Perspectives on "Control of Ca release from within the cardiac sarcoplasmic reticulum"

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Every time the heart beats, increases and decreases in cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyto}$) switch on and off the mechanochemical reaction that produces movement and force. These $[Ca^{2+}]_{cyto}$ transients are generated by the interplay of several different mechanisms, including (1) Ca^{2+} entry into the cell (largely via $Ca_V1.2$ channels), (2) Ca^{2+} release from the SR (a specialized intracellular organelle), and (3) cytosolic Ca^{2+} removal processes (i.e., various pumps and ion exchangers) that extrude Ca^{2+} from the cell or return it back into the SR. Mitochondrial Ca^{2+} stores, and various other organelles and Ca^{2+}-binding molecules, also contribute (Bers, 2001). However, the primary source of the transient rise in $[Ca^{2+}]_{cyto}$ is release from the SR. The following five Perspective articles focus on the control of Ca release from within the SR and, in particular, the mechanisms whereby cardiac SR Ca^{2+} release is altered by changes in $[Ca^{2+}]_{SR}$. The articles reveal that this crucial function is indeed dynamically modulated by the changing contents of the store and that multiple mechanisms are at work in the modulation, even if their respective impacts remain controversial.

Ca^{2+} release from the SR occurs at discrete sites (i.e., Ca^{2+} release units [CRUs]) and is mediated by RyR2 channels deployed in specialized SR junctional membranes, called the terminal cisternae. The term "couplon" (Stern et al., 1997, 1999; Franzini-Armstrong et al., 1999), which refers to the set of proteins that work in interaction, conveys the idea that the device operating here comprises multiple channels (Ca_Vs and RyRs) and various closely associated molecules, the concerted function of which defines the duration and magnitude of the local Ca^{2+} flux.

The RyR channels bridge two compartments with markedly different Ca^{2+} regimens. The SR is a small-volume Ca^{2+} storage compartment, where resting free $[Ca^{2+}]$ is near 1 mM and where Ca^{2+} buffering has high capacity and low affinity. The cytosol is instead a compartment of much larger volume, where the free $[Ca^{2+}]$ is four orders of magnitude lower and buffering has multiple sites, affinities, and kinetic rates. Because these regimens are so different, a well-behaved system would benefit from control by dual sensors—a "toe" in each compartment—to monitor, feedback, and modulate the system accordingly. The cytosolic sensor would have to react to small and rapid Ca^{2+} stimuli, whereas the intra-SR (luminal) sensor should be tuned to detect Ca^{2+} depletion.

Nature does even better. The RyR channel actually has multiple sensors in each compartment, all of which react differently to local Ca^{2+} changes to govern the degree by which RyRs open. Closed RyRs open when Ca^{2+} binds to a site, now apparently identified (des Georges et al., 2016), on the cytosolic side on the RyR protein. The triggering Ca^{2+} signal normally arises first from voltage-dependent $Ca_V1.2$ channels in the plasma membrane and is then reinforced by Ca^{2+} from open RyRs. The result is Ca^{2+}-induced Ca^{2+} release, or CICR (Fabiato, 1983; Näbauer et al., 1989). Another Ca^{2+}-binding site on the cytosolic side of the RyR mediates Ca^{2+}-dependent inactivation, or CDI, which appears to be more marked in skeletal muscle than in the heart (Meissner et al., 1986; Fill and Copello, 2002). Additionally, several lines of evidence appear to show that the RyR channel also reacts to luminal Ca^{2+} concentration ($[Ca^{2+}]_{SR}$) changes (e.g., Terentyev et al., 2003; Jiang et al., 2007; Laver, 2007).

The mechanisms by which Ca^{2+} release from the SR is controlled are important for normal heart function. To begin with, every heartbeat requires a cytosolic Ca^{2+} transient of finite duration; therefore, CICR must reliably terminate. Isn't this termination dictated simply by the finite duration of the action potential, which turns on and off the Ca^{2+} entry into the cells that triggers RyR opening during systole? Not really. Researchers have long identified the possibility that Ca^{2+} release from open RyR channels will feed back positively, on the same channels or its neighbors, in a self-sustaining and potentially explosive process. Therefore, some CICR termination mechanism or mechanisms must exist, although there is no agreement as to their nature. Insufficient termination will not just distort the immediate

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mechanical output, but the extra Ca^{2+} in the cytosol will drive excess plasmalemmal Na–Ca exchange, depolarizing the cell (Noble et al., 1996). Such extemporaneous depolarization will in turn perturb the vital pattern of cardiac action potentials (Sipido, 2006).

Although SR Ca^{2+} depletion is an inescapable consequence of Ca release, $[Ca^{2+}]_{SR}$ may increase or decrease depending on the pattern and frequency of action potentials, circumstantially leading to Ca^{2+} overload. The putative sensors of SR depletion, now facing the opposite change, appear to induce more channel opening activity, which compounds with the increased $[Ca^{2+}]$ gradient to produce disproportionately greater Ca^{2+} release flux, again with potentially deleterious consequences (MacLennan and Chen, 2009).

The molecular underpinnings of this continuous sensor action, from negative to positive, are the focus of the issues discussed in the present articles. Another equally central matter is whether the observed actions are direct (i.e., mediated by intra-SR sensor sites) or indirect (i.e., meditated by Ca^{2+} flowing out through an open RyR channel and binding to cytosolic-facing sites on the same or a neighboring RyR). The indirect actions are so-called feed-through mechanisms (Tripathy and Meissner, 1996); they reflect $[Ca^{2+}]_{SR}$ because this concentration establishes the trans-SR Ca^{2+} driving force.

A third focus of interest is whether the truly luminal $[Ca^{2+}]_{SR}$ actions are caused by Ca^{2+} binding to a RyR-resident site or to other couplon proteins (Terentyev et al., 2006). The latter possibility gained favor with the discovery that RyR gating defects are associated with calsequestrin mutations. These mutations, in humans or when induced in model animals, are linked to CPVT2, a disease of cardiac rhythm mediated by extemporaneous Ca^{2+} release (Faggioni and Knollmann, 2012).

These questions are discussed in articles in this issue of JGP by Blatter, Cannell and Kong, Györke et al., Jones et al., and Sobie et al. In addition to collectively providing an updated and complete panorama of the field, these perspectives give these leading groups an opportunity to recall earlier ideas and their own salient contributions to the field.

Thus, Cannell and Kong (2017) reprise their influential suggestion of an "induction decay" mechanism for termination (Laver et al., 2013). Their paper is also notable for facing quantitatively the crucial question of the depth of depletion of $[Ca^{2+}]_{SR}$ that occurs during normal function. This dynamic variable cannot be directly measured because of the size of the organelle, which is below the spatial resolution of the optical techniques available. Cannell and Kong (2017) reexamine here the elegant numeric estimation that they have used to approach this issue (Kong et al., 2013).

The ideas of Sobie and colleagues, on the role of SR depletion as a determinant of control of Ca release, were extremely influential when first proposed ∼15 yr ago (Sobie et al., 2002). Upon reexamination, and given the substantial new information at hand, they now revisit and amend their original proposal. Sobie et al. (2017) also tackle the questions posed by the coexistence of excitatory effects on both sides of the SR membrane, where the changes in $[Ca^{2+}]$ are initially of opposite signs. This duality of effector sites poses problems for the understanding and quantitative prediction of basic function. The interplay of effects becomes an issue of life and death when the sites are altered.

The convergence of transient cytosolic Ca^{2+} increase and SR Ca^{2+} depletion, which occurs every time the RyR channels open, as well as the potential consequences of malfunction are brought into sharp focus in the article by Blatter (2017), which describes these phenomena in atrial cells and adds an unexpected twist. In atria, the interference of defective SR Ca^{2+} release with action potential conduction critically alters cardiac rhythm. Atria present an additional conundrum: because most atrial cells lack transverse tubules, many of their SR release sites (in so-called "corbular" SR) have no nearby source of trigger Ca^{2+}. How are they activated? Simple CICR will not do because the sensitivity of RyRs is exquisitely tuned for activation by open Ca_Vs nearby, and no such channels exist for corbular RyRs. This article smartly answers the question, recalling the observation by Blatter and Maxwell of a brief local increase in $[Ca^{2+}]_{SR}$ ahead of the activation of RyR channels that advances toward the atrial cell axis (Maxwell and Blatter, 2012, 2017). Thus, the lucid theoretical contemplation of two-way changes in $[Ca^{2+}]$ in the article by Sobie et al. (2017) comes to life in the experimental observations of Blatter's laboratory and their theory of "tandem activation."

The two final perspectives tackle molecular aspects of intra-SR Ca^{2+} sensing and control. Jones et al. (2017) endeavor to review the evidence on control by intra-SR Ca binding to luminal sites on the RyR (Jiang et al., 2007; Chen et al., 2014). This task is now more pressing and potentially more rewarding as the molecular structure of RyRs has become known at increasing resolution (des Georges et al., 2016; Peng et al., 2016). Jones et al. (2017) take this opportunity to reexamine their influential description of Ca release induced by store overload (SOICR). As with other effects of the changes in luminal Ca^{2+}, SOICR can be caused by a direct action on luminal effector sites or be a consequence of the heightened Ca^{2+} flux through release channels that open stochastically. In turn, the putative luminal effector sites could be on different SR-resident proteins. Jones et al. (2017) focus on the evidence for effector sites on the RyR.

Györke et al. (2017) instead discuss the evidence supporting intra-SR Ca^{2+} sensing and control through the association between RyRs and calsequestrin. Inside the SR of skeletal muscle, calsequestrin is polymerized and

depolymerizes as $[Ca^{2+}]_{SR}$ changes (Manno et al., 2017). This structural change (probably occurring in the heart as well) may provide a conformational signal to gate the SR channel, while also reducing the Ca^{2+} buffering power of this storage protein. To examine the ways in which these mechanisms may condition Ca^{2+} release, Györke et al. (2017) recall their influential work on the relevance of intra-SR buffering in general and calsequestrin as both buffer and potential regulator of RyR gating (Terentyev et al., 2003, 2006). The article by Györke et al. (2017) is also notable for placing the changes in RyR gating within the general phenomena of refractoriness (with their mechanical and electrical aspects). They describe various mechanisms whereby abnormal control of RyR channel gating reduces the interval of refractoriness; in turn, this reduction may lead to synchronized Ca^{2+} release from multiple channels, a condition needed for the ensuing depolarization to elicit an ectopic action potential.

While revealing considerable agreement on basic issues, the articles expose different opinions on the relevance of the multiple mechanisms. As just one example of the controversies that remain, Jones et al. (2017) stress the functional relevance of Ca^{2+} binding on RyR sites accessible from the SR lumen, whereas both Cannell and Kong (2017) and Sobie et al. (2017) point at the near-sufficiency of the mechanisms that rely on the reduction in unitary Ca^{2+} current to assure termination of flux. Likewise, based on the success of "pernicious attrition"/"induction failure" to account for Ca^{2+} release termination, they question the relative importance of calsequestrin as a sensor.

Perspectives are not written to contemplate all possible control mechanisms or face every controversy of the chosen topic. Therefore, many questions remain. *We invite readers to submit their own contributions to these and related matters in the form of articles providing alternative views. These articles should be written as Perspectives (see http://jgp.rupress.org/about#article) and will be subject to the same editorial consideration and peer review as the articles in this issue.* Perspectives relating to this series should be submitted electronically at www.jgp.org.

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