Commentaries on Viewpoint: The cardiac contraction cycle: Is Ca\(^{2+}\) going local?

**MITOCHONDRIAL CONTROL OF Ca\(^{2+}\) IN THE DYADIC CLEFT**

TO THE EDITOR: In this Viewpoint, Fowler and Smith (2) outline the importance of endogenous buffer systems to limiting the spatial and temporal spread of a Ca\(^{2+}\) signal in the highly controlled dyadic cleft without proper consideration of the Ca\(^{2+}\) buffering capacity of neighboring mitochondria. In addition to the close juxtaposition of t-tubules and the SR membrane, mitochondria are also ensnared in the SR network and are bounded at each end by the junctional Ca\(^{2+}\) release sites. Whether this spatial association of mitochondria with the SR is of relevance for local control of SR Ca\(^{2+}\) release events has been highly controversial for many years. Using electron tomography for three-dimensional imaging of dyadic clefts, the work of Masahiko Hoshijima and colleagues (3) recently lent support to the hypothesis that mitochondria are functionally coupled to this Ca\(^{2+}\) microdomain identifying electron-dense structures that link SR or t-tubules with mitochondrial outer membranes at unexpectedly high densities. It appears that these structures stabilize the proximity of the organelles, which could give the mitochondria access to the high Ca\(^{2+}\) concentrations in the microdomain. Besides the high capacity of sarcolemmal Ca\(^{2+}\) binding sites in the cleft (1) mitochondria may serve as additional buffers for Ca\(^{2+}\), maintaining the refractoriness of Ca\(^{2+}\)-releasing sites, and regulating the propagation of Ca\(^{2+}\) release events (5). Furthermore, the high Ca\(^{2+}\) concentrations of a Ca\(^{2+}\) microdomain appear to be essential for activation of the mitochondrial Ca\(^{2+}\) uniporter [K0.5 –20 mM (4)] to supply sufficient Ca\(^{2+}\) for the dehydrogenases of the tricarboxylic acid cycle.

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**DYADIC GEOMETRY IN NORMAL AND FAILING CARDIOMYOCYTES**

TO THE EDITOR: While Fowler and Smith (1) focused largely on the importance of endogenous buffering systems in regulating local Ca\(^{2+}\) levels, they also correctly point out that the physical constraints of the dyad are critical. New evidence suggests that previous estimations of dyadic geometry should be reexamined. With the use of electron tomography, Hayashi et al. (2) recently reported that cleft size is highly variable, but that more than one-third of dyads are smaller than 1.5E05 nm\(^3\). This represents a striking 10-fold reduction in dyadic cleft volume from earlier estimates (3). Such tight geometric constraints could have considerable implications for local Ca\(^{2+}\) regulation, by promoting Ca\(^{2+}\)-sensitive feedback between nearby Ca\(^{2+}\) flux pathways. As well, strict diffusional limitations would likely promote Na\(^+\) accumulation in the dyad, thus increasing reverse-mode Na\(^+\)-Ca\(^{2+}\) exchange, and the likelihood that the resulting Ca\(^{2+}\) influx can trigger SR Ca\(^{2+}\) release.

Accurate analyses of cleft geometry are required to understand alterations in excitation-contraction coupling in pathological conditions. T-tubule disorganization in heart failure can lead to the formation of “orphaned” ryanodine receptors (4, 5), but more subtle alterations in cleft geometry may also occur. While SR Ca\(^{2+}\) release is dysynchronous in failing myocytes (4, 5), it is also delayed (4), which may result from a widening of the dyadic cleft. An expansion of the cleft volume may also contribute to reduced efficiency of Ca\(^{2+}\)-induced Ca\(^{2+}\) release in this condition and profoundly impact local Ca\(^{2+}\) feedback systems and dyadic Na\(^+\) homeostasis, as described above.

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nance. Little is known about the mechanisms underlying dyad formation at the cell surface and most importantly at the t-tubules where most of the Ca cycling occurs (1). We know that t-tubules are labile: absent in neonatal cells, decreasing during cell culture, and are disorganized in some pathologies like heart failure (1). It is therefore essential to understand the mechanisms involved in the biogenesis and maintenance of t-tubules and the formation of dyads. Some hints about t-tubules formation were recently provided (3); however, this field remains largely unexplored. The last 15 years have shown a profusion of data about the function of local calcium signaling at the dyadic space (4); it is now time to address the challenging question of the structure of the dyad.

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STRUCTURAL DETERMINANTS OF DYADIC JUNCTIONS

TO THE EDITOR: Since Stern (4) proposed the “local control” models of cardiac excitation-contraction coupling 17 years ago, significant progress have been made on the characterization of local Ca2+ events, demonstration of functional coupling of L-type Ca2+ channels and ryanodine receptors, measurement of microdomain Ca2+ fluxes, modeling of Ca2+ dynamics in the dyadic junctions, as well as the detection of coupling defects in cardiac hypertrophy and heart failure. These advances are outlined clearly by Fowler and Smith (2) in this Viewpoint article. However, the molecular determinants supporting the micro-architecture of dyadic junctions have not been discussed. Takeshima et al. (5) identified junctophilins (JH) as a class of proteins spanning the membrane of junctional sarcoplasmic reticulum (jSR) and interacting with t-tubular membrane. Junctophilin-2 (JH-2) knockout mice showed deficiency in cardiac junctional complexes, disrupted Ca2+ transients, and embryonic lethality. Expression of JH-2 was found downregulated in hypertrophic and cardiomyopathic mouse models, and mutation of JH-2 gene was detected in patients of hypertrophic cardiomyopathy (3). Furthermore, jSR proteins, including triadin, may also play important roles in maintaining the integrity of dyadic junctions. Ablation of triadin gene decreased the expression of all junctional proteins including ryanodine receptor-2, calsequestrin-2, junctin, JH-1 and JH-2; caused fragmentation of jSR; reduced contacts of jSR and t-tubules; altered Ca2+ release; and exaggerated ventricular arrhythmias (1). Local Ca2+ events have been well researched; however, the roles and interactions of junctional proteins supporting the structures for local Ca2+ signaling deserve extensive future investigations.

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LOCAL CONTROL OF CARDIAC Ca2+ SIGNALING: MODELS, NANO-STRUCTURE, AND CHALLENGES

TO THE EDITOR: The timely Viewpoint by Fowler and Smith (3) emphasizes the critical role of local Ca2+ signaling and highlights the utility of exogenous buffers to identify the scale over which Ca2+-dependent protein-protein interactions occur. Our understanding of local control has clearly made progress in recent years and this is reflected in computationally efficient mathematical models that attempt to capture the complex interactions between local and global Ca2+ signaling (4, 5). Such models provide a rigorous way in which we can state our hypotheses on how the system works and serve to expose remaining gaps in our understanding.

As pointed out in the article, the geometry of the junction, its protein environment and surroundings are the primary determinants of the local nature of Ca2+ signaling (3). It is therefore exciting to witness the development of new fluorescence microscopy techniques that will allow the direct observation of cardiac signaling structures at the nanometer scale (1, 2). This should help clarify the location of accessory proteins and the detailed junctional morphology. It is conceivable that these techniques can be extended to live cell imaging (2) and allow direct observation of local signaling over nanometer distances.

While the insight into the local nature of Ca2+ signaling has resolved some of the apparent “paradoxes” of cardiac Ca2+ signaling, fundamental questions remain about the (normally robust) termination of the local release events, i.e., Ca2+ sparks. Several candidate mechanisms have been identified but we lack definitive answers on their relative importance, species differences, alterations in pathology, and effects on global signaling.

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SNAPTORNE FORMS OF CALCIUM RELEASE THAT STAY LOCAL

TO THE EDITOR: “The Cardiac Contraction Cycle: Is Ca\(^{2+}\) Going Local?” is a valuable “summation” of cellular mechanisms that contribute to spatio-temporal Ca\(^{2+}\) regulation within cardiac cells (3). While the opening scene highlights spontaneous stochastic Ca\(^{2+}\) releases via ryanodine receptors of the sarcoplasmic reticulum, most of the subsequent focus shifts to action potential-triggered RyR Ca\(^{2+}\) release, i.e., synchronized form of RyR Ca\(^{2+}\) release. It is a pity that, within the context of “Ca\(^{2+}\) Going Local,” the functional importance, in health and disease, of spontaneous RyR Ca\(^{2+}\) release, i.e., a localized, unsynchronized form of RyR Ca\(^{2+}\) release, is not addressed. For example, in cardiac pacemaker cells, local submembrane RyR Ca\(^{2+}\) releases are a crucial mechanism for normal automaticity (5). In ventricular myocytes, the effectiveness of inotropic interventions (e.g., stimulation of adrenergic receptors) that increase myocyte SR Ca\(^{2+}\) loading plateaus at the very moment when localized spontaneous Ca\(^{2+}\) releases begin to occur between externally triggered AP cycles (2). As Ca\(^{2+}\) loading continues to occur, these spontaneous Ca\(^{2+}\) releases grow in magnitude and become partially synchronized (1). Partially synchronized spontaneous local Ca\(^{2+}\) releases can trigger abnormal action potentials, impart a Ca\(^{2+}\)-diastolic tone and reduce the magnitude of AP-triggered Ca\(^{2+}\) release, via localized SR depletion, localized RyR inactivation and localized L-type Ca current inactivation (4). Students of pacemaking heart failure mechanisms ought not to lose sight of these functional sequelae of unsynchronized Ca\(^{2+}\) release, i.e., a form of Ca\(^{2+}\) going “local” in their experimental vision.

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NEIGHBORHOOD AND THE NEIGHBORS IMPACT THE LOCAL Ca\(^{2+}\) SIGNALS

TO THE EDITOR: In their Viewpoint, Fowler and Smith emphasize the importance of localized Ca\(^{2+}\) signaling in the modulation of cardiac contractility. In cardiomyocytes, while majority of the LTCCs are localized to T-tubules (3) forming couplings with the RYR2 contributing to generation of spatial and temporal Ca\(^{2+}\) microdomains, at the same time it needs to be recognized that the LTCCs are precisely regulated by associated proteins indirectly contributing to localized Ca\(^{2+}\) signals. Both its “neighborhood” as well as associations impacts this precise regulation of the LTCC. For example, in support of localized cardiac Ca\(^{2+}\) signaling and differential \(I^{\beta}\) adrenergic regulation of LTCC, we have demonstrated that a population of LTCC localized to caveolae forms a macromolecular signasome consisting of components of \(I^{\beta}2AR/AC/PKA\) signaling cascade critical to its regulation and generation of Ca\(^{2+}\) microdomains (1). Important functional consequences of location and associations of the LTCC are exemplified by differential Ca\(^{2+}\) signaling through the \(I^{\beta}-\) ARs. Sustained stimulation of \(I^{\beta}\) receptors mediated Ca\(^{2+}\) influx through LTCC via activation of CaMKII leads to myocyte apoptosis (5). In contrast, \(I^{\beta2}\) activation of LTCC via Gi is protective against apoptotic signals (2, 4).

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