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An intelligent sarco-endoplasmic reticulum Ca^{2+} store: Release and leak channels have differential access to a concealed Ca^{2+} pool

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ABSTRACT

Simultaneous recording of cytosolic and sarco-endoplasmic reticulum (SR/ER) luminal free calcium concentrations ($[Ca^{2+}]_i$ and $[Ca^{2+}]_L$, respectively) supports the notion that release channels (RyRs and IP_3Rs) use a concealed Ca^{2+} source, likely to be associated with intra-SR/ER Ca^{2+} binding proteins, whereas SR/ER Ca^{2+} leak channels can only access free luminal Ca^{2+}. We hypothesize that Ca^{2+} is trapped by oligomers of luminal Ca^{2+}-binding proteins and that the opening of release channels induces the rapid liberation of this "concealed" Ca^{2+} source associated with intra-ER Ca^{2+} buffers. Our hypothesis may also clarify why SERCA pumps potentiate Ca^{2+} release and explain quantal characteristics and refractory states of Ca^{2+} release process.

1. Introduction

Calcium ions (Ca^{2+}) modify cell behavior by stabilizing conformational states of effector proteins, which include enzymes (kinases, phosphatases, proteases, phospholipases, etc.), ion channels and transporters [1,2]. An excess of Ca^{2+} ions is, however, toxic [3], for which reason cells spend substantial energy to keep the activity of this ion in the cytosol at a low level (near 100 nM). Physiological Ca^{2+} signals are spatially restricted and transient in nature, while cell-wide elevations in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) occur due to summation of these localized Ca^{2+} events [4]. Physical and chemical signals induce increases in the $[Ca^{2+}]_i$ from two main sources, the external milieu and the internal Ca^{2+} stores. The latter are mainly associated with the endoplasmic reticulum (ER) in non-muscle cells and the sarcoplasmic reticulum (SR) in muscle cells. This review is focused on recent studies that employed simultaneous recordings of the $[Ca^{2+}]_i$ and the SR/ER luminal free Ca^{2+} concentration ($[Ca^{2+}]_L$). These studies, performed in many different cell types, resulted in several important discoveries: (i) physiological Ca^{2+} release results in relatively small decrease in $[Ca^{2+}]_L$ [5,6]; (ii) there is substantial rise in the $[Ca^{2+}]_i$ before any significant decrease in the $[Ca^{2+}]_L$ [7,8] and (iii) release channels (RyRs and IP_3Rs) switch from a large to a small Ca^{2+} buffer capacity source in the course of Ca^{2+} release process [8]. It has been shown that the $[Ca^{2+}]_i$ response does not mirror the changes in the $[Ca^{2+}]_L$ [7,8], which implies an apparent violation of the law of mass conservation (as was initially perceived by Rios' group [7]). For this reason we propose that ER Ca^{2+} release channels have preferential access to a concealed source of Ca^{2+} that is not in rapid equilibrium with $[Ca^{2+}]_L$. We will review the data supporting the presence and the possible nature of this concealed source of Ca^{2+} which is used primarily by Ca^{2+} release channels whereas SR/ER leak channels/pathways do not have a direct access to this source. We also discuss data suggesting that the connection between release channels and the concealed Ca^{2+} source is transient in nature and does not recover immediately after the return of $[Ca^{2+}]_L$ to the resting level. Moreover, there is evidence suggesting that this connection is facilitated by active SERCA pumps. This model may explain the transient nature of elementary release events, such as Ca^{2+} sparks and puffs, and also the apparent compartmentalization of the sources of Ca^{2+} seen by activation of these release channels in an otherwise continuous SR/ER.

2. The sarco-endoplasmic reticulum

The SR/ER is a multifunctional organelle formed by highly fusogenic network of an interconnected series of tubules and cisternae distributed throughout the eukaryotic cell [9]. The SR/ER membrane (the endomembrane) is endowed with SERCA pumps and Ca^{2+} release channels, the former accumulate Ca^{2+} in the lumen against its electrochemical gradient, whereas the latter allow Ca^{2+}

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to diffuse out of the store on opening of the channels. There are two main types of Ca^{2+} release channels, the ryanodine receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP_3Rs). The Ca^{2+} buffer capacity of the SR/ER is determined by high capacity, low affinity Ca^{2+}-binding proteins, whereas the steady state $[Ca^{2+}]_L$ depends on the balance between SERCA pumping and the endomembrane Ca^{2+} leak, the nature of which has not been fully established [10]. The continuous lumen of the SR/ER possesses diffusion barriers neither for Ca^{2+} nor for proteins in secretory cells [5], neurons [11], heart [12], and smooth muscle cells [13]. However, the SR/ER is spatially heterogeneous because Ca^{2+} often accumulates only in certain regions of this organelle [14,15] and SERCA pumps and release channels are not evenly distributed in the endomembrane [16–18].

3. SERCA pumps

Sarco/endoplasmic reticulum Ca^{2+} ATPase is a P type ATPase with a stoichiometry of 2 Ca^{2+} ions transported at the expense of the hydrolysis of one molecule of ATP [19]. Since only 2–3 protons counterbalance four charges of the two incoming Ca^{2+} ions [20], the activity of SERCA pumps should be electrogenic rendering a positive ER membrane potential and higher intraluminal pH in respect to the cytoplasm. However, whether the ER membrane is tight enough to keep these gradients until release channels are open is not clear; the endomembrane contains both K^+ and Cl^- channels that could, in principle, dissipate the ER membrane potential created by the activity of SERCA pumps [21].

These pumps not only reload the SR/ER with Ca^{2+} but also facilitate the Ca^{2+} release process via either RyRs [8,22–27] or IP_3Rs [8,26,28,29]. Indeed, inhibition of SERCA pumps with thapsigargin slowed down agonist-induced Ca^{2+} waves in pancreatic acinar cells despite the absence of any evidence for a reduction in $[Ca^{2+}]_L$ [29]. In smooth muscle cells, inhibition of SERCA pumps with thapsigargin resulted in slower and smaller RyR- and IP_3R-mediated $[Ca^{2+}]_i$ responses without any obvious reduction in the $[Ca^{2+}]_L$ [26]. Moreover, simultaneous recording of $[Ca^{2+}]_L$ and $[Ca^{2+}]_i$ in single smooth muscle cells showed that carbachol-induced $[Ca^{2+}]_i$ response was halved following SERCA inhibition with thapsigargin; although, paradoxically carbachol triggered a larger reduction in the $[Ca^{2+}]_L$ [8]. It has been shown that rapid inhibition of SERCA pumps by photolysis of a thapsigargin precursor decreased the rate of Ca^{2+} wave propagation in heart cells [27]. Additionally, histamine-induced $[Ca^{2+}]_i$ response is potentiated by active SERCA pumps in HeLa cells [28]. Taken together, these data indicate that active SERCA pumps participate in the Ca^{2+} release mechanism by facilitating activation of Ca^{2+} release channels. Since the SERCA pump turnover rate is too slow in comparison to the rate of ion flux via Ca^{2+}-release channels and because the number of SERCA pumps is not sufficient to provide Ca^{2+} directly to these channels, we have hypothesized that SERCA pumps assist Ca^{2+} release by facilitating the communication between release channels and luminal Ca^{2+}-binding proteins [8,26]. Indeed, it has been shown that there is a direct communication between RyRs and SERCA pumps in heavy SR vesicles of skeletal muscle [22–25,30]. All the data reviewed here suggest that this type of communication is not exclusive of skeletal muscle but is present in other cell types.

4. Luminal SR/ER proteins have high capacity and low affinity for Ca^{2+} and may form a concealed Ca^{2+} pool

The main SR/ER Ca^{2+} binding proteins such as calsequestrin and calreticulin have a high capacity and a low affinity for Ca^{2+} ions. It has been reported that calsequestrin polymerizes into large filaments in the lumen of the SR in skeletal muscle and it has been proposed that Ca^{2+} drives this polymerization [31]. Furthermore, different degrees of calsequestrin complexity, proceeding from monomer, to dimer to multimer, correlate with an increase in Ca^{2+} binding capacity and a decrease in the Ca^{2+} binding affinity [32]. Thus, it is expected that the transition from dimer to monomer should produce liberation of Ca^{2+} ions and therefore the reduction of the complexity of calsequestrin can liberate large amounts of Ca^{2+} to the SR lumen. Different accessibility of release versus leak channels to the concealed Ca^{2+} source may result from distinct kinetics of interaction of these channels with the SR/ER Ca^{2+} binding proteins, where the opening of release channels causes much faster depolymerization and massive Ca^{2+} liberation from the SR/ER Ca^{2+} buffer. This in turn might explain how release channels gain access to a large concealed source of Ca^{2+} [7,8]. Whether this is also true for calreticulin, the major Ca^{2+} binding proteins in the ER, needs to be investigated. Incidentally, monitoring of Ca^{2+} release in heart cells isolated from calsequestrin knock-out mice suggests an apparent redundancy in the function of luminal Ca^{2+} binding proteins as sources of Ca^{2+} for release channels [33].

5. Reticulum release channels

The Ca^{2+} gradient between the SR/ER lumen and the cytoplasm is so large that the opening of release channels should reduce $[Ca^{2+}]_L$ immediately. However, simultaneous recordings of $[Ca^{2+}]_L$ and $[Ca^{2+}]_i$ show that the process of Ca^{2+} release is more complex than previously thought [7,8]. Such complexity is evident from phase diagrams, which compare the amplitude of the change in the $[Ca^{2+}]_i$ with the reduction in the $[Ca^{2+}]_L$ at the same time point (Fig. 1). This type of analysis shows that Ca^{2+} release in smooth muscle cells involves four different phases as indicated by the numbers in Fig. 1A. The first three phases occur while the release channels are open and phase 4 develops when the release channels are closed. Phase 1 is characterized by a large increase in $[Ca^{2+}]_i$ with minimal decrease in $[Ca^{2+}]_L$. Since there is no reduction in the $[Ca^{2+}]_L$ and yet the $[Ca^{2+}]_i$ response is due to Ca^{2+} emerging from the SR/ER, phase 1 implies that the SR/ER has an exceptionally large Ca^{2+} buffer capacity. In fact, phase 1 is a flat line, which indicates an almost infinite buffer capacity of the Ca^{2+} source. This certainly is not possible because of a contradiction to the principle of mass conservation [7]. Thus, the most feasible explanation for these data is the presence of a concealed Ca^{2+} source that is not in rapid equilibrium with the $[Ca^{2+}]_L$ but is accessed by release channels. Phase 2 shows the opposite relationship; a large decrease in the $[Ca^{2+}]_L$ produces relatively small changes in the $[Ca^{2+}]_i$ (Fig. 1A). Phase 2 implies that the release channels suddenly experience a low capacity Ca^{2+} source (i.e. a large decrease in $[Ca^{2+}]_L$ produces limited changes in the $[Ca^{2+}]_i$). The transition from phase 1 to phase 2 demonstrates that both types of release channels switch from a high to low capacity Ca^{2+} source while still being open [8]. Phase 3 (Fig. 1A) occurs when the internal Ca^{2+} store has been functionally exhausted so the supply of Ca^{2+} is terminated and now the cytosolic Ca^{2+} removal mechanisms become relevant. Phase 4 (Fig. 1A) is initiated by the removal of the stimulus (in this case caffeine), which closes release channels allowing the SERCA pumps to restore the $[Ca^{2+}]_L$ [8]. These four phases of the Ca^{2+} release process are present in various cell types; and in particular the phase 1, which is characterized by maximal $[Ca^{2+}]_i$ responses with minimal reduction in the $[Ca^{2+}]_L$. To illustrate this, we have generated phase diagrams from published data for simultaneous recording of $[Ca^{2+}]_i$ and $[Ca^{2+}]_L$ in three other type of cells (Fig. 1B–D). In skeletal muscle [7] the comparison between the Ca^{2+} spark and the corresponding luminal Ca^{2+} decrease ("skrap" – see [7]) shows the phase 1, whereas phases 2 and 3 are overlapping because of an extremely fast rate of cytosolic Ca^{2+} clearance in these cells (Fig. 1B). Simi-