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Cell Calcium 42 (2007) 379–387

cell calcium

www.elsevier.com/locate/ceca

A guide to sparkology: The taxonomy of elementary cellular Ca^{2+} signaling events

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Received 11 February 2007; received in revised form 18 February 2007; accepted 19 February 2007
Available online 10 April 2007

Abstract

Since the discovery of the Ca^{2+} spark as an elementary event of cellular Ca^{2+} signaling almost 15 years ago, the family of newly described Ca^{2+} signal entities has been ever growing. While scientists working in Ca^{2+} signaling may have maintained an overview over the specifics of this nomenclature, those outside the field often make the complaint that they feel hopelessly lost. With the present review we collect and summarize systematic information on the many Ca^{2+} signaling events described in a variety of tissues and cells, and we emphasize why and how each of them has its own importance. Most of these signals are taking place in the cytosol of the respective cells, but several events have been recorded from intracellular organelles as well, where they may serve their own specific functions. Finally, we also try to convey an integrated view as to why cellular microdomain signaling is of fundamental biological importance.

Keywords: Ca^{2+} signaling; Microdomain; Subcellular Ca^{2+} signals; Ca^{2+} sparks

1. Introduction

Historically, humans have always been keen to explore the big and the small things in their world. While the search for the big things seems to have found a natural frontier with the universe and with infinity, the end of the search for the small things has not yet reached an analogous limit. In biological research, the quest for ever smaller and smaller objects for research has moved the focus from systems to organisms, to organs and to single cells and finally to subcellular signals and single molecules. This strategy has a strong motivation in the idea that small parts of a system are easier to understand than a complex system itself, a concept, which is the foundation of the reductionistic approach. However, this approach has not always been as helpful as we expected, mainly because the complexity of even the smallest and most elementary events had been underestimated or because the ultimate goal, to put the small pieces back together to see the big picture, has been a dauntingly difficult task.

However, the discovery and characterization of elementary cellular Ca^{2+} signaling events and the lessons we have learned from dissecting this signaling system into its parts and pieces are a good example to make a case that the reductionistic approach can work. Based on knowledge obtained from the analysis of these elementary events we have indeed achieved a better understanding of far more complex systems, such as all kinds of muscle cells, neurons, but also non-excitable cells (for reviews see Refs. [1–6]).

1.1. Why did nature invent elementary signaling events?

In face of the ever growing collection of Ca^{2+} signaling events, one wonders why this field has expanded so rapidly and why such a variety of different elementary Ca^{2+} signals exist. Is there a universal principle behind the multitude of discovered elementary Ca^{2+} signals? One common fundamental feature of all these events is the basic concept of "microdomain Ca^{2+} signaling" [4]. This is a means to

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0143-4160/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ceca.2007.02.010

direct Ca^{2+} signals in precise ways to very specific subcellular targets. It can be seen as an alternative way to encode Ca^{2+} signals spatially, in addition to the widely used amplitude and frequency-dependent coding schemes for second messenger signaling. The combination of all these coding schemes allows the use of a single second messenger, such as Ca^{2+}, in a very versatile way [5,6]. In recent years, it became clear that this principle is not only used to control the second messenger Ca^{2+}, but is also implemented in other signaling pathways that rely on diffusible messengers, such as cAMP [7] or nitric oxide (NO) [8]. While the spatial focusing and confinement of Ca^{2+} microdomain signals occurs by virtue of strong buffering and slow diffusion, cAMP is locally restrained by phosphodiesterases shielding the site of cAMP production from the remainder of the cell. NO is inherently unstable and shortlived, and therefore can only act over very short distances.

In addition, these spatial coding schemes allow for more complex Ca^{2+} signaling systems than amplitude and frequency coding alone. As a classical problem, cardiac muscle Ca^{2+} signal amplification by Ca^{2+}-induced Ca^{2+} release (CICR) faced the difficulty of being instable at the required Ca^{2+} signaling gain, with potentially catastrophic consequences via induction of Ca^{2+} oscillation triggering cardiac arrhythmias. Nature solved this problem by not relying on a Ca^{2+} signal amplification within the common pool of the cytosol, but rather limiting the space of amplification to the "Ca^{2+} synapse" of the microdomain in the dyadic cleft, as detailed below [9].

With the present review, we try to compile a list of the Ca^{2+} signaling events established so far, in a variety of tissues and cells, and even within some specific organelles. Each of the elementary Ca^{2+} signals discovered during the last 15 years has its own "signature" in terms of spatial and temporal features of the resulting Ca^{2+} transient. This signature depends on the cell type, on the subcellular microarchitecture of the signaling microdomain, and on the type and number of channels underlying the Ca^{2+} flux. This review will summarize all these finding and put them together in a table for easy comparison (see Table 1).

2. Members of the family

2.1. The Ca^{2+} spark

The first discovered subcellular Ca^{2+} signal has been termed a "Ca^{2+} spark" [10]. This finding was published in 1993 and has only been made possible by the introduction of state-of-the-art confocal microscopic imaging instrumentation to Ca^{2+} signaling research. Confocal microscopes minimize out-of-focus fluorescence, by virtue of their optical sectioning capabilities, which is particularly beneficial when imaging relatively thick cells, such as muscle cells.

Sparks and other local Ca^{2+} signals are commonly described and quantified by their amplitude (expressed as either $\Delta[Ca^{2+}]$ or as normalized increase of fluorescence, $\Delta F/F_0$), by their duration (measured as full duration at half maximal amplitude; FDHM), by their width (measured as full width at half maximal amplitude, FWHM). Sometimes the rise time and the half time or the τ of the decay are also analyzed. Obviously, the Ca^{2+} signal during a spark only indirectly reflects the real events in the microdomain, where the concentration changes are much larger and occur faster, as predicted by computer modeling [11,12]. In the simple case (i.e. without release channel reopenings during spark decay), the rise time of the spark corresponds to the duration of the release of Ca^{2+} from the intracellular store, the amplitude and width (together also referred to as "Ca^{2+} signal mass") correlate with the amount of Ca^{2+} released. The spark decay is mainly governed by diffusional dissipation of the Ca^{2+} signal away from its source, although some participation by Ca^{2+} transporters such as the SR Ca^{2+} pump has been noted [13]. Importantly, it has to be kept in mind that some of these parameters are prone to distortions due to the fact that many Ca^{2+} sparks originate in locations that are outside the confocal optical section and Ca^{2+} subsequently diffuses into the focal plane [14,15]. When the distance from the focal plane is too large, the sparks will eventually no longer be detectable [15]. While it was initially believed that Ca^{2+} sparks are very stereotypical events, it was later noted that the parameters mentioned above can show subtle changes. Such changes seem to depend on the preparation, on the isoform of the involved channels (i.e. cardiac RyR2 versus skeletal RyR1) and on the prevailing conditions, such as the Ca^{2+} content of the SR/ER. It was also observed that the "signature" of the elementary Ca^{2+} signals can change during certain diseases or in the presence of pharmacological modifiers of RyR channel gating (e.g. ryanodine, FK506 or imperatoxin [16]).

2.1.1. Ca^{2+} sparks in cardiac muscle

Several reviews have been published summarizing the general, if not universal, importance of the Ca^{2+} sparks in cardiac muscle cells [1,2,6]. As already described above, for the framework of cardiac excitation–contraction coupling the Ca^{2+} spark was the functional signal underpinning the "local control" theory with a biological reality [17]. This theory and the associated findings also supported the earlier notion that RyRs are relatively insensitive to incoming Ca^{2+} triggers [18] and only respond to the large and highly localized Ca^{2+} signals prevailing in the dyadic cleft. Thereby, the local Ca^{2+} control mechanism solved the Ca^{2+} signaling paradox for cardiac CICR. The paradox arose because the amplification inherent in CICR could not be implemented in mathematical "common pool" models without creating a highly unstable system with a tendency to oscillate [9]. With a model containing elementary signal transmission events (the mathematical representations of Ca^{2+} sparks) this problem could be solved. Each element could then have a very high gain and essentially correspond to an all-or-none event. Despite the high degree of positive feed-back, the overall cellular CICR system was

Table 1
Summary of published parameters describing elementary Ca^{2+} signaling events. For each event we present the ranges of values reported by several groups

Event (name)	Tissue	Species	Cell type	Compartment	Amplitude	Width (μm)	Duration (ms)	Rise time (ms)	Reference
Spark	Heart	Rat Dog Mouse Rabbit	Ventricular myocyte	Cytosol	100–300 nM Up to ~ $2\,\Delta F/F_0$	~ 2	20–40 FDHM	~ 10	[10,13,19] [87] [76] [21]
	Skeletal muscle	Frog	Muscle fiber	Cytosol	~ 100 nM Up to ~ $2\,\Delta F/F_0$	1–2	~ 10 FDHM	~ 5	[23,25,26,57]
		Rat	Muscle fiber	Cytosol	Up to ~ $2\,\Delta F/F_0$	~ 2	~ 15 FDHM	~ 8	[28,33]
	Vessel Ileum Ureter	Rat Guinea pig	Smooth muscle cells	Cytosol	50–200 nM Up to $4\,\Delta F/F_0$	~ 2	30–50 half time of decay	20–100	[37,38,45] [43] [44]
Quark	Heart	Guinea pig	Ventricular myocyte	Cytosol	30 nM	~1	–	~ 5	[55]
Ember	Skeletal muscle	Frog Rat	Muscle fiber	Cytosol	~ $0.2\,\Delta F/F_0$	~1.4	100–200 full duration	~ 20	[29,54,57] [33,58]
Spike	Heart	Rat	Ventricular myocyte	Cytosol	~ $0.5\,\Delta F/F_0$	–	~ 10 half time of decay	15	[64]
Sparklet	Heart	Rat	Ventricular myocyte	Cytosol	~ 20 nM ~ $0.5\,\Delta F/F_0$ (for 0.5 pA I_{Ca})	~1	–	–	[66]
Puff	Ovary Epithelium	Toad Human	Oocyte HELA	Cytosol	100–200 nM	~ 5	~ 200 half time of decay	50–80	[67] [70]
Blip	Ovary Endothelium Epithelium	Toad Bovine Human	Oocyte CPAE HELA	Cytosol	10–30 nM	1–3	50–200 half time of decay	25–50	[72] [73] [70]
Mark	Heart	Rat	H9C2	Mitochondria	200–400 nM ~ $0.6\,\Delta F/F_0$	~ 0.5	~ 170 FDHM	–	[79]
Scrap	Heart	Rabbit	Ventricular myocyte	SR	~ 25% percent of reduction in SR $[Ca^{2+}]$	–	~ 100–200 τ recovery	~ 100 time to nadir	[86,89]
Blink	Heart	Rabbit	Ventricular myocyte	SR	~ 0.05 reduction in $\Delta F/F_0$	~ 1	~ 30 τ recovery	~ 20 time to nadir	[89]
Skrap	Skeletal muscle	Frog	Muscle fiber	SR	~ 7% percent of reduction in SR $[Ca^{2+}]$	~ 1	~ 250 τ recovery	~ 50 time to nadir	[91]

Quantitative comparisons among different events need to be done carefully, as the experimental and recording conditions often vary widely.

stable and Ca^{2+} signals did not spread as waves or perpetuate as oscillations [19]. In such a system, the amplitude of the whole-cell Ca^{2+} transient is regulated by recruiting fewer or more Ca^{2+} sparks [20,21], which will then sum up to shape the cellular Ca^{2+} transient.

2.1.2. Ca^{2+} sparks in skeletal muscle

After the description of Ca^{2+} sparks in cardiac muscle the search for analogous elementary signals initially focused on cells which were known to have Ca^{2+} signaling pathways similar to heart. Indeed, within a short period of time, Ca^{2+} sparks were also discovered in skeletal muscle [22,23]. Most of the data on skeletal muscle Ca^{2+} sparks was collected from amphibian (frog) muscle fibers [24–26]. Only later it was found, as a big surprise, that in normal and healthy mammalian skeletal muscle fibers Ca^{2+} sparks are very infrequent [27,28] and that upon depolarization Ca^{2+} release occurs in a homogeneous fashion, with signals termed "precursors" of Ca^{2+} sparks (also referred to as "eventless", "small-event" release or "ember", see below) [29]. Several explanations have been proposed why this could be the case [30] but we are still far from a complete understanding of the involved mechanisms. Developing myotubes exhibit Ca^{2+} sparks until the EC-coupling machinery is fully differentiated and is in place. Because in myotubes the Ca^{2+} sparks can only be seen in subcellular regions in which voltage-dependent Ca^{2+} release is not yet established, it was proposed that the mechanical link between voltage-sensors and RyRs is not only required for the activation of SR Ca^{2+} release, but normally also suppresses Ca^{2+} sparks [31,32]. More recently, it has been observed that mammalian fibers with impaired or challenged metabolism (e.g. after skinning [33], following mitochondrial substrate removal [34], or subsequent to stress, like osmotic shock or strenuous exercise [35]) develop Ca^{2+} signals reminiscent of sparks. Also, in some muscle diseases, such as muscle dystrophy, the prevalence of Ca^{2+} sparks is dramatically increased, particularly after mechanically stressing the fiber [36]. In any case, it appears that in mammalian skeletal muscle the Ca^{2+} sparks are normally suppressed but are important in various pathophysiological states of this tissue.

2.1.3. Ca^{2+} sparks in smooth muscle

In smooth muscle cells Ca^{2+} sparks were discovered 2 years after the initial report in cardiomyocytes [37] (see Refs. [38,39] for reviews). Subsarcolemmal Ca^{2+} sparks were proposed to underlie the "spontaneous transient outward currents" (STOCs), caused by activation of sarcolemmal Ca^{2+}-dependent K^+ channels. This was later confirmed in several studies, some measuring the electrical and Ca^{2+} signals simultaneously [40–45]. Relaxation of smooth muscle cells by Ca^{2+} is a nice example of how the same messenger can have different, even opposite, downstream effects depending on its spatial targeting. Whole-cell or global elevations of the cytosolic Ca^{2+} concentration will lead to contraction of the smooth muscle cell, while subsarcolemmal Ca^{2+} sparks will induce relaxation by a hyperpolarization of the cell membrane.

2.2. The Ca^{2+} macrospark

The "Ca^{2+} macrospark" seems to correspond to simultaneous or near simultaneous Ca^{2+} release from two (or more) closely located spark sites [46]. It can also be interpreted as a very short Ca^{2+} wave propagating as a reaction–diffusion system driven by CICR [47]. Macrosparks have already been described in early reports of Ca^{2+} sparks [10,48], but have later also been observed in smooth muscle cells where they were associated with spontaneous transient outward currents (STOCs) too large in amplitude to be explained by a single Ca^{2+} spark [40].

2.3. The Ca^{2+} quark

Initial calculations of the Ca^{2+} signal mass of Ca^{2+} sparks had suggested that each event resulted from opening of only one (or very few) RyRs. Uncertainties inherent in such calculations arise from the unknown Ca^{2+} buffer capacity of the cytosol, especially on the level of the microdomain. In addition, the Ca^{2+} flux of the RyR *in situ* had probably been overestimated, based on channel reconstitution experiments in lipid bilayers (to be around 4 pA). Estimates of Ca^{2+} flux *via* single RyRs under conditions approaching the physiological composition of the cytosol, in particular regarding Mg^{2+} and ATP, suggested significantly smaller fluxes of less than 0.6 pA [49]. Obviously, the real *in situ* Ca^{2+} flux via a single RyR is still unknown because is has not been measured. There were also early observations of cellular Ca^{2+} signals suggesting that a Ca^{2+} spark may actually result from the opening of a group of RyRs (for review see Ref. [50]). For example, flash photolytic activation of SR Ca^{2+} release in cardiac myocyte resulted in a Ca^{2+} release signal that appeared to be spatially homogeneous, i.e. not containing events detectable as Ca^{2+} sparks [51]. Based on this observation, it was proposed that a Ca^{2+} signaling event smaller than a Ca^{2+} spark may exist, which then would correspond to the opening of a single RyR, and it was termed a "nanoscopic Ca^{2+} release event" or "Ca^{2+} quark" [51]. Similarly, heterologously expressed RyRs generated functional Ca^{2+} release in Chinese hamster ovary (CHO) cells, but again release was eventless and could not be resolved as Ca^{2+} sparks [52,53]. It was concluded that in this cell system the RyRs would most likely not reproduce the highly specialized microarchitecture and channel assemblies found in muscle and therefore operate independently and as single channels. Homogeneous, or eventless, Ca^{2+} release has also been observed in adult mammalian skeletal muscle [54] or in frog skeletal muscle during voltage clamp when CICR was suppressed by tetracain [29,54]. Later, two-photon photolytic activation of localized Ca^{2+} release in cardiac myocytes revealed tiny events with a signal mass that was around 40 times smaller than a Ca^{2+} spark (see Fig. 1), but it remained unclear whether these events were in fact Ca^{2+}

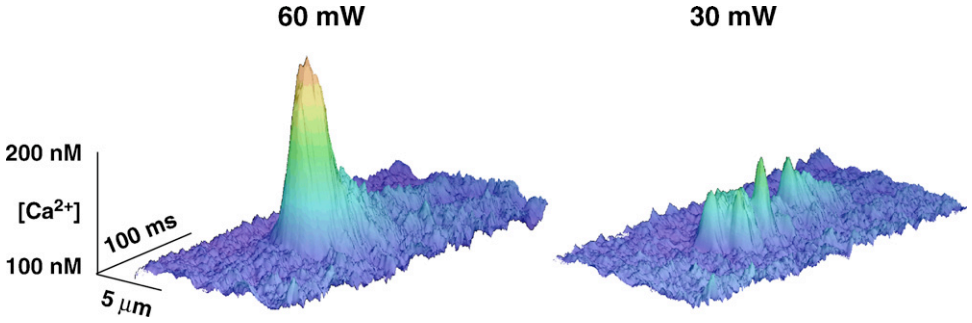

Fig. 1. Two-photon photolytic activation of CICR in a guinea-pig ventricular myocyte. Photolysis of DM-nitrophen with a high-power laser pulse elicits a Ca^{2+} spark-like signal (left panel). A low-power pulse is followed by several tiny Ca^{2+} release events exhibiting a signal mass that is around 40-fold smaller than the spark, possibly corresponding to Ca^{2+} quarks (figure adapted from Ref. [55]).

quarks [49,55]. Recent computer modeling of Ca^{2+} quarks suggests that usually such single channel openings would not be resolved in the noise of confocal images [56].

2.4. The Ca^{2+} ember

In skeletal muscle, the trigger for Ca^{2+} release from the SR is mediated by an allosterical interaction between RyRs and voltage sensors. The initial Ca^{2+} release is thought to be subsequently amplified by CICR. Non-sparky localized intracellular Ca^{2+} signals were first detected in amphibian skeletal muscle under very low voltage depolarizations or in the presence of RyR channel blockers [29]. They were referred to as precursors of Ca^{2+} sparks. Later, it was shown that Ca^{2+} sparks elicited by larger voltage depolarizations have a persistent component of a constant and relatively small amplitude, named "the ember" (see Fig. 2). Visibility of embers was enhanced when CICR was inhibited and it was proposed that the Ca^{2+} ember reflects opening of RyR channel(s) activated directly through the mechanical interaction with voltage sensors. The ember provides the trigger Ca^{2+}that can later be amplified by CICR, producing Ca^{2+}sparks [33,57]. Ca^{2+} ember-like events were also detected in cut mammalian fibers under voltage clamp [58] and after permeabilization [33]. The absence of Ca^{2+} sparks in mammalian skeletal muscle under physiological conditions and the presence of embers is considered to be a strong indication for a limited role of CICR in normal mammalian excitation–contraction coupling.

2.5. The Ca^{2+} spike

The signal mass of Ca^{2+} sparks is a complex function of release flux duration and release wave-form, but also of Ca^{2+} buffering, compartmentalization and diffusion. For many studies the SR Ca^{2+} release flux would be the parameter of choice to analyze. Previously, mathematical models have been developed to calculate whole-cell SR Ca^{2+} release flux from skeletal and cardiac muscle by determining Ca^{2+} removal rates by various systems for any possible Ca^{2+} concentration and back-extrapolating to Ca^{2+} release [59,60]. However, applying a similar approach to elementary Ca^{2+} signaling events has been notoriously difficult, mostly because of signal-to-noise problems [14,61,62]. Thus, averaging and low-pass filtering of the signals was often needed, partly obscuring the desired information. Another approach is to dramatically increase the capacity of the Ca^{2+} removal function, by adding a large amount of Ca^{2+} buffers [63]. Under these conditions, the local fluorescence signals will nearly perfectly reflect the Ca^{2+} release flux. Indeed, this approach was successfully applied to cardiac myocytes and the local Ca^{2+} signals observed under these conditions were termed "Ca^{2+} spikes" [64]. These Ca^{2+} spikes should not be confused with the kinetic Ca^{2+} spikes observed after flash photolysis of caged Ca^{2+} com-

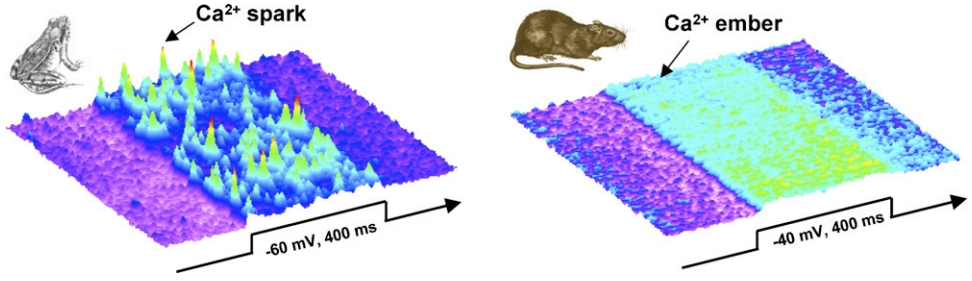

Fig. 2. Sparks and embers in skeletal muscle. Moderate depolarizations elicit Ca^{2+} sparks in amphibian and Ca^{2+} embers in mammalian muscles (figure adapted from Ref. [30]).

pounds [65] or with neuronal Ca^{2+} spikes (see Ref. [3] for review).

2.6. The Ca^{2+} sparklet

In extremely challenging experiments a breakthrough has been made with the simultaneous recording of single L-type Ca^{2+} channel currents and the resulting Ca^{2+} signals in cardiac myocytes [66]. The recorded tiny Ca^{2+} signals were termed "Ca^{2+} sparklets". These results are important because, for the first time, both the total amount of Ca^{2+} delivered to the microdomain and the resulting concentration change were recorded at the same time. Thus, the known single channel current via the L-type Ca^{2+} channel and the resulting Ca^{2+} signal could be used as a ruler to quantify the much larger Ca^{2+} flux from the SR via the RyRs during a Ca^{2+} spark. It was determined that the SR Ca^{2+} release flux underlying a typical Ca^{2+} spark corresponds to approximately 2.1 pA in these experiments, and that the number of open RyRs is 4–6 (derived from RyR single channel conductance measurements in lipid bilayer studies [49]).

2.7. The Ca^{2+} puff

In non-excitable cells, localized Ca^{2+} signals that are initiated by the second messenger $InsP_3$ were first described as "Ca^{2+} puffs" [67]. The Ca^{2+} puffs recorded in Xenopus oocytes after photolytic liberation of $InsP_3$ from a caged compound probably defined the foundation for the general concept that subcellulary localized Ca^{2+} signals can be all-or-none events while the cell can still generate a response that depends in amplitude on the stimulus intensity in a graded way [68]. Ca^{2+} puffs seem to involve activation of several $InsP_3$ receptor channels which can show some local propagation, leading to a large range of Ca^{2+} puff durations [69]. In the meantime, Ca^{2+} puffs have been described in a large range of cells, such as, for example, HeLa cells [70] and PC12 neuronal cells [71].

2.8. The Ca^{2+} blip

It was noted that Ca^{2+} puffs were often triggered by yet smaller Ca^{2+} signals, "Ca^{2+} blips" [69,70,72,73]. Ca^{2+} blips are thought to arise from openings of a single $InsP_3$ receptor channel [74], and they would thus be equivalent to Ca^{2+} quarks *via* RyRs.

To add another layer of complexity, some cells express both types of Ca^{2+} release channels, RyRs and $InsP_3$Rs on their Ca^{2+} stores (e.g. cardiac atrial cells [75,76] and PC12 cells [71]). In most cases both types of channels have access to the same Ca^{2+} store compartment, but a complete separation between $InsP_3$Rs and RyR Ca^{2+} storage pools is also conceivable. Very recently, $InsP_3$-dependent Ca^{2+} signaling within a microdomain inside the cell nucleus has been implicated in excitation–transcription coupling [77] while very long lasting Ca^{2+} release signals have been observed to occur from the nuclear envelope which may represent a Ca^{2+} store with a relatively large local volume [78].

2.9. The Ca^{2+} mark

In the cardiac H9C2 cell line miniature Ca^{2+} signals have been recorded from individual mitochondria using the Ca^{2+} indicator rhod-2 [79]. These signals, "Ca^{2+} marks", were thought to be triggered by Ca^{2+} sparks occurring close to the respective mitochondria. Based on measurements with the chemoluminescent Ca^{2+} indicator aequorin targeted to mitochondria, such a preferential access of SR Ca^{2+} release to mitochondria has been proposed before imaging of mitochondrial Ca^{2+} signals was performed [80]. Through which pathway the Ca^{2+} marks travel from the SR/ER into the mitochondria is not yet established, but seems to involve some "tunneling" of Ca^{2+} which is not easily accessible from the cytosolic space [81,82].

2.10. The Ca^{2+} scrap

Signals reporting SR Ca^{2+} depletion during Ca^{2+} release in rabbit cardiomyocytes were termed "Ca^{2+} scraps". While the local control theories and the resulting new concepts of CICR have greatly facilitated our understanding of the processes by which Ca^{2+} signaling is activated in a variety of systems, tissues and cells, the other side of the coin has been much more challenging to tackle experimentally. What are the mechanisms leading to the termination of Ca^{2+} release events? Several possibilities have been proposed in the past, such as Ca^{2+} induced inactivation of the release, stochastic attrition among Ca^{2+} release channels, but also functional depletion of the stores. In this respect, it would be helpful to be able to follow the Ca^{2+} concentration of the store directly. While this has been possible in a variety of cell types for some time by using low-affinity Ca^{2+} indicators (often originally designed to be Mg^{2+} indicators) [83,84], this kind of experiments has been very difficult in skeletal and cardiac muscle, possibly because of the small fractional volume of the Ca^{2+} store in these cells, and the large fraction which is occupied by mitochondria. Only recently have the first Ca^{2+} signals from within skeletal [85] and cardiac SR, the Ca^{2+} scraps, been resolved [86], using loading of the cell with the AM-ester form of the low affinity Ca^{2+} indicator fluo-5-N. This technique has also been applied to obtain further mechanistic insight into the pathophysiology of arrhythmogenic conditions related to congestive heart failure [87] and mutations of the Ca^{2+} binding protein calsequestrin [88].

2.11. The Ca^{2+} blink

As an extension to Ca^{2+} measurements inside the cardiac SR on the level of the cell (see Ca^{2+} scraps) it has recently become possible to record the local functional SR Ca^{2+} depletion and refilling resulting from single Ca^{2+} sparks, a signal that has been termed a "Ca^{2+} blink" [89]. These

measurements are at the edge of signal-to-noise limitations but are very valuable because it is still not clear which mechanism(s) actually terminate Ca^{2+} sparks. Since a local functional depletion with rapid recovery is assumed to be critical [61,90], measuring the sarcoplasmic signals during and after Ca^{2+} sparks may be the ideal approach to clarify this point.

2.12. The Ca^{2+} skrap

Recently, a microscopic intra-SR Ca^{2+} depletion signal has also been recorded from skeletal muscle fibers ("Ca^{2+} skrap"), by using an a ingenious signal detection approach with an indicator that changes both, the excitation and emission spectrum upon Ca^{2+} binding [91]. By using shifted excitation and emission ratio (SEER) imaging of Mag-Indo-1, used as a low affinity Ca^{2+} indicator, local intrastore depletions of SR Ca^{2+} could be recorded. Interestingly, these events were seen when mitochondrial Ca^{2+} uptake was suppressed. It is well known that mitochondria take up Ca^{2+} indicators that are loaded using the AM-ester form. Thus, Mag-Indo-1 entrapped in the mitochondria will report some increase of mitochondrial Ca^{2+} during the sparks (see Section 2.9), thereby obscuring the Ca^{2+} skraps.

3. Discussion and outlook

The discovery of Ca^{2+} sparks undoubtedly had a huge impact on the field of cellular and subcellular Ca^{2+} signaling research, in a way comparable to the inspiration which the first single-channel recordings created for cellular electrophysiology. This Ca^{2+} signaling research field virtually exploded during the last 10 years and today the Medline database lists 2818 papers containing the word "spark" in their title or abstract. This number does not yet include most of the publications describing anologous Ca^{2+} release events appearing under their own name, as listed above. While a few more Ca^{2+} signaling events with specific features are being described and characterized even now (e.g. "Ca^{2+} glow" [92], "Ca^{2+} syntillas" [93] and others), we think that the general concept of microdomain signaling with diffusible messengers, but also with the related but more structurally defined macromolecular complexes, will develop even further, gain momentum and attract significant attention from the research community. Again, understanding the inner molecular workings occurring in these tiny spaces will be a challenging task for future generations of scientists from various fields and with different backgrounds. Interdisciplinary approaches combining all state-of-the-art techniques borrowed from biophysics, biochemistry, molecular biology, bioinformatics, nanotechnology and materials sciences seem to be the most promising strategy to solve all these questions and to put the findings together into a comprehensive framework of the system of the cell, the organ, and the organism [94].

Acknowledgements

We acknowledge grant support from the following sources: Swiss National Science Foundation, Muscular Dystrophy Association, Swiss Foundation for Research on Muscle Diseases, Swiss State Secretariat for Education and Research, UMDNJ & Sigrist Foundations.

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