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New and Notable: On the importance of ryanodine receptor subunit cooperativity in the heart

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Intracellular calcium (Ca^{2+}) homeostasis in cardiac myocytes is an important determinant of both contractile and electrophysiological function, being responsible for excitation contraction coupling (ECC) through the process of Ca^{2+}-induced-Ca^{2+}-release (CICR). Cardiomyocytes contain an intracellular structure which facilitates rapid and robust CICR: type 2 ryanodine receptors (RyRs), responsible for Ca^{2+} release from the intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR), are distributed throughout the intracellular volume in clusters closely juxtaposed (< 20nm) with the sarcolemmal membrane and the L-type Ca^{2+} channels which reside therein, forming dyads (Scriven *et al.*, 2013). RyR dysfunction may directly impair CICR, and thus ECC, resulting in inhibited or inefficient cellular contraction; moreover, many experimental reports have indicated that a change of RyR characteristics is a major contributor to arrhythmia associated with various conditions, such as atrial fibrillation (Voigt *et al.*, 2012) and heart failure (Dobrev & Wehrens, 2014).

To understand the role of RyRs at multiple scales (i.e., from molecular- to organ-level), a physiologically accurate mathematical description of RyR kinetics in health and disease is needed. An ideal RyR model should not only replicate the experimental observations of RyR kinetics but also conduct robustly in the context of the multiple interacting components of the subcellular or cell-level computational models of Ca^{2+} homeostasis. Multiple well-established (2-, 3-, and 4-state) RyR Markov models have been commonly used to replicate RyR kinetics (i.e., opening, closing, inactivation, and refractoriness) and systolic/diastolic Ca^{2+} regulation (e.g., SR leak, Ca^{2+} sparks, and Ca^{2+} waves). All the models are designed to replicate part of the RyR kinetics and Ca^{2+} dynamic features, but may be parameterised for different purposes, e.g., maintaining stable Ca^{2+} homeostasis, or reproducing spontaneous Ca^{2+} sparks at appropriate frequency. Despite the fundamental importance of RyR dynamics for cellular function, the development of a mathematical description of the RyR which is both determined from experimental channel observations and simultaneously robustly reproduces the full range of physiological and pathophysiological behaviours remains a major challenge (Colman *et al.*, 2022).

A better understanding of structure-function relationships at the molecular scale may help to develop such a robust description. Combined with more high-resolution RyR structure images in the recent decade (Yuchi & Van Petegem, 2016), RyR conformations with ligand-binding and RyR-RyR interaction are better understood (Williams *et al.*, 2018). In addition, experimental data suggest that the neighboring subunit-subunit interactions in RyR tetrameric structure substantially regulate RyR opening under not only physiology but also pathological conditions (Yuchi & Van Petegem, 2016). However, such subunit interactions have yet to be systematically assessed in regards to impact on channel and cellular function. To explore these interactions, Greene, Lucko and Shiferaw in this issue of *Biophysical Journal* (Greene *et al.*, 2023) present a theoretical study which mathematically models the RyR with four explicit subunits, applied to study single channel dynamics and whole-cell homeostasis.

The theoretical and numerical analysis reveals that 1) the minimal number of open-state subunits for RyR opening, and 2) the balance of cooperative interactions between neighboring subunits, are both important for regulating RyR opening and closure, and thus stable but efficient Ca^{2+} cycling. A major feature of RyR kinetics which was required to be captured was the simultaneous ability to rapidly respond to sub-millisecond changes to Ca^{2+} (i.e., to be triggered on activation of the L-type Ca^{2+} channels to facilitate CICR), to robustly close during electrical excitation to end CICR, and to demonstrate stability (i.e., low noise) at resting (diastolic) Ca^{2+} levels. Such function necessitates a steep dependence on intracellular Ca^{2+} concentration, which could be a result of a minimum number of required subunits to be simultaneously open before a flux is permitted.

The authors found that in the case where three or all four of the subunits were required to be in the open state in order to permit flux, the RyR can be both reliably shut at low Ca^{2+} levels while being able to rapidly respond to high Ca^{2+} concentrations (i.e., be a robust Ca^{2+} sensor) within a relatively wide parameter space of subunit-subunit interaction strengths (Figure); if two subunits were required to be simultaneously open, then the RyR can still reproduce this function but within a much more restricted region of cooperativity parameter space; for just one subunit, it was not possible to simultaneously fulfil all of these criteria.

The study also assessed the dependence of Ca^{2+} spark dynamics on the relationship between cooperativity energy penalties for the open or closed states, revealing that only under appropriate balance of these parameters could robust and self-terminating triggered Ca^{2+} sparks be induced: favouring the open state led to long-lasting (i.e., not self-terminating) Ca^{2+} sparks, whereas favouring the closed state led to unreliable Ca^{2+} sensitivity (sparks were unable to be robustly initiated). The size of the region of this parameter space became narrowed as the number of required subunits was reduced from four to two, and when just one subunit was required, only long-lasting (not self-terminating) sparks could be induced. Finally, spontaneous Ca^{2+} spark frequency was also highly sensitive to both the number of required subunits and the cooperativity parameter space, demonstrating again that the requirement for fewer subunits led to less stable behaviour, reflected in a higher frequency of spontaneous sparks.

By considering simultaneously multiple parameter spaces and features of Ca^{2+} spark dynamics, this study provides valuable insight into the fundamental physiology of RyR structure-function relationships. This paper highlights the importance of keeping proper subunit-subunit interaction energy and the number of open-state subunits for RyR opening. Meanwhile, this paper shows the potential of building a pipeline to study RyR dynamics from protein structure to a thermodynamic-based theoretical model with biophysics properties, similar to recent models developed for other ion channels (e.g., Silva *et al.*, 2009; DeMarco *et al.*, 2021). Overall, this study lends new insight into the effects of RyR subunits' interaction on Ca^{2+} signaling and will benefit the field.

Multi-scale RyR structural changes (i.e., binding-site, subunit-subunit, RyR-RyR, RyR cluster) underly myriad pathophysiological conditions, such as alternans and triggered activity. Meanwhile, the RyR conformations are regulated by varying targets (e.g., EGTA, Ca, Caffeine-ATP) and clustering (e.g., RyR-RyR regulation and subcellular distribution) (Williams *et al.*, 2018; Galice *et al.*, 2018). To understand the effects of RyR structure changes on disease mechanisms, this model could be extended with disease-specific structure information and be integrated into the cardiac cell model with subcellular spatial details.

Future works could incorporate further RyR regulations such as Calsequestrin (Restrepo *et al.*, 2008), RyR-RyR interactions (Sobie *et al.*, 2002), cluster size and distribution (Sutanto *et al.*, 2018; Zhang *et al.*, 2022). Meanwhile, disease-induced changes in RyR properties (e.g., hyperphosphorylation and disrupted distribution) could also be studied under the same pipeline from structural information to Ca^{2+} cycling.

Figure

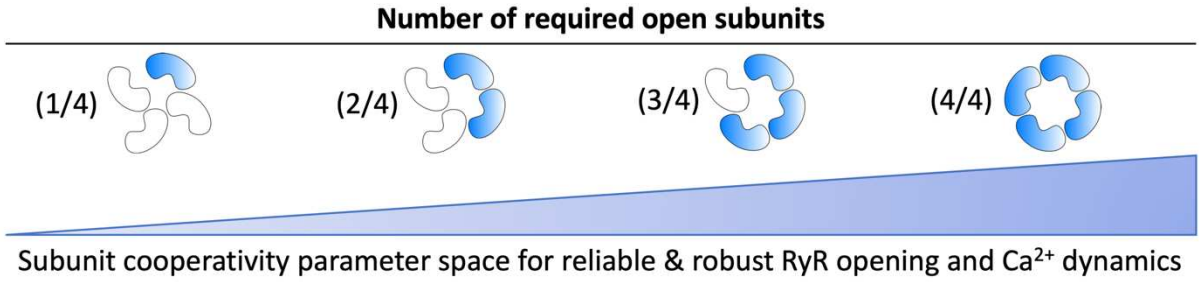

Figure. Illustration of the relationship between the minimum number of subunits required for release flux and the size of the parameter space of subunit cooperativity that fulfils robust RyR function.

Author contributions

All authors conceived, designed, drafted, and edited the manuscript, and prepared figures.

Declaration of interests

The authors declare no competing interests.

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