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Review

Cardiac myocytes and local signaling in nano-domains

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ABSTRACT

It is well known that calcium-induced calcium-release in cardiac myocytes takes place in spatially restricted regions known as dyads, where discrete patches of junctional sarcoplasmic reticulum tightly associate with the t-tubule membrane. The dimensions of a dyad are so small that it contains only a few Ca^{2+} ions at any given time. Ca^{2+} signaling in the dyad is therefore noisy, and dominated by the Brownian motion of Ca^{2+} ions in a potential field. Remarkably, from this complexity emerges the integrated behavior of the myocyte in which, under normal conditions, precise control of Ca^{2+} release and muscle contraction is maintained over the life of the cell. This is but one example of how signal processing within the cardiac myocyte and other cells often occurs in small "nano-domains" where proteins and protein complexes interact at spatial dimensions on the order of ~1–10 nm and at time-scales on the order of nanoseconds to perform the functions of the cell. In this article, we will review several examples of local signaling in nano-domains, how it contributes to the integrative behavior of the cardiac myocyte, and present computational methods for modeling signal processing within these domains across differing spatio-temporal scales.

Contents

1. Introduction

It is becoming increasingly clear that cellular signal processing often occurs in small "nano-domains", where proteins and protein complexes interact at spatial dimensions ranging from 1's to 10's of nanometers, and on time-scales of nanoseconds to microseconds. This mode of signaling in fact appears to be a common design motif in all cells, and is certainly true of the cardiac myocyte. In this article, we will present examples of the important role of such local signal processing in the cardiac myocyte. We will focus on the integration of Ca^{2+} and Na^{+} signals in and near the cardiac dyad.

Calcium-induced calcium-release (CICR) in cardiac myocytes takes place within dyads, where discrete patches of junctional sarcoplasmic reticulum (jSR) tightly associate with the t-tubule membrane. Dyad volume, diameter, and height are estimated to be $\sim 3 \times 10^5$ nm^3, 100–200 nm, and 10–15 nm, respectively (Franzini-Armstrong et al., 1999; Hayashi et al., 2009). L-type

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Ca^{2+} channels (LCCs) are preferentially located in the t-tubule membrane, in close apposition to the jSR membrane. As our initial example of signal processing in nano-domains, we will consider the remarkable, recent experimental and modeling evidence indicating that LCCs are able to sense both local Ca^{2+} concentration, as well as global Ca^{2+} signals. This signal processing results from the differing temporal dynamics of conformational changes due to Ca^{2+} binding to the C- versus N-lobe of calmodulin (CaM), which is tethered to LCCs and mediates Ca^{2+}-dependent inactivation. It is a compelling example of how an important regulatory signal can be sensed by a pair of molecules.

Because of the small physical dimensions of the dyad, modeling studies indicate that at any instant of time there are only a few ($\sim$1–100) Ca^{2+} ions present, and that Ca^{2+} signaling may be influenced significantly by the physical shape and size of the dyad, the relative placement of dyad proteins such as ryanodine receptors (RyRs) and LCCs, local membrane buffering of Ca^{2+} by negatively charged phospholipid headgroups, and the dyadic electric field. Our second case study will review how nano-scale models of Ca^{2+} signaling in the dyad that are based on the fundamental physical principles governing the trajectories of individual Ca^{2+} ions and their binding to RyRs, provide insights into the nature of dyadic Ca^{2+} signaling and CICR. Additional signal processing occurs in the dyad. Examples include signaling mediated by protein kinase A (PKA) and Ca^{2+}/CaM-dependent protein kinase II (CaMKII). These signaling molecules are part of complex macromolecular assemblies that help guide their phosphorylation of target proteins in the dyad. These signaling molecules also exert global whole-cell level actions. Our third case study will review computational models of the actions of these important signaling molecules.

Extra-dyadic processes operating at the length-scale of 10's to possibly 100's of nms are also likely to influence signal processing in the dyad by establishing "boundary conditions". Imaging studies have revealed that the Na^{+}-Ca^{2+} exchanger (NCX) is localized within and/or near the dyad, and experimental evidence suggests that Ca^{2+} entry via reverse-mode NCX activity may either trigger or modulate CICR. Recent evidence also indicates that the membrane adaptor protein ankyrin-B (ANK-B) binds to NCX, the Na^{+}-K^{+} ATPase (NKA), and the inositol 1,4,5-phosphate operated Ca^{2+}-releasing channel ($InsP_3R$) in the T-tubules, creating the substrate for a macromolecular signaling complex that may play a crucial role in modulating CICR and regulation of Na^{+} and Ca^{2+} levels in the cardiac myocyte (Mohler et al., 2005). We will review experimental data regarding the functional role of extra-dyadic Na^{+} and Ca^{2+} transporters and their possible organization into protein complexes, and suggest directions for future modeling of these systems.

Finally, as an additional example of extra-dyadic processes and their influence on dyad signal processing, we will consider the effects of close, physical approximation of mitochondria with the jSR. It is now known that mitochondria are localized within a few 10's of nms of, and are tethered to, the jSR. Mitochondria take up Ca^{2+} via the Ca^{2+} uniporter (mCU) and extrude Ca^{2+} through the mitochondrial Na^{+}-Ca^{2+} exchanger (mNCE). Recent data suggest that mCU transport in cardiac myocytes is fast, raising the possibility that positioning of mitochondria near the dyad may play a significant role in setting the boundary conditions regulating CICR and local Ca^{2+} transients on a beat-to-beat basis.

2. Signal processing in the cardiac dyad

2.1. Sensing of local and global Ca^{2+} signals by calmodulin molecules at the site of the L-type Ca^{2+} channel

Ca^{2+} is a ubiquitous signaling molecule in the heart (as well as other organ systems) that plays a role in regulating a large variety of processes. In order to achieve coordinated signaling at the whole-cell level, cardiac myocytes must have mechanisms that allow for sorting of global and local Ca^{2+} signals in order to regulate processes such as gradation of contraction and heart rate (sensitive to global Ca^{2+}) as well as processes involved in energy production, transcription, and cell survival (sensitive to localized Ca^{2+}) (Anderson and Mohler, 2009). The cardiac dyad nano-domain is an example where a molecular Ca^{2+} sensor mediating Ca^{2+}-dependent inactivation (CDI) of the L-type Ca^{2+} current (I_{CaL}) is positioned in very close proximity to Ca^{2+} sources (LCCs and RyRs), allowing for privileged Ca^{2+} signaling. Recent studies (Dick et al., 2008; Tadross et al., 2008) have shed new light on the function of CaM as a sensor for CDI of I_{CaL}, demonstrating the remarkable processing of the Ca^{2+} signal that can be performed by individual molecules within the dyad in order to sense local versus global Ca^{2+}.

Tadross et al. (2008) used a combination of elegant models and experiments to elucidate the mechanisms underlying Ca^{2+} channel CDI, by which CaM can sense and decode local and global Ca^{2+} signals. CaM continuously complexes with LCCs and serves as the cytosolic Ca^{2+} sensor for CDI (Erickson et al., 2001). CaM is a bi-lobed protein, where both the N- and C-lobes bind Ca^{2+}, but with different affinities. Despite the fact that both lobes are in very close proximity to the LCC Ca^{2+} flux (i.e. $\sim$10 nm from the channel pore), each lobe responds selectively to either the local (brief high-amplitude spikes in [Ca^{2+}]) or global (a longer lasting pedestal of [Ca^{2+}]) component of the Ca^{2+} signal. The ability of the LCC to respond selectively to either of these Ca^{2+} signals was explained by interpretation of a variety of experiments using the model shown in Fig. 1. This model shows the states for the association of a single lobe of CaM with a Ca^{2+} channel. Transitions from state 1 through 4 represent dissociation of apoCaM from the Ca^{2+} channel, binding of 2 Ca^{2+} ions to a lobe of CaM, and binding of Ca^{2+}/CaM to the effector site for CDI. If the transitions between states 2 and 3 are slow relative to channel gating, then brief high-amplitude spikes in Ca^{2+} arising from channel gating will be sufficient to cause accumulation of channels in state 3, and subsequent transition to state 4 (CDI). This is the hypothesized mechanism for local Ca^{2+} sensing by the C-lobe of CaM. However, if the transition between states 3 and 2 is fast relative to the Ca^{2+} fluctuations associated with channel gating (in conjunction with slow transitions between states 1 and 2, and states 3 and 4), then global selectivity emerges (see Tadross et al. (2008) for details). This yields a mechanism that is sensitive to fractional presence of Ca^{2+} over time (i.e. highly sensitive to weak but sustained Ca^{2+} signals), but insensitive to the intensity of the Ca^{2+} signal, and can explain N-lobe sensitivity. Furthermore, N-lobe selectivity can be switched between global and local properties by altering the function of an additional Ca^{2+}/CaM binding site on the N-lobe called NSCaTE (that normally restricts CaM to respond to local Ca^{2+} in Cav1 channels) (Dick et al., 2008). This mechanism for the selective molecular sensing of local versus global Ca^{2+} signals is without question remarkable. The implications of this selective Ca^{2+}-sensing mechanism on our

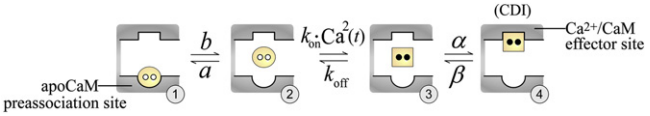

Fig. 1. Basic states for a lobe of CaM in complex with a Ca^{2+} channel (from (Tadross et al., 2008)). In state 1, apoCaM (yellow circle) is bound to the apoCaM site (round pocket). In state 2, apoCaM is a transiently dissociated. In state 3, CaM binds two Ca^{2+} ions (black dots) to become Ca^{2+}/CaM (yellow square), which can then bind the Ca^{2+}/CaM effector site (square pocket), yielding CDI (state 4).