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Review article

Pernicious attrition and inter-RyR2 CICR current control in cardiac muscle

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ABSTRACT

In cardiac muscle cells, ryanodine receptor (RyR) mediated Ca^{2+} release from the sarcoplasmic reticulum (SR) drives the contractile apparatus. Spontaneous bouts of inter-RyR Ca^{2+} induced Ca^{2+} release (CICR) generate an elemental unit of SR Ca^{2+} release called a spark. Sparks are localized events that terminate soon after they begin. The local control of sparks is not clearly understood. In this article, we review the potential regulatory role that the changing single RyR Ca^{2+} current may play. Moreover, we aggregate RyR data into a working scheme of inter-RyR CICR current control of sparks and a potential inter-RyR CICR termination mechanism that we call pernicious attrition. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

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1. Introduction

In cardiac muscle, the action potential activates dihydropyridine receptor (DHPR) Ca^{2+} channels across the surface membrane and transverse tubule membrane. Each DHPR mediates a small Ca^{2+} influx that activates underlying cardiac ryanodine receptor (RyR2) Ca^{2+} release channels on the sarcoplasmic reticulum (SR). Each open RyR2 mediates a relatively large Ca^{2+} flux into the cytosol. This DHPR-RyR2 Ca^{2+}-induced Ca^{2+} release (CICR) initiates cardiac excitation–contraction coupling (ECC). RyR2s are clustered at discrete SR release sites and just some are associated with DHPRs. Non-DHPR linked RyR2s are activated via inter-RyR2 CICR. The combined result DHPR- and inter-RyR2 CICR is the large cytosolic Ca^{2+} transient that drives the contractile apparatus.

During diastole, RyR2 channels are predominantly closed. There is, however, always some probability that a RyR2 will spontaneously open. When one does, it mediates a Ca^{2+} flux into the cytosol and this Ca^{2+} can trigger inter-RyR2 CICR, generating a Ca^{2+} spark [1]. If not, the Ca^{2+} released represents non-spark RyR-mediated SR Ca^{2+} leak [2,3]. The cytosolic Ca^{2+} signal generated by a typical spark is usually insufficient to activate RyR2s at neighboring release sites and thus sparks are local spatially-confined events. Abnormally large (or frequent sparks) can activate RyR2s at neighboring release sites, generating propagating SR Ca^{2+} waves that travel across the cells during diastole [4].

Sparks are thought to be normal events in cardiac muscle cells that contribute to the normal SR Ca^{2+} leak and consequently govern overall SR Ca^{2+} load [5]. Spontaneous Ca^{2+} waves are abnormal events. During a wave, some of the released Ca^{2+} will be extruded from the cell via Na–Ca^{2+} exchange (NCX) activity. Since NCX function is electrogenic, this may depolarize a cell during diastole sufficiently to trigger an action potential and possibly initiate a life-threatening arrhythmia. Despite decades of effort, the mechanisms that control the inter-RyR2 CICR underlying sparks and waves remain poorly understood. For example, the local negative control that counters the inherent positive

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feedback of CICR is unknown. Also, we know that sparks are critically controlled by local intra-SR (luminal) Ca^{2+} levels but exactly how is unclear. Here, we describe the role of the single RyR2 Ca^{2+} current in CICR local control and its possible contribution to the luminal Ca^{2+} sensitivity and termination of sparks.

This review briefly describes how the luminal Ca^{2+} sensitivity of local SR Ca^{2+} release may rise in mammalian cardiac muscle. It describes the changes in single RyR2 Ca^{2+} current amplitude that naturally occur during a spark as well as the local cytosolic Ca^{2+} signal generated by a single RyR2 opening event. The potential role of this signal in modulating local RyR2 activity is discussed and we coin the term pernicious attrition to describe the failure of local inter-RyR2 CICR as the signal becomes smaller. An attempt was made to present this growing complex body of information in a readable easily-understood way. It was impractical to describe and include all the quality studies that comprise this field but we hope this effort helps general interest readers understand local CICR control in heart better.

2. Luminal Ca^{2+} sensitivity

Spark frequency increases dramatically as SR Ca^{2+} load rises [6] and sparks terminate when the local SR load (intra-SR free Ca^{2+} level) falls to a critical level [7–9], the termination threshold. It is thought that single RyR2 channels somehow "sense" changes in local SR load. This intra-SR Ca^{2+} sensing may arise from Ca^{2+} binding to the luminal side of the RyR2 protein itself or to an intra-SR protein that is closely associated with the RyR2 [10]. The most popular RyR2 luminal Ca^{2+} sensing mechanism involves the calsequestrin (CASQ), triadin (TD) and junctin (JN) proteins [11–14]. Indeed, there are naturally occurring RyR2, CASQ and TD mutations [15–19] linked to catecholaminergic polymorphic ventricular tachycardia (CPVT), a form of wave-evoked arrhythmia associated with abnormal RyR2 luminal Ca^{2+} sensitivity. It is commonly believed that intra-SR Ca^{2+} sensing mechanisms entirely explain the luminal Ca^{2+} sensitivity of SR Ca^{2+} sparks. There is, however, another way that local SR Ca^{2+} load may control local Ca^{2+} release.

The local SR Ca^{2+} load defines the magnitude of the trans-SR Ca^{2+} driving force. Thus, local load determines the single RyR2 Ca^{2+} current amplitude. The single RyR2 Ca^{2+} current provides the local Ca^{2+} signal that drives the inter-RyR2 CICR underlying the spark. As SR load rises, this local Ca^{2+} signal will become larger because the current is bigger. The larger local Ca^{2+} signal intuitively will increase the likelihood of inter-RyR2 CICR (i.e. sparks). As load falls, the local Ca^{2+} signal will become smaller and the likelihood that inter-RyR2 CICR starts (or continues) becomes less. The possible contribution of single RyR2 Ca^{2+} current in CICR local control is an embedded element, typically not emphasized, in several modeling studies [20–24]. Recently, Sato & Bers [24] have even described current control of sparks with impressive quantitative detail and current control seems to be gaining in popularity as a potentially significant load-dependent process that helps govern sparks and waves [8,9,25,26]. Until recently, the current control scenario above has been experimentally difficult to verify because single RyR2 Ca^{2+} current and local SR load are difficult to differentially manipulate in cells. We recently overcame this obstacle and experimentally showed that single RyR2 Ca^{2+} current amplitude does indeed substantially contribute to spark local control [27]. Thus, RyR2 luminal Ca^{2+} controls of sparks and waves through a composite of processes that include intra-SR Ca^{2+} sensing mechanisms and single RyR2 current control of inter-RyR2 CICR.

3. The single RyR2 Ca^{2+} current

Single RyR2 function has been defined by incorporating the channel into planar lipid bilayers [10]. The RyR2 is a poorly selective Ca^{2+} channel [28] and multiple RyR2-permeable cations (K^+, Mg^{2+} and Ca^{2+}) are present in cells. There are normally no trans-SR K^+ or Mg^{2+} gradients and the high SR K^+ permeability holds the SR at 0 mV [29], the K^+ equilibrium potential. When the RyR2 opens, all RyR2-permeable ions enter the poorly selective pore. There is a net SR Ca^{2+} efflux (at 0 mV) because there is a trans-SR Ca^{2+} gradient. Without K and Mg present, the single RyR2 Ca^{2+} current (at 0 mV) can be as large as ~4 pA when super-physiological luminal Ca^{2+} levels (e.g. 10–50 mM) are present [30]. In cell-like salt solutions (i.e. 120 mM K, 1 mM free Mg and <100 μM free Ca^{2+} in the cytosol; 120 mM K, 1 mM free Mg and 1 mM free Ca^{2+} in the SR lumen), single RyR2 channel Ca^{2+} current amplitude at 0 mV is ~0.35 pA [27,31–33]. Since robust SR countercurrent assures that the SR Vm never strays far from 0 mV [29,33,34], the amplitude of the single RyR2 channel Ca^{2+} current is likely near 0.35 pA in cells if local intra-SR free Ca^{2+} is 1 mM.

Measurements suggest the local free Ca^{2+} level in the SR falls roughly 50% (to ~0.5 mM) during a spark [7,35,36]. Mathematical modeling, however, suggests that it may fall even further (90%) depending on the speed of release termination and rate of Ca^{2+} transfer between regions inside the SR [8,9]. Our extensive single RyR2 permeation works [33,34,37–41] indicate that single RyR2 Ca^{2+} current amplitude (at 0 mV) will vary almost proportionally with local SR Ca^{2+} load. Thus, single RyR2 Ca^{2+} current (at 0 mV) may be 0.35 pA when a spark begins but would fall to 0.175 pA if local load decreased by 50% (or to 0.035 pA if it decreased by 90%) when the spark terminates.

4. The local cytosolic Ca^{2+} signal generated by a single RyR2 opening

The local cytosolic Ca^{2+} signal generated around a Ca^{2+} point source, like an open RyR2 conducting a 0.35 pA Ca^{2+} current, can be calculated using standard diffusion theory [42–44]. Fig. 1 plots the steady state local Ca^{2+} concentration around a 0.35 pA point source as a function of distance (thick solid curve). For the RyR, these steady state concentrations are likely established in <10 μs [45,46] and are sustained as long as the current is sustained. The elevation in local cytosolic Ca^{2+} generated by a 0.175 and 0.035 pA current is also shown (thin solid curves). The dashed curve is the elevation in local cytosolic Ca^{2+} level with the 0.35 pA current in the presence of 1 mM cytosolic BAPTA, a fast-binding Ca^{2+} buffer. To help interpret these curves, 3 adjacent RyR2s are depicted at the bottom of Fig. 1. The adjacent RyR2s shown roughly reflect the known physical dimensions of the RyR2 [47,48]

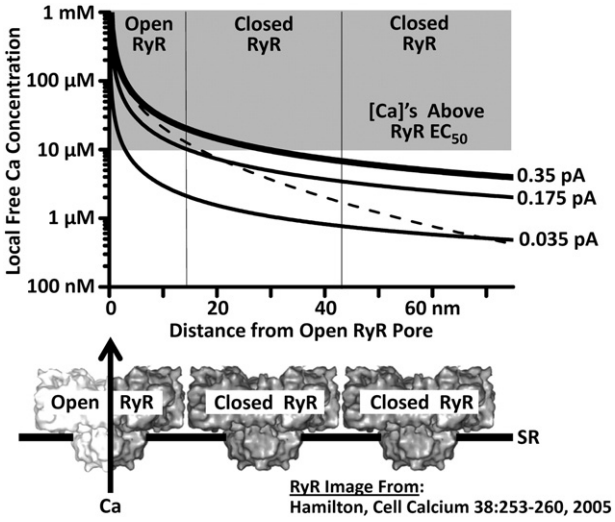

Fig. 1. Local Ca^{2+} diffusion around one open RyR2 channel. Local free Ca^{2+} concentration is plotted as a function of distance from the center of a Ca^{2+} point source of 0.35, 0.175 or 0.035 pA. Local Ca^{2+} concentrations were calculated as specified elsewhere [44]. Three RyR channels (one open, two closed) are illustrated at the bottom to provide interpretive context. The Ca^{2+} activation EC_{50} of the single cardiac RyR channel in cell-like salt solution is ~10 μM [30] and super-EC_{50} Ca^{2+} concentrations are indicated by the shaded area on the plot. The dashed line represents local Ca^{2+} levels when there is a 0.35 pA Ca^{2+} current with 1 mM cytosolic BAPTA present.