CARDIAC MUSCLE

Ca^{2+} sparks in heart muscle

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Overview

In heart, there is an intracellular calcium concentration ($[Ca^{2+}]_i$) transient that is responsible for triggering each heart beat (Bers, 2002) The elevation of $[Ca^{2+}]_i$ activates contractile proteins that lead to cellular shortening and pumping of blood by the heart. In this presentation, the molecular regulation of the $[Ca^{2+}]_i$ transient will be discussed. Each heart cell is organized to enable local Ca^{2+} release events to be individually triggered and also for the overall cellular signal to be appropriately responsive to the electrical activity of the heart. Because of the combination of local control of Ca^{2+} and the mixture of local and global (i.e. cellular and heart-wide) control of electrical activity and hormonal regulation, the heart is able to be an extremely reliable pump.

Structural organization of the SR–TT junction

The cellular and subcellular organization of the heart muscle cell (cardiac myocyte) is a central feature in its operation. Each cell is organized into contractile units (sarcomeres) that span the muscle striations region from one "z line" to the next "z-line" (Bers, 2001). At each z-line, transverse tubules (TT) penetrate into the depth of the cell from the surface membrane. These invaginations of the surface membrane enable the cardiac action potential (AP) to invade the depths of each heart cell and affect channels and transporters. L-type Ca^{2+} channels are plentiful on the sarcolemmal plasma membrane and on the TT membranes. When the membrane is depolarized during the AP, L-type Ca^{2+} channels (DHPRs or dihydropyridine receptors) open briefly to permit the entry of Ca^{2+}. When the DHPRs happen to be located near or over the region of contact between the sarcoplasmic reticulum (SR) and the TT, they can affect the SR Ca^{2+} release channels (ryanodine receptors or RyR2s). At the SR–TT junction, there are clusters of RyR2s (between 10's and 100's (Franzin-Armstrong, 1999; Franzini-Armstrong *et al.,* 1999) and these channels are sensitive to local $[Ca^{2+}]_i$. When the local $[Ca^{2+}]_i$ is elevated by the opening of a DHPR, the nearby RyR2s may be activated. If so, then a local SR Ca^{2+} release event occurs and such events are called Ca^{2+} sparks.

The cardiac calcium transient and Ca^{2+} sparks

Ca^{2+} sparks are the elementary SR Ca^{2+} release events and can occur in a cell at rest (diastolic Ca^{2+} sparks) or as part of the $[Ca^{2+}]_i$ transient (systolic Ca^{2+} sparks) (Cheng *et al.*, 1993, 1994, 1995; Cannell *et al.*, 1994, 1995; Santana *et al.,* 1996; Sobie *et al.*, 2002). During diastole, resting $[Ca^{2+}]_i$ is about 100 nM and activates RyR2s at a low rate, estimated to be about 10^{-4} s^{-1}. However, when an AP activates DHPRs, the rate may increase dramatically. In the 10–20 ms of the initial part of the AP, approximately half of the potential Ca^{2+} spark site may be activated. This means that during that brief period approximately 100,000–500,000 RyR2s may be activated (out of a population of 1,000,000). This is roughly a 10^5 fold increase over the rate at rest. There are many biophysical issues of interest with regard to Ca^{2+} sparks. We know only a small amount about how they are triggered. We do not know what the quantitative relationship is between the local increase in $[Ca^{2+}]_i$ and the triggering of Ca^{2+} sparks. Another area of active research is how Ca^{2+} sparks are modulated. While the $[Ca^{2+}]_i$ transient is known to increase following activation by β-adrenergic agonists, it is not clear exactly how that happens. Such protein-kinase A dependent signaling is interesting and recent investigations have uncovered macromolecular complexes that are important. These complexes include those involving RyR2. One of the more interesting issues being investigated is how is it possible for the Ca^{2+} sparks to stop. How do Ca^{2+} sparks terminate? These issues and related recent discoveries will be presented.

Ca^{2+} signaling defects in cardiac disease

Many investigators have examined the contractile defects in diverse forms of heart disease. These include many models of cardiac dysfunction that span cardiac hypertrophy to heart failure and arrhythmias. A brief discussion of some of the more interesting recent findings will be presented. There is, however, a theme that is seen in normal and dysfunctional heart cells. The subcellular organization of channel proteins, signaling proteins, regulatory proteins and cytoskeletal proteins is important to normal cellular function. Several examples will be presented to illustrate our evolving understanding of the links between electrical activity, Ca^{2+} signaling and heart cell function (Gómez *et al.*, 1997, 2001;Esposito *et al.*, 2000; Choi *et al.*, 2002; Mohler *et al.*, 2003; Reiken *et al.*, 2003).

Summary

The spatial organization of the cardiac myocyte is critical to its function. Cardiac Ca^{2+} signaling depends on interplay between membrane currents, local concentration changes of diverse ions and the specialized structures. The Ca^{2+} spark, the elementary unit of excitation–contraction coupling in heart, is thus a product of both global and local processes. Our understanding of both normal and pathological cellular behavior in heart is enhanced by studying Ca^{2+} sparks.

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Calcium regulation of single cardiac ryanodine receptor channels

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In cardiac muscle, Ca^{2+}-sensitive ryanodine receptor (RyR) Ca^{2+} release channels mediate the intracellular Ca^{2+} signals that govern contraction. The RyR channels exist and operate in tightly organized arrays at discrete Ca^{2+} release sites on the sarcoplasmic reticulum (SR). These Ca^{2+} release sites are juxtaposed to groups of dihydropyridine receptor (DHPR) Ca^{2+} channels located in the transverse tubule (T-tubule) membrane. It is believed that during the action potential Ca^{2+} entry through DHPRs activates the RyR channels in nearby Ca^{2+} release site. This process is called Ca^{2+}-induced Ca^{2+} release (CICR).

Intuitively, the CICR process should be self-regenerating because of its intrinsic local positive feedback (i.e. Ca^{2+} released triggering further Ca^{2+} release). *In vivo*, however, the CICR process is precisely controlled. To reconcile this paradox, it is assumed that there must be some local negative feedback mechanism acting to counter the inherent positive feedback of CICR. Several candidate negative feedback mechanisms have been proposed. These include Ca^{2+} dependent inactivation, stochastic attrition, adaptation and 'fateful' inactivation.

Studies to date on intact or permeabilized cells present contradictory results concerning the identity of the mechanism(s) that provide the negative feedback required to control the CICR process. Likewise, single channel studies have failed to clearly establish the identity of the local negative control mechanism(s) responsible for 'turning off' the RyR channel in cells.

The function of single RyR channels is typically defined by incorporating the channel into artificial planar lipid bilayers. In cells, RyR channels operate in a restricted space, are subject to regulation by numerous soluble regulatory factors, and respond to large fast trigger Ca^{2+} stimuli. These parameters are generally missing in single RyR channel bilayer studies. Here, we combine the conventional bilayer method, flash photolysis and near-field epifluorescence Ca^{2+} detection to test how single RyR channels respond to large fast Ca^{2+} stimuli.

Our working hypothesis is that the RyR channel protein itself does not have a its own Ca^{2+} dependent negative control mechanism. Instead, the negative control that terminates Ca^{2+} release in cells may be a function of the cellular geometry and/or regulatory factors missing at the single RyR channel level. To test this idea, single cardiac RyR channels were incorporated into bilayers and a novel flash-stir method was used to generate fast Ca^{2+} transients on the cytosolic side of the channel. The same high numerical aperture, multimode optic fiber that carries the photolytic UV flash was used to carry excitation/emitted light for local Ca^{2+} detection by Rhod-2. A standard fast transient Ca^{2+} stimulus induced a brief burst of channel activity. We found that as the interval between two standard stimuli becomes shorter (< 300 ms), the channel's response to the 2nd stimulus becomes larger. Regardless of what the underlying mechanism turns out to be, this use-dependent preconditioning of single RyR channel function is an