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·述 评· Editorial

Calcium Sparks in Heart Muscle Cells

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Abstract: As a ubiquitous intracellular messenger, Ca^{2+} plays important roles in vital physiological processes, such as muscle contraction, synaptic transmission, hormone secretion, metabolism, gene transcription, fertilization, cell survival and cell death[1-4]. It has been increasingly appreciated that spatial and temporal patterning endows Ca^{2+} signal with efficiency, specificity, and unparalleled versatility, such that the same ions fulfill different and even opposing physiological functions in a given cell. Thus, direct visualization of Ca^{2+} microdomain (0.1 – 10 μm) holds the promise to dissect out the space-time architecture of Ca^{2+} dynamics, and thereby to elucidate Ca^{2+} signaling mechanisms in different physiological contexts. Since our first recording of Ca^{2+} sparks[5] in cardiac myocytes in 1993, imaging microdomain Ca^{2+} in muscles has offered unique insights into molecular mechanisms of excitation-contraction (EC) coupling, and revolutionized our understanding of Ca^{2+} handling and signal transduction (for reviews, see Reference 7). In this mini-review, we intend to present a brief introduction on Ca^{2+} sparks in cardiac myocytes and other cell types.

Key words: Ca^{2+} sparks; signal transduction; cardiac myocytes; excitation-contraction (EC) coupling

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心肌细胞中的钙火花

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摘 要:细胞离子钙是最普遍、最重要的第二信使之一,参与包括肌肉收缩、突触传递、激素分泌、基因转录以及细胞分裂、受精、代谢和凝血等多种生理过程。对心肌细胞来说,离子钙将电活动与收缩联系起来,即介导细胞的兴奋-收缩耦联。1993 年我们首次在激光共聚焦显微镜下发现心肌细胞胞内钙释放的基本单位——钙火花。历经十几年的研究,人们对钙火花和胞内钙信号的本质及兴奋-收缩耦联的微观机理的认识日趋深刻。特别是,新发展起来的非紧密封接膜片钳和激光共聚焦显微镜成像合成技术可以激活单个 *L* 型钙通道,通过钙致钙释放机制,诱发钙火花。此文简要介绍心肌细胞中自发和诱发钙火花的研究及其意义,并对有关研究领域的前景作一展望。

关键词:钙火花;信号传导;心肌细胞;兴奋-收缩耦联

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1 Visualization of spontaneous Ca^{2+} sparks

With the advent of confocal microscope and the new generation of fluorescent indicator, fluo-3, micro-domain Ca^{2+} in muscles was first visualized as Ca^{2+} sparks in cardiac myocytes[5]. In quiescent cardiac cells, a Ca^{2+} spark reflects a transient increase in local Ca^{2+} due to the spontaneous activation of ryanodine receptors (RyRs) in a single Ca^{2+} release unit (CRU) in the sarcoplasmic reticulum (SR). It appears abruptly amidst seemingly featureless background, reaches its peak within 10 milliseconds, and dissipates in another 20 milliseconds. Under physiological conditions a Ca^{2+} spark remains solitary, and is confined to ~2.0 microns in diameter[5,6].

The existence of Ca^{2+} sparks tells us something that had not been appreciated before. First, the SR Ca^{2+} release occurs in a stochastic and discrete manner. Mapping the origin of Ca^{2+} sparks revealed that spark-generating sites, coincided with T-tubules, are separated by ~1.8 μm in the longitudinal direction and 0.5 – 1.5 μm in the transverse direction[7]. Genesis of Ca^{2+} spark at T-SR junctions during small depolarization has been shown to be governed by Poisson statistics[8]. Second, the rate of occurrence of spontaneous Ca^{2+} sparks suggests that RyRs in situ is surprisingly insensitive to Ca^{2+}, validating an important premise of the local control theory[9], a theory which was first advanced by Stern in 1992 to explain the mechanism for EC coupling. In a typical myocyte, approximately 100 Ca^{2+} sparks ignite every second[5]. This translates into an open frequency of 0.0001 s^{-1} or a mean close time of 10 000 s for RyRs in cells, differing by orders of magnitude from those in the planar lipid bilayer[7]. Third, the brevity of Ca^{2+} sparks indicates that regenerative Ca^{2+}-induced Ca^{2+} release (CICR)[7,10] within a spark-generating unit must be somehow terminated promptly. Together, the spontaneous termination, the spatial confinement of Ca^{2+} sparks, and the low Ca^{2+} sensitivity of RyRs provide important bases for the local control theory[7]. Confocal imaging of Ca^{2+} sparks has provided a novel and powerful means by which RyR, an intracellular Ca^{2+} channel, can be observed optically in intact functioning cells[7].

2 Visualization of evoked Ca^{2+} sparks

It is now generally accepted that evoked Ca^{2+} sparks constitute the elementary events underlying cardiac EC coupling[7]. During full-fledged cardiac EC coupling, Ca^{2+} sparks overlap in space and time, and summate into a global Ca^{2+} transient. It is estimated that about 104 sparks are activated within a few tens of milliseconds in a single myocyte during each cardiac cycle[11], raising cytosolic $[Ca^{2+}]$ to ~1 mmol · L^{-1}.

Ca^{2+} sparks with similar properties as the spontaneous ones can be evoked by Ca^{2+} influx through voltage-operated L-type Ca^{2+} channels (LCCs)[7,12,13], via the CICR mechanism. To resolve individual events, Ca^{2+} sparks should be activated at a low density, by near-threshold (–60 mV ~ –40 mV) or very brief depolarization[8,14], with reduced extracellular $[Ca^{2+}]$[15], or under conditions when LCC availability is reduced pharmacologically[7,11,14,16], However, most methods to trigger Ca^{2+} sparks suffered from the out-of-focus blurring problem[12]. Confocal sampling theory states that, when out-of-focus events are registered, the optical blurring will render reduced amplitude, broadened spatial spreading, and blunted kinetics, with the degree of distortion depending on the relative positioning[17,18]. This problem has hindered our effort to investigate the nature about elementary Ca^{2+} release events.

Fortunately, we have recently provided a solution to this problem by controlled activation of Ca^{2+} sparks from a known in-focus CRU, using "loose-seal" patch-clamp combined with confocal Ca^{2+} imaging[12,13]. Specifically, a low-resistance (20 – 50 MΩ) seal was

formed by gently pressing the patch pipette against the surface membrane without disrupting the exquisite EC coupling machinery. Population of in-focus Ca^{2+} sparks can then be evoked by repeated patch depolarization. The more rigorous characterization of spark properties revealed that the true amplitude of Ca^{2+} sparks exhibits a broad, modal distribution[12, 13], in contrast to the notion that Ca^{2+} sparks are all-or-none or stereotypical[5, 8, 12]. Polymorphism of Ca^{2+} sparks in terms of variable amplitude is evident even for events from a single CRU[12]. Importantly, the rising rate, a reporter of the underlying Ca^{2+} release flux, displays a strong negative correlation with spark rise time, an index of Ca^{2+} release duration[12]. These led to the proposal of a new model for cardiac spark genesis: a cohort of variable RyRs in a CRU are activated to generate a spark; an ensuing negative feedback overrides the regenerative CICR among RyRs, thereby contributing to termination of the spark[12].

3 Ca^{2+} sparks in other cell types

High Ca^{2+} microdomains due to intracellular Ca^{2+} release are widely found in many other cell types. To date, Ca^{2+} sparks are shown also to be present in skeletal and smooth muscle cells containing different isoforms of RyRs[7]. Ca^{2+} sparks of both RyR and IP3 receptor (IP_3R) origin are present in non-excitable cells such as glial cells and endothelial cells, too[7]. Analogous IP3R Ca^{2+} sparks, namely "Ca^{2+} puffs" or "Ca^{2+} blips", have also been extensively characterized in xenopus oocytes[7]. Ca^{2+} sparks as the universal building blocks of Ca^{2+} signaling fulfill distinctly different physiological roles in these cells.

4 Perspective

In spite of many advancements in microdomain Ca^{2+} imaging, there are ample examples that physiologically relevant Ca^{2+} signaling mechanisms are concealed in nanodomains (1 – 100 nanometers). In this regard, a fundamental limitation of the optical approach is in its spatial resolution, which is ~200 nanometers at best, as in total internal reflection fluorescent microscopy (TIRFM)[19]. Visualization of nanodomain Ca^{2+} would require *supra-optical resolution*, which demands new and innovative approaches. A possible strategy might involve tethering or docking Ca^{2+} indicators, through molecular and genetic manipulations, at molecularly defined locations.

It is predictable that next generation of Ca^{2+} indicators and imaging methods will be called upon to perfect cellular Ca^{2+} imaging. Likewise, new areas of cellular Ca^{2+} signaling will be vigorously explored in the near future. Particularly, targeted expression of protein-based Ca^{2+} indicators to subcellular compartments[20], in conjunction with time-lapsed Ca^{2+} imaging, holds a great promise to uncover new dimensions of the space-time architecture of cellular Ca^{2+} dynamics and to elucidate roles of Ca^{2+} in long-term regulation of non-contractile cellular functions (e.g., myocyte differentiation, growth, hypertrophy, migration and apoptosis). We eagerly await grander horizons that lie ahead.

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