RESEARCH HIGHLIGHT

The dynamic dyad: cardiac ryanodine receptors on the move

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This research highlight focuses on new developments in our understanding of the structure and function of the mammalian dyad, and of the type 2 ryanodine receptor (RyR2) in particular. Recent investigations have challenged the view of dyads as static and repetitive structures with one functioning much as the next. New data has revealed that dyads have diverse molecular architectures and are dynamic structures where the organization of their RyR2 can be changed by changes in the local environment.

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The last 15 years has seen a remarkable evolution in our understanding of how the cardiac dyad is structured. Until recently, that structure was thought to be somewhat similar to that of the skeletal muscle triad. Skeletal muscle triads have a rigid array of type 1 ryanodine receptors (RyR1) positioned in a checkerboard pattern with the associated calcium channels (Ca_v 1.1) coupled to every second RyR1 pair. In contrast, early freeze-fracture studies of the cardiac dyad indicated that the myocardial calcium channel (Ca_v 1.2) was randomly positioned opposite the type 2 ryanodine receptors (RyR2), which also formed a regular checkerboard array of a hundred or more tetramers covering the junctional sarcoplasmic reticulum [1]. As in skeletal muscle, the RyR2 configuration was thought to be fixed, reflecting intrinsic properties of the protein and providing the structural basis for inter-protein allosteric interaction [2, 3, 4]. Later immunofluorescence analyses refined this view, positioning the Ca_v1.2 more centrally over their underlying RyR2 clusters, as well as finding numerous smaller clusters of RyR2 without adjacent Ca_v1.2 - a grouping whose function remains unknown[5]. Other than differences in size, individual dyads were thought to be largely equivalent in both structure and function [6]. New imaging techniques have changed this perception.

Dyads can be differentiated on the basis of at least three characteristics; their molecular constituents [7, 8, 9], their position within the cell [7, 8, 10] and the latest discovery, which is the topic of this research highlight, the distribution of their ryanodine receptors [11, 12, 13].

Different labs, one using electron tomography and the other super-resolution immunofluorescence microscopy, published near simultaneous papers demonstrating that the RyR2 tetramer array did not span the entire dyad [12, 13]. The tetramers were instead distributed in small clusters of ~1-20 members that were separated from each other by ~50-100 nm. These results enabled a re-analysis of Ca^{2+} dynamics and spark formation, making the polymorphism of the latter much easier to explain[14].

Another landmark paper studied purified RyR1 in lipid

bilayers and demonstrated that the tetramers could spontaneously arrange themselves in one of two ways, dependent on the free Mg^{2+} concentration: in very low Mg^{2+} they were arranged in the expected checkerboard, overlapping each other by about 50% (~14 nm) and in physical contact with their neighbors, while in 4mM Mg^{2+} they adopted a packed side-by side arrangement where adjacent tetramers were not in physical contact[15, 16]. These results demonstrated that the tetramers' relative positions might be malleable, and combined with early data from our lab, led us to investigate RyR2 tetramer distribution in vivo.

Using dual-tilt electron tomographic techniques, coupled with custom-written image analysis software, we examined the distribution of hundreds of RyR2 tetramers in rat and human myocytes and found comparable results in both species; the RyR2 tetramers were neither uniformly nor regularly arranged[11]. Their distributions could be roughly categorized as equal parts checkerboard and side-by-side with a few being isolated or having partners in both configurations. The distance between the tetramers was highly variable and the overall impression was one of disorder. If there is positive allosteric interaction between the tetramers it is unlikely to occur via the tetramers' clamp domains, as this would require a fixed inter-protein distance [2].

Using permeabilized rat ventricular myocytes and the same Mg^{2+} concentrations used by Lai's group we discovered that the tetramers in situ behaved similarly to those in the bilayers; 0.1 mM Mg^{2+} produced a largely checkerboard configuration, while 4 mM Mg^{2+} produced a largely side-by-side configuration. A phosphorylation cocktail that activated both protein kinase A (PKA) and Ca^{2+}-calmodulin-dependent protein kinase II (CamKII) also arranged the tetramers into a largely checkerboard configuration. The mixed arrangement seen in human and rat myocytes would seem to reflect both the ventricular myocyte's intermediate Mg^{2+} concentration (~1 mM) and the tetramers' basal phosphorylation state.

These results imply that the tetramers' positions are dynamic and that they move in response to changes in their local environment. The disordered RyR2 distributions we observed in the rat and human myocytes are best interpreted as a snapshot in which the relative positions of the tetramers reflect their phosphorylation state and the amount of bound Mg^{2+} among other factors, at the moment they were fixed. Importantly, we also found that the tetramers' distribution was correlated with their Ca^{2+} spark frequency, a measure of their open probability; side-by-side tetramers had the lowest spark frequency, checkerboard the highest while the mixed distribution of control cells produced values roughly double that seen in 4 mM Mg^{2+}. These results suggest that not all of the tetramers within a given dyad have the same open probability, and that a tetramer's open probability is reflected, in part, by its position relative to its neighbors. We don't yet know how the tetramers are moved, whether they must be moved to change their open probability, or the effect(s) of other regulatory factors such as nitrosylation.

In conclusion, our view of the dyad has changed from that of a fixed and rigid structure to a dynamic one whose Ca^{2+} release is modulated by numerous, local, factors, which vary across the width of the myocyte and from one dyad to the next. The result is that we can no longer consider dyads to be either structurally or functionally equivalent, or all the RyR2 within them to have the same Ca^{2+} sensitivity. These results open new avenues of investigation and possibly a new understanding of the mechanisms that regulate RyR2 and therefore cardiac contractility.

Conflicting interests

The authors have declared that no competing interests exist.

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