of the ECG T-wave. Such electrical alternans has been shown in numerous clinical and experimental studies to be a robust indicator of arrhythmia risk (reviewed in [19]). Moreover, electrical alternans is thought to originate with alternans in Ca^{2+} transient amplitude [20] and [21]. The data of Korneyev et al. therefore suggest that Casq2 knockout may predispose hearts to certain types of Ca^{2+} -dependent arrhythmias while simultaneously protecting hearts against other types of Ca^{2+} -dependent arrhythmias. Indeed, previous studies, both experimental [22] and theoretical [23], have shown that slow recovery of SR $[Ca^{2+}]$ can increase the risk of alternans, although in these studies kinetics of SR $[Ca^{2+}]$ were altered due to changes in the rate of SR Ca^{2+} uptake rather than changes in buffering. Together, these observations therefore suggest that the “Goldilocks Principle” may apply to Ca^{2+} release restitution in heart cells: recovery that is either too fast or too slow may be detrimental, whereas a rate of recovery between the potentially dangerous extremes may be ideal.

Clearly additional work must be performed to test this interesting idea. For one thing, it will be important to confirm that altering Ca^{2+} release restitution affects arrhythmia risk not only in mice, but also in larger animals that bear a greater resemblance to human physiology. It will also be critical to investigate how Ca^{2+} release restitution is altered not only by Casq2 knockout, but in disease states such as heart failure, where multiple changes that influence Ca^{2+} release occur. Overall, however, studies such as that of Korneyev et al. [7] point to the need for a quantitative rather than simply a qualitative understanding of how perturbations influence cardiac Ca^{2+} release restitution. To gain such a quantitative understanding and unravel mechanisms, techniques both at the whole heart level and at the level of the Ca^{2+} spark are likely to be valuable.

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Abbreviations

[Ca ²⁺] _i	intracellular calcium concentration
SR	sarcoplasmic reticulum
JSR	junctional sarcoplasmic reticulum
RyR	ryanodine receptor
CICR	Ca ²⁺ -induced Ca ²⁺ release
Casq2	type 2 (cardiac) calsequestrin, SR Ca ²⁺ buffer
CPVT	catecholaminergic polymorphic ventricular tachycardia

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Fig. 1.

Mathematical modeling of Ca^{2+} spark restitution after Casq2 knockout. (A) As illustrated schematically, Ca^{2+} spark restitution is simulated by opening an individual RyR at time $t = 0$, then opening a second RyR at a specified interval. Running repeated trials and collecting statistics allows us to predict how Ca^{2+} spark amplitude and triggering probability evolve as a function of the interval. The stochastically-gating Ca^{2+} release unit is a cluster of 28 RyRs, with parameters as in [6]. Model parameters were previously adjusted to describe Ca^{2+} spark restitution in myocytes from healthy rats. Here, we assume that spark restitution in wild-type ($Casq2^{+/+}$) mice is similar to that in healthy rats. Probabilities and amplitudes at each interval are mean values based on at least 500 trials. (B) Simulated recovery of normalized Ca^{2+} spark amplitude in $Casq2^{+/+}$ (solid line) and $Casq2^{-/-}$ (dashed line) mice. Amplitude restitution in the knockout (time constant = 13 ms) is considerably faster than in the WT mouse (time constant = 94 ms). (C) Recovery of normalized Ca^{2+} spark triggering probability in $Casq2^{+/+}$ (solid line) and $Casq2^{-/-}$ (dashed line) mice. Triggering probability

recovery is dramatically faster in the knockout (half time = 24.6 ms) compared with the WT mouse (half time = 193 ms). In simulated $\text{Casq2}^{-/-}$ mice, $[\text{Casq2}]$ in the junctional SR was reduced from 30 mM to 0 mM. Thus, we assumed that Casq2 knockout caused a decrease in SR Ca^{2+} buffering and no additional alterations.

Table 1No physiological role for Ca²⁺-dependent inactivation of RyRs?

Experimental evidence against Ca ²⁺ -dependent inactivation of RyRs		
Result		Reference
Spontaneous Ca ²⁺ waves persist at sustained cytosolic [Ca ²⁺] of 50 μM		[24]
Ca ²⁺ spark release flux does not influence level of SR [Ca ²⁺] at termination		[25]
RyR sensitization does not affect recovery of Ca ²⁺ spark amplitude		[6]
Casq2 knockout accelerates Ca ²⁺ transient restitution		Current study
Mathematical models that assume strong Ca ²⁺ -dependent inactivation of RyRs		
Authors	Year	Reference
Wang, Stern, Rios and Cheng	2004	[26]
Shannon, Wang, Puglisi, Weber and Bers	2004	[27]
Lee and Keener	2008	[28]
Liang, Hu and Hu	2009	[29]
Krishna, Sun, Valderrabano, Palade and Clark	2010	[30]
Hashambhoy, Greenstein and Winslow	2010	[31]
Rovetti, Cui, Garfinkel, Weiss and Qu	2010	[32]
Mathematical models that omit Ca ²⁺ -dependent inactivation of RyRs		
Authors	Year	Reference
Sobie, Dilly, Cruz, Lederer and Jafri	2002	[11]
Restrepo, Weiss and Karma	2008	[23]
Hartman, Sobie and Smith	2010	[33]
Gaur and Rudy	2011	[34]
Williams, Chikando, Tuan, Sobie, Lederer and Jafri	2011	[35]