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ELEMENTARY AND GLOBAL ASPECTS OF CALCIUM SIGNALLING

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Summary

Calcium is a ubiquitous second messenger used to regulate a wide range of cellular processes. This role in signalling has to be conducted against the rigid homeostatic mechanisms that ensure that the resting level of Ca^{2+} is kept low (i.e. between 20 and 100 nmol l^{-1}) in order to avoid the cytotoxic effects of a prolonged elevation of [Ca^{2+}]. Cells have evolved a sophisticated signalling system based on the generation of brief pulses of Ca^{2+} which enables this ion to be used as a messenger, thus avoiding its toxic effects. Such Ca^{2+} spikes usually result from the coordinated release of Ca^{2+} from internal stores using either inositol 1,4,5-trisphosphate or ryanodine receptors. Using Ca^{2+} imaging techniques, the opening of individual channels has now been visualized and models have been proposed to explain how these elementary events are coordinated to generate the global Ca^{2+} signals that regulate cellular activity.

Key words: calcium, inositol trisphosphate, ryanodine receptors, calcium-induced calcium release, sparks, puffs, waves.

Introduction

The ubiquitous second messenger Ca^{2+} is responsible for regulating a wide range of cellular processes (Clapham, 1995). It is used at the beginning of life to mediate the process of fertilization and then is brought into play to regulate some of the cell cycle events during early development. As cells differentiate to perform specific functions, Ca^{2+} is once again called upon to regulate processes as diverse as muscle contraction, exocytosis, energy metabolism, chemotaxis and synaptic plasticity during learning and memory. Ca^{2+} is an unlikely candidate to perform this role of a universal messenger because prolonged elevations of [Ca^{2+}] result in irreversible damage as occurs during cardiac or cerebral ischaemia (Trump and Berezesky, 1995). Because of its cytotoxicity, the intracellular level of Ca^{2+} in resting cells is normally held within a narrow range of 20–100 nmol l^{-1}. The signalling functions of Ca^{2+} have to be performed against this background of a tightly controlled Ca^{2+} homeostasis.

Another consequence of this rigid homeostatic control over Ca^{2+} is that this messenger has a very low diffusibility in cytoplasm. Distributed throughout the cytoplasm is an extensive array of Ca^{2+} pumps (Carafoli, 1994) which rapidly sequester Ca^{2+}, thus restricting its diffusion. In order to overcome the twin problems of an inherent cytotoxicity and low diffusibility, cells have evolved an ingenious mechanism of signalling based on presenting Ca^{2+} as brief spikes often organized as regenerative waves (Cheek, 1991; Berridge, 1993; Clapham, 1995). To understand this spatiotemporal organization of Ca^{2+} signalling, it is necessary to describe the properties of the Ca^{2+} channels that regulate the entry of Ca^{2+} into the cytoplasm.

The structure and function of Ca^{2+} channels

Cells have access to two sources of signal Ca^{2+}. First, it can enter from the outside (Ca^{2+}_o). Ca^{2+} enters from the outside through a variety of channels such as the voltage-operated channels (VOCs), receptor-operated channels (ROCs) or store-operated channels (SOCs). Second, it can be released from internal stores (Ca^{2+}_s) (Fig. 1). Which of these sources is used varies somewhat from cell to cell. In most cells, it is the internal stores which provide most of the signal Ca^{2+} so attention has focused on the intracellular Ca^{2+} channels, of which there are two main types (Berridge, 1993; Clapham, 1995). First, there is the ryanodine receptor (RYR) family comprising three members: RYR1 found in skeletal muscle and certain neurones (e.g. Purkinje cells), RYR2 found in cardiac muscle, brain and some other cells, and RYR3 found in smooth muscle, brain and other cells (Bennett *et al.* 1996; Giannini *et al.* 1995). Second, the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) family has a number of members (Furuichi and Mikoshiba, 1995; Taylor and Traynor, 1995; Bezprozvanny and Ehrlich, 1995). There are four $InsP_3R$ genes, and further diversity results from alternative splicing. These two receptor families must have evolved from a common ancestor since they display considerable sequence homology which is matched by a number of physiological similarities, particularly with regard to the control of channel opening (Taylor and Traynor, 1995).

Cytosolic Ca^{2+} homeostasis in resting cells is achieved by balancing the leak of Ca^{2+} (entering from the outside or from the stores) by the constant removal of Ca^{2+} using pumps either on the plasma membrane or on the internal stores (Fig. 1). These pumps ensure that cytoplasmic [Ca^{2+}] remains low and that the stores are loaded with signal Ca^{2+}. The brief burst of

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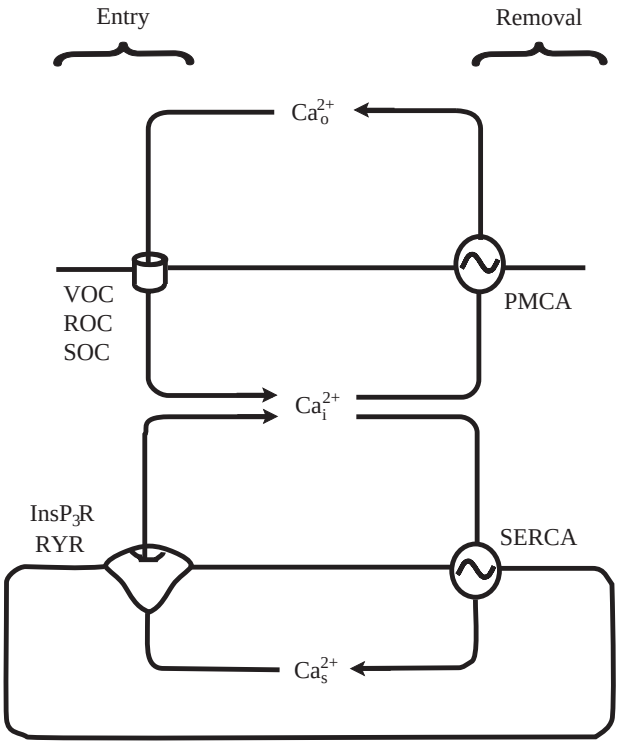

Fig. 1. Intracellular Ca^{2+} (Ca_i^{2+}) homeostasis is achieved by balancing entry and removal of both external Ca^{2+} (Ca_o^{2+}) and Ca^{2+} stored in the endoplasmic/sarcoplasmic reticulum (Ca_s^{2+}). VOC, voltage-operated channel; ROC, receptor-operated channel; SOC, store-operated channel; PMCA, plasma membrane Ca^{2+}-ATPase; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; $InsP_3R$, $InsP_3$ receptor; RYR, ryanodine receptor.

Ca^{2+} responsible for cell activation is usually produced by the coordinated opening of either the RYRs or the $InsP_3Rs$. Perhaps their most important property is their sensitivity to Ca^{2+}, i.e. they display the phenomenon of Ca^{2+}-induced Ca^{2+} release (CICR) which is of major significance for the generation of complex signals. Ca^{2+} has a biphasic effect on the RYRs and $InsP_3Rs$: as its concentration is increased, it initially exerts a positive feedback effect by enhancing the opening of the channels (i.e. CICR), but as soon as the concentration reaches a certain level the feedback switches from positive to negative and Ca^{2+} then inhibits the channel (Bezprozvanny and Ehrlich, 1995). This negative feedback effect ensures that just enough Ca^{2+} is released to give a meaningful signal, thus avoiding the cytoplasm from being swamped with this potentially cytotoxic agent.

The fact that Ca^{2+} release is regenerative has important implications for signalling because it provides one of the mechanisms for coordinating the activity of individual receptors, i.e. they can communicate with each other using Ca^{2+} as a messenger (Bootman and Berridge, 1995). A specific region within the cell usually functions as an initiation site in that it is the first to release Ca^{2+} which then diffuses outwards to excite neighbouring receptors, thereby setting up a Ca^{2+} wave. A global Ca^{2+} signal is created by coordinating release from all the receptors using Ca^{2+} as the messenger. A more specialized mechanism of coordination is found in skeletal and cardiac muscle, where the opening of the RYRs is tightly coupled to the action potential sweeping over the plasma membrane (Cannell *et al.* 1995; Lopez-Lopez *et al.* 1995).

Using a regenerative process is inherently dangerous because it is liable to be triggered by the stochastic opening of a single channel. To avoid such random triggering of regenerative Ca^{2+} waves, cells have developed mechanisms for regulating the excitability of these intracellular receptors such that they are turned off in resting cells but become increasingly excitable when Ca^{2+} signals are being generated. In the case of the $InsP_3Rs$, excitability is regulated by the agonist-dependent generation of $InsP_3$ by cell surface receptors. This $InsP_3$ binds to the $InsP_3Rs$, greatly enhancing their sensitivity to the stimulatory action of Ca^{2+}. In effect, the $InsP_3R$ is under the dual regulation of two agonists – $InsP_3$ and Ca^{2+}. The primary function of the former is to increase the Ca^{2+} sensitivity of the $InsP_3R$. Similarly, the RYR may also be under dual regulation, at least in some cell types (Lee, 1994; Galione and White, 1994). The putative second messenger cyclic ADP ribose (cADPR) is able to enhance the Ca^{2+} sensitivity of the RYRs.

In summary, through the ability of $InsP_3$ or cADPR to enhance the sensitivities of the $InsP_3Rs$ and RYRs respectively, these messengers convert the quiescent cytoplasm into an excitable medium in which these intracellular channels can communicate with each other to generate global Ca^{2+} signals.

Elementary events of Ca^{2+} signalling

Recent advances in image analysis using confocal microscopy have enabled the operation of either single or small groups of these intracellular channels to be visualized (Bootman and Berridge, 1995). The brief opening of these channels gives rise to localized pulses (approximately 2 μm in diameter) such as the sparks in cardiac muscle (Cheng *et al.* 1993) or the blips and puffs in *Xenopus* oocytes (Yao *et al.* 1995; Parker and Yao, 1996). These elementary events of Ca^{2+} signalling have a characteristic time course: the concentration of Ca^{2+} builds up rapidly but once the channel closes, because of the negative feedback effect described earlier, the concentration falls more slowly as the Ca^{2+} gradually disperses by passive diffusion (Fig. 2). Ca^{2+} channels in the plasma membrane display similar elementary events such as the bumps in *Drosophila* receptors (Hardie, 1991) or the quantum emission domains (QEDs) in squid giant synapses (Sugimori *et al.* 1994). Attention is now focused on how these elementary events contribute to various aspects of Ca^{2+} signalling.

Contribution to the resting level of Ca^{2+}

The spontaneous opening of Ca^{2+} channels has been observed in resting cells and this input of Ca^{2+} can contribute to the resting level of Ca^{2+}. In smooth muscle cells, the RYRs

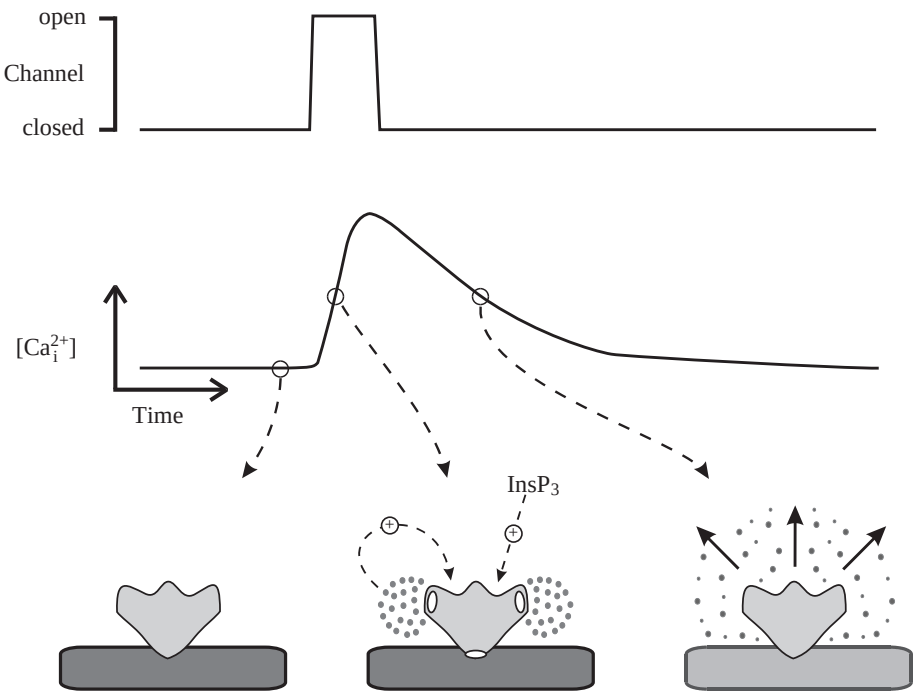

Fig. 2. Elementary event of Ca^{2+} signalling. The figure illustrates how the transient opening of an inositol 1,4,5-trisphosphate ($InsP_3$) receptor on the endoplasmic reticulum gives rise to an elementary event that is characterized by a rapid rising phase facilitated by the positive feedback effect of Ca^{2+}. Once the channel closes, the slower recovery phase is due to the passive diffusion of Ca^{2+} away from the channel.

display elementary events referred to as sparks (Nelson *et al.* 1995). When these sparks occur close to the plasma membrane, they act on Ca^{2+}-sensitive K^+ channels to produce a spontaneous transient outward current (STOC). The occurrence of STOCs in coronary smooth muscle was found to induce small fluctuations in the resting level of Ca^{2+} (Ganitkevich and Isenberg, 1996). Similarly, low levels of stimulation can increase the frequency of these elementary events, resulting in an increase in the resting level of Ca^{2+} as has been described in *Xenopus* oocytes (Parker and Yao, 1996) and in HeLa cells (Bootman and Berridge, 1996).

Localized action of elementary events

Evidence is beginning to emerge that elementary events might be capable of exerting a highly localized signalling function in addition to their role in contributing to the global elevation of Ca^{2+} levels described in the next section. An interesting case concerns the smooth muscle STOCs which can cause relaxation through membrane hyperpolarization (Nelson *et al.* 1995). What is remarkable, therefore, is that the same messenger Ca^{2+} is able to control both contraction and relaxation. This ability of Ca^{2+} to mediate opposing responses can be explained by the spatial organization of the Ca^{2+} signalling system. Localized high-concentration pulses of Ca^{2+} near the membrane cause relaxation, whereas contraction results from the global elevation of $[Ca^{2+}]$ that occurs when a large proportion of the channels release Ca^{2+} synchronously using one of the mechanisms described below.

Global Ca^{2+} signalling

The primary role of elementary events is to contribute to the global Ca^{2+} signals responsible for controlling the diverse range of cellular processes described earlier (Bootman and Berridge, 1995). Such global responses depend upon the temporal coordination of a sufficient number of these elementary events so that their individual contributions will sum to give an increase in the cytosolic level of Ca^{2+}. As mentioned earlier, cells have evolved two main coordinating mechanisms. First, the opening of RYRs in various muscle cells is synchronized by being tightly coupled to the action potential in the plasma membrane. Second, synchronization is achieved by the channels communicating with each other using Ca^{2+} as a coupling factor. These two mechanisms will be illustrated by describing some specific examples. The evoked release of Ca^{2+} in muscle depends upon the dihydropyridine receptor (DHPR) in the plasma membrane functioning as a voltage sensor which detects the depolarization and then transfers the information to the underlying RYRs. In skeletal muscle, the DHPRs are directly coupled to the RYR1s, and information is transferred by a process of conformational coupling (Tsugorka *et al.* 1995; Klein *et al.* 1996). The DHPR responds to membrane depolarization by undergoing a change in conformation which is transmitted to the RYR1, inducing the latter to gate Ca^{2+}. Through this tight coupling, the action potential is capable of a near simultaneous recruitment of all the RYR1s to give the explosive release of Ca^{2+} responsible for the contraction of skeletal muscle.

Since the DHPRs do not associate directly with the RYR2s in cardiac cells, coupling is achieved using Ca^{2+} as an intermediary. The action potential opens the DHPRs which gate a small pulse of Ca^{2+} that is then greatly amplified as it stimulates a small group of RYR2s (Cannell *et al.* 1995; Lopez-Lopez *et al.* 1995). This coupling unit consisting of one DHPR plus approximately four RYR2s represents an autonomous

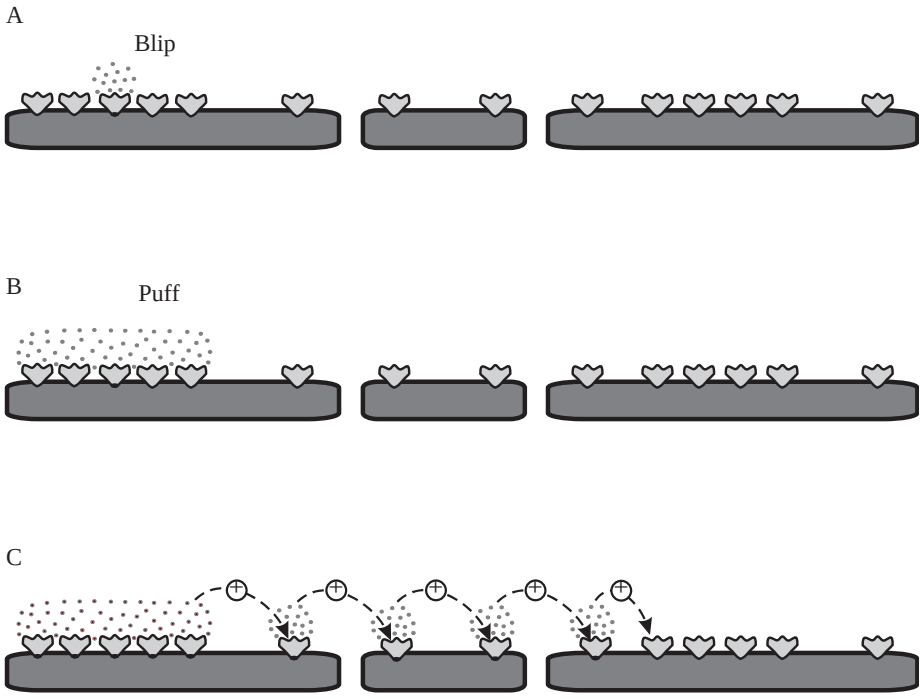

Fig. 3. Elementary and global events of Ca^{2+} signalling as exemplified in *Xenopus* oocytes. (A) At low $InsP_3$ concentrations, a few receptors release Ca^{2+} as elementary events referred to as blips (see Fig. 2). (B) At intermediate $InsP_3$ concentrations, groups of $InsP_3$ receptors release Ca^{2+} to form puffs which fail to spread because neighbouring receptors are less excitable. (C) At high concentrations of $InsP_3$, all the receptors are excitable and puffs then act as initiation sites to spawn a regenerative wave that spreads through the process of Ca^{2+}-induced Ca^{2+} release.

elementary event of cardiac Ca^{2+} signalling. Ca^{2+} imaging has revealed that these units can fire spontaneously and independently of each other to produce sparks which fail to activate neighbouring units because of their low Ca^{2+} sensitivities (Cheng *et al.* 1993; Lopez-Lopez *et al.* 1995; Cannell *et al.* 1995). The fact that these elementary events can function independently of each other explains the long-standing paradox that Ca^{2+} release in cardiac cells can be graded with membrane potential. For each unit, the process of CICR operates between the DHPRs and the RYR2s, whereas the latter do not communicate with each other. However, the RYRs in the separate units can begin to communicate with each other when the cardiac cell becomes overloaded with Ca^{2+} as a consequence of an increase in the sensitivity of the RYR2s. In effect, this Ca^{2+} overload converts the cytoplasm into an excitable medium such that a Ca^{2+} spark can ignite neighbouring units, thereby setting up a regenerative Ca^{2+} wave (Cheng *et al.* 1993). Although these Ca^{2+} waves in overloaded cardiac cells are somewhat abnormal and can result in cardiac arrhythmias, they represent the synchronization mechanism used normally by most other cells as described below.

A characteristic feature of the Ca^{2+} signal in many non-muscle cells is that it is often organized as a wave similar to that observed in overloaded cardiac cells. There appear to be pre-determined areas which act as initiation sites where the signal first appears before spreading through the cell as a wave. This behaviour has been well-characterized in *Xenopus* oocytes responding to increasing concentrations of $InsP_3$ (Fig. 3) (Yao *et al.* 1995; Parker and Yao, 1996). At very low $InsP_3$ concentrations, there is a gradual increase in the resting level of Ca^{2+} which seems to result from the random openings of individual $InsP_3$ receptors to give brief pulses of Ca^{2+} called blips (Parker and Yao, 1996). As described earlier, the appearance of these elementary events represents the first indication of an increase in excitability and is associated with an increase in the resting level of Ca^{2+} (Parker and Yao, 1996; Bootman and Berridge, 1996). With further increases in stimulation, the blips in *Xenopus* oocytes grow into larger puffs which represent the initiation sites for the onset of Ca^{2+} waves (Fig. 3B). Such waves represent the mechanism of coordinating the release of Ca^{2+} by all the $InsP_3$ receptors. If the latter are sufficiently sensitized, they will respond to the Ca^{2+} diffusing away from a puff site and thereby propagate the signal through a process of CICR (Fig. 3C).

Conclusion

Cells maintain a rigid control over the intracellular level of Ca^{2+}, thus ensuring that the level is kept low during periods of inactivity. In order to use Ca^{2+} as a messenger, cells overcome this tight homeostatic control by using sophisticated mechanisms to release Ca^{2+} in brief bursts using either $InsP_3$ or ryanodine receptors. These receptors display a unique autocatalytic process of Ca^{2+}-induced Ca^{2+} release (CICR) which overwhelms the normal homeostatic mechanisms by producing an explosive release of Ca^{2+} to give a brief pulse of Ca^{2+} often organized as a wave that sweeps through the cytoplasm. Such waves may occur repetitively, thus establishing an oscillation whose frequency is sensitive to agonist concentration. There is reason to believe, therefore, that Ca^{2+} signalling might be frequency-modulated. By encoding information in the form of brief all-or-none spikes, the cell can use Ca^{2+} as a messenger while retaining a tight control over Ca^{2+} homeostasis.

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