Biochimica et Biophysica Acta 1820 (2012) 1185–1194

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Fundamental properties of Ca^{2+} signals ☆

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ARTICLE INFO

Article history:
Received 12 September 2011
Received in revised form 16 October 2011
Accepted 17 October 2011
Available online 25 October 2011

Keywords:
Calcium dynamics
Mathematical modeling
Cell signaling
Stochastic process

ABSTRACT

Background: Ca^{2+} is a ubiquitous and versatile second messenger that transmits information through changes of the cytosolic Ca^{2+} concentration. Recent investigations changed basic ideas on the dynamic character of Ca^{2+} signals and challenge traditional ideas on information transmission.
Scope of review: We present recent findings on key characteristics of the cytosolic Ca^{2+} dynamics and theoretical concepts that explain the wide range of experimentally observed Ca^{2+} signals. Further, we relate properties of the dynamical regulation of the cytosolic Ca^{2+} concentration to ideas about information transmission by stochastic signals.
Major conclusions: We demonstrate the importance of the hierarchal arrangement of Ca^{2+} release sites on the emergence of cellular Ca^{2+} spikes. Stochastic Ca^{2+} signals are functionally robust and adaptive to changing environmental conditions. Fluctuations of interspike intervals (ISIs) and the moment relation derived from ISI distributions contain information on the channel cluster open probability and on pathway properties.
General significance: Robust and reliable signal transduction pathways that entail Ca^{2+} dynamics are essential for eukaryotic organisms. Moreover, we expect that the design of a stochastic mechanism which provides robustness and adaptivity will be found also in other biological systems. Ca^{2+} dynamics demonstrate that the fluctuations of cellular signals contain information on molecular behavior. This article is part of a Special Issue entitled Biochemical, biophysical and genetic approaches to intracellular calcium signaling.

1. Introduction

The Ca^{2+} signaling pathway translates external signals into intracellular responses by increasing the cytosolic Ca^{2+} concentration in a stimulus dependent pattern. The concentration increase can be caused either by Ca^{2+} entry from the extracellular medium through plasma membrane channels, or by Ca^{2+} release from internal storage compartments. In the following, we will focus on inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release from the endoplasmic reticulum (ER), which is the predominant Ca^{2+} release mechanism in many cell types. The signal cascade starts typically at a plasma membrane G-protein coupled receptor [1–3]. Due to binding of an agonist, the receptor activates phospholipase C (PLC), which in turn produces IP_3 at the cell membrane. IP_3 diffuses in the cytosol and binds to IP_3 receptor channels (IP_3Rs), where subsequent binding of Ca^{2+} to activating Ca^{2+} binding sites switches the channel to a state with high open probability [4]. This positive feedback of Ca^{2+} on its own release channel is called Ca^{2+}-induced Ca^{2+}-release (CICR). Opening of an IP_3R triggers a Ca^{2+} flux into the cytosol due to the large concentration differences between the two compartments [4–7], which is in the range of 3 to 4 orders of magnitude. The released Ca^{2+} is removed from the cytosol either by sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs) into the ER or by plasma membrane Ca^{2+} ATPases into the extracellular space.

IP_3Rs are spatially organized into clusters of up to about fifteen channels, which are scattered across the ER membrane with distances of 1 to 7 μm [8–12]. The coupling between channels is achieved through CICR based on Ca^{2+} diffusion. Given that the diffusion length of Ca^{2+} is less than 2 μm, the coupling between channels in a cluster is much stronger than the coupling between channels in adjacent clusters [13]. The structural hierarchy of IP_3R organization, from the single channel to clusters, is also reflected in the dynamic responses of the intracellular Ca^{2+} concentration, as revealed through fluorescence microscopy and simulations [12, 14–17]. Openings of single IP_3Rs ('blips') may trigger collective openings of IP_3Rs within a cluster ('puffs'), while Ca^{2+} diffusing from a puff site can activate neighboring clusters, eventually leading to a global, i.e. cell wide, Ca^{2+} spike [15]. Marchant and Parker followed the signal generation from its origin at a single channel cluster to the global Ca^{2+} wave, which corresponds

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doi:10.1016/j.bbagen.2011.10.007

to a concentration spike in whole cell recordings [15, 18]. Importantly, repetitive sequences of these Ca^{2+} spikes encode information that is used to regulate many processes in various cell types [19–21].

Cellular spike sequences exhibit a refractory period after a spike [22–26]. The refractoriness has often been related to the negative feedback of high Ca^{2+} concentrations on the open probability of IP_3Rs, as observed in patch clamp experiments [27–29] (see Refs. [5, 30, 31] for reviews). Together with the positive feedback of CICR at small Ca^{2+} concentrations, this negative feedback leads to a bell shaped dependence of the stationary open probability of IP_3Rs on cytosolic Ca^{2+} [6, 32]. The negative feedback causes an almost fixed (or deterministic) refractory period of several tens of seconds in the global signals. However, such a recovery timescale has not been observed with the local puff dynamics of IP_3R clusters. Interpuff intervals (IPIs) exhibit a relative refractory period of a few seconds only [12, 14, 15, 18, 33–35]. Hence, the negative feedback that determines the time scale of interspike intervals (ISIs) is different from the feedback contributing to IPIs and requires global (whole cell) release events.

Here, we review the dynamic properties of Ca^{2+} spike sequences. An important property of ISIs (and IPIs) is that they form a distribution. Rather than having a single value for the ISI, cells exhibit a spread of times between consecutive spikes. The very existence of a distribution of ISIs hints at the presence of fluctuations somewhere in the spike generation process. Broadly speaking, these fluctuations can arise from two different types of processes. With the first one, the variations arise from deviations around a constant ISI. In this context, we would assume that the fundamental process giving rise to the constant ISI is a deterministic oscillator. With the second one, the emergence of each spike is completely random. In this case, the dynamics are truly stochastic. The first process would generate regular spike sequences without fluctuations, the second process would not generate any spikes without fluctuations. The distinction between a deterministic and a stochastic spike generation mechanism is not only important for the choice of the appropriate mathematical description, but also points towards the fundamental biological processes that are involved in shaping ISIs.

To test the two approaches, we take advantage of the fact that they make different predictions with respect to the dependence of spike characteristics on cellular parameters. For example, stochastic models reproduce the sensitive dependence of the average ISI on the diffusional properties of the cytosol, while deterministic models predict independence of the average ISI from diffusion coefficients and buffer concentrations [26, 36]. Similar considerations apply to other correlations [16, 26, 37–40]. Stochastic models reconcile dissociation constants of the Ca^{2+} regulatory binding sites on the IP_3R measured *in vitro* with the dynamic behavior and local concentrations *in vivo*[17], and they offer straightforward explanations for the large measured cell-to-cell variability of the average ISI [36, 41]. Moreover, the standard deviation of ISIs within a single spike sequence is in many cases of the same order of magnitude as the average value, and these fluctuations are an additional source of information. As we will see below, the standard deviation presents a better indicator for the IP_3R open probability than the average ISI. The former is governed by the randomness of the spike generation mechanism, while the latter is mostly determined by a global feedback.

Deterministic models have contributed substantially to the development of concepts and ideas in the field. One of the first theoretical considerations was undertaken by Meyer and Stryer in 1988 [42], who used a nonlinear dependence of the release flux on the IP_3 concentration and suggested a feedback through IP_3 oscillations. This hypothesized feedback could not be verified experimentally in general and led to further model development. A prominent class of models for the IP_3 receptor (see Refs. [5, 30, 31, 43] for reviews) considers one site for IP_3 binding that sensitizes a subunit for Ca^{2+} binding, one for Ca^{2+} that activates a subunit, and another one for Ca^{2+} that dominantly inhibits a subunit. In the DeYoung–Keizer model [27], it is assumed that a channel opens if at least 3 subunits of the tetrameric IP_3R are in the active state. The different affinities for Ca^{2+} binding to the activating and inhibiting binding sites lead to a bell shaped stationary open probability of an IP_3R [5, 32]. Another conceptually important model was introduced by Goldbeter et al. [44, 45]. It is based on the existence of two Ca^{2+} pools representing the ER and the cytosol, respectively. Increasing IP_3 triggers Ca^{2+} release from the ER into the cytosol inducing further CICR by a positive feedback. After emptying the ER, Ca^{2+} is pumped back by SERCAs into the ER. Repeating this scenario leads to oscillations with similar properties as those observed in experiments.

With the improvement of experimental techniques, a growing number of measurements was published that could not be explained within the framework of deterministic models, which neglected the spatial arrangement of IP_3R clusters. More precisely, these models did not incorporate the Ca^{2+} concentration gradients around an open IP_3R cluster and the weaker diffusive coupling between clusters, as compared to within clusters (see below). Furthermore, the averaging procedure that leads from the mathematical description of all the individual channels in a cell (master equation) to the rate equations of deterministic models identifies *small* probabilities with *small* currents. If the cell is in a state with small open probability, a small fraction of channels is open and causes a small release flux. On the contrary, stochastic models that take channel clustering into account identify small probabilities with *rare* events that cause locally large concentration changes and have the potential to initiate global spikes [16, 36, 41]. We will see below that this hierarchic cascade of events (Fig. 1) gives rise to dynamical properties and parameter dependences different from those in deterministic models.

2. The dynamics of IP_3R clusters

The local Ca^{2+} concentration at open channels is orders of magnitude larger than the spatially averaged bulk concentrations [46]. Single open channels may cause Ca^{2+} concentrations in a volume of the size of the IP_3R channel vestibule of 20–70 μM, several open channels of up to 220 μM [13]. The concentration at a distance of only 1 μm from the cluster is orders of magnitude smaller.

These large Ca^{2+} gradients around an open cluster of IP_3Rs also raised the question whether the nonlinear interplay between Ca^{2+} release and Ca^{2+} uptake suffices to reproduce the Ca^{2+} spike sequences observed in experiments. To answer this question, we studied a deterministic model of a single cluster in a three dimensional cytosolic environment [17]. The flux through a Ca^{2+} liberating cluster was chosen according to realistic simulations [13] and determined by the number of open channels. To compute this number, we employed the DeYoung–Keizer model as a prototypical framework for IP_3R dynamics [27]. Based on Ca^{2+} fluxes that lead to realistic Ca^{2+} concentrations at a releasing cluster [13], the deterministic cluster model does not generate oscillations. After an initial release phase, all channels within the cluster close and remain inactive forever.

We may understand this behavior by considering the impact of the large Ca^{2+} concentrations on the nonlinear feedback functions that regulate Ca^{2+} liberation. Assuming that these functions are of Hill type (as is the case in most IP_3R models), a good measure of the dynamic range of the feedback is the dissociation constant K_D. Generally speaking, feedback only exists if the Ca^{2+} concentration is in the range of the dissociation constant. For Ca^{2+} activation, the dissociation constant is in the order of 100–500 nM, while Ca^{2+} inhibition is governed by a K_D of around 2 μM [47, 48]. Keeping in mind that Ca^{2+} concentrations at a releasing cluster reach peak values of 20–220 μM [13], all feedback processes saturate. Almost all channels in a cluster become inhibited, they remain inactive even when the Ca^{2+} concentration drops an order of magnitude. A concentration keeping most channels inhibited can be maintained with a tiny fraction of channels that remain active. Although inhibition is removed at basal