Chapter 9

ELEMENTARY Ca\(^{2+}\) RELEASE EVENTS: RYANODINE RECEPTOR Ca\(^{2+}\) SPARKS

W. J. Lederer, Eric A. Sobie, Silvia Guatimosim, and Long-Sheng Song
Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, MD

INTRODUCTION

The Ca\(^{2+}\) spark is the elementary Ca\(^{2+}\) signaling event in heart muscle that underlies excitation-contraction (EC) coupling.\(^{240,274-276}\) Each Ca\(^{2+}\) spark reflects the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR) that was either “triggered” by a brief local increase in [Ca\(^{2+}\)]\(_i\) or that occurred spontaneously due to a variety of factors. Ca\(^{2+}\) sparks occur at distinctive sites within the heart cell, mainly at the junctions between the SR and the surface membrane or between the SR and the transverse tubule (TT) membrane.\(^{277-280}\) At these junctions, specialized regions of the SR, the “junctional SR” or “jSR” contain RyR2 clusters that are organized in arrays. The L-type Ca\(^{2+}\) channels (dihydropyridine receptors, DHPRs) located in the sarcolemmal (SL) or TT membranes are thought to be located near the jSR so that Ca\(^{2+}\) flux through the DHPRs can influence the [Ca\(^{2+}\)]\(_i\) at the jSR. The complex of the jSR, the associated array of RyR2s, the DHPRs, the TT or SL membrane and all of the proteins that are associated with these elements form a unit called “the couplon”.\(^{279}\) The primary input to the EC coupling machinery in heart muscle is the membrane voltage or “action potential” (AP) and the primary elementary output is the Ca\(^{2+}\) spark. The summation of Ca\(^{2+}\) sparks produces the cardiac Ca\(^{2+}\) transient.\(^{281}\) There are, of course, many factors that are also important and we seek to discuss them in this review. However, the primary focus here is a discussion of RyR2s, Ca\(^{2+}\) sparks and our understanding of EC coupling.
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THE Ca\(^{2+}\) SPARK AND THE [Ca\(^{2+}\)]\(_{i}\) TRANSIENT

High-speed imaging of [Ca\(^{2+}\)]\(_{i}\) within a quiescent heart cell reveals the occurrence of subcellular elevations of local Ca\(^{2+}\) that occur at a rate of about 100 per cell per second. These elevations, called Ca\(^{2+}\) sparks, are viewed using a confocal microscope and the Ca\(^{2+}\)-sensitive indicator fluo-3, fluo-4 or rhod-2. Fig. 9-1 shows a line-scan image of Ca\(^{2+}\) sparks from a rat heart cell loaded with fluo-3. In an XY image, each Ca\(^{2+}\) spark appears to be nearly spherical with a diameter of about 2 micrometers (\(\mu\)m, microns).

The Ca\(^{2+}\) sparks arise from a quiescent [Ca\(^{2+}\)]\(_{i}\) of about 100 nM and reveal local peak levels of 200 to 300 nM. The slight eccentricity that may be seen in the XY image of Ca\(^{2+}\) sparks may reflect the asymmetric distribution of diverse proteins in the transverse plane (along the z-lines and parallel to the TTs) versus the longitudinal plane (along the long axis of the cell and parallel to the contractile filaments). Fig. 9-1 A depicts signal averaged line scan images of Ca\(^{2+}\) sparks. The Ca\(^{2+}\) sparks rise to a peak in about 10 ms and fall with a half-time of decay of about 20 ms.

Because Ca\(^{2+}\) sparks arise as a cluster of RyR2s are activated at a couplon, they are predominantly located along the Z-lines of the sarcomere and at the TTs which reside on the Z-line. Fig. 9-1 B shows a signal-averaged view of the TTs obtained at the same time as the Ca\(^{2+}\) sparks and reveals the location of the Ca\(^{2+}\) sparks along the TTs. The longitudinal TTs (TT extensions that are parallel to the long axis of the cell and that connect Z-line TTs) are relatively sparse compared to the transverse TTs and the abundance of couplons and Ca\(^{2+}\) sparks along these longitudinal TTs remains unstudied. Fig. 9-1 C shows a surface plot of Ca\(^{2+}\) sparks from Fig. 9-1 A with respect to the TTs (Fig. 9-1 B).

At “rest” or under non-stimulated conditions Ca\(^{2+}\) sparks arise in heart cells due to the spontaneous opening of RyR2s in the cluster at the couplon. With roughly a million RyR2s within a single rat heart cell, a Ca\(^{2+}\) spark rate of 100 per cell per second is equivalent to a spontaneous opening rate of an isolated RyR2 (e.g. in a planar lipid bilayer) of about \(10^{-4}\) per second. For a single RyR2, this would mean an opening once every 10,000 seconds. Thus, the spontaneous Ca\(^{2+}\) spark rate is consistent with the hypothesis that these Ca\(^{2+}\) sparks are due to the spontaneous openings of RyR2s within the cluster and that these openings of RyR2s are sufficient to activate the entire couplon to produce a Ca\(^{2+}\) spark.