The organisation and functions of local Ca^{2+} signals

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Summary

Calcium (Ca^{2+}) is a ubiquitous intracellular messenger, controlling a diverse range of cellular processes, such as gene transcription, muscle contraction and cell proliferation. The ability of a simple ion such as Ca^{2+} to play a pivotal role in cell biology results from the facility that cells have to shape Ca^{2+} signals in space, time and amplitude. To generate and interpret the variety of observed Ca^{2+} signals, different cell types employ components selected from a Ca^{2+} signalling 'toolkit', which comprises an array of homeostatic and sensory mechanisms. By mixing and matching components from the toolkit, cells can obtain Ca^{2+} signals that suit their physiology. Recent studies have demonstrated the importance of local Ca^{2+} signals in defining the specificity of the interaction of Ca^{2+} with its targets. Furthermore, local Ca^{2+} signals are the triggers and building blocks for larger global signals that propagate throughout cells.

Key words: Calcium, Signalling, Channel

Introduction

Over the past decade, there has been an increasing awareness of local interactions between Ca^{2+} and its target molecules. The term 'local' has been variously used to describe microdomains of Ca^{2+} (dimensions ~10-100 nm) at the mouth of channels or larger subcellular Ca^{2+} signals spanning several micrometers. All of these signals are local in the sense that they are spatially restricted rises in Ca^{2+} concentration within a cell. However, the sources and targets of the Ca^{2+} signals are usually very different.

Most cells utilise Ca^{2+} influx from the extracellular space and Ca^{2+} release from intracellular stores to generate intracellular signals. When activated, both Ca^{2+} entry and Ca^{2+} release channels can give rise to brief pulses of Ca^{2+} that form a small plume around the mouth of the channel before diffusing into the cytoplasm (reviewed by Neher, 1998). Such signals can remain localised and activate effectors within the immediate vicinity of the channels, they can recruit effectors (for example, see Maasch et al., 2000) or they can be summated to yield global increases that propagate throughout and between cells. Local Ca^{2+} signals therefore serve to activate specific targets before diffusion or regenerative mechanisms spread the Ca^{2+} across a cell.

Non-excitable cells

Ca^{2+} puffs

A diverse array of local Ca^{2+} signals have been visualised in electrically non-excitable cells (Bootman, 1996). In addition, spatially restricted Ca^{2+} signals below the resolution of visible imaging techniques have been inferred from the activity of Ca^{2+}-dependent processes. One type of local Ca^{2+} signal that appears to operate in many, if not all, electrically non-excitable cells is 'Ca^{2+} puffs' (Fig. 1). These elementary events have amplitudes typically ranging from ~50-600 nM, a spatial spread of ~6 μm and a total duration of ~1 second. These Ca^{2+} puff dimensions indicate that there are <100 such sites in small somatic cells. Such events were first observed in *Xenopus* oocytes (for example, see Yao et al, 1995) but have subsequently been observed in HeLa cells (Bootman et al., 1997a), PC12 cells (Reber and Schindelholz, 1996; Koizumi et al., 1999) and endothelial cells (Hüser and Blatter, 1997). The non-stereotypic nature of Ca^{2+} puffs indicates that they probably arise from sites containing variable numbers of inositol 1,4,5-trisphosphate receptors (Ins(1,4,5)P_3Rs; Sun et al., 1998; Thomas et al., 1998). The temporally and spatially coordinated recruitment of Ca^{2+} puffs is responsible for the generation of repetitive Ca^{2+} waves and oscillations observed during hormonal stimulation (Bootman et al., 1997b; Marchant et al., 1999; reviewed by Berridge, 1997). Although different non-excitable cell types give rise to distinct global Ca^{2+} spikes, similar Ca^{2+} puffs can be recorded from various cell lines expressing different combinations of the three Ins(1,4,5)P_3R isoforms (Tovey et al., 2000). This suggests that cell-specific recruitment of a generic elementary signal underlies different global signals.

In HeLa cells (Thomas et al., 2000) and *Xenopus* oocytes (Marchant and Parker, 2001), Ca^{2+} puff sites that have a higher sensitivity to Ins(1,4,5)P_3 consistently trigger Ca^{2+} waves (Fig. 1). What gives these pacemaking Ca^{2+} puff sites their enhanced sensitivity is unclear. In the case of somatic cells, the pacemaker sites tend to be distributed in a perinuclear region (Lipp et al., 1997), which raises the possibility that they can send Ca^{2+} signals specifically into the nucleus.

Apical Ca^{2+} spikes in pancreatic acinar cells

Another well-known local Ca^{2+} signal occurs in the apical region of secretory cells such as pancreatic acinar cells (reviewed by Petersen et al., 1999). In common with the pacemaker sites described above, the Ins(1,4,5)P_3Rs that underlie the apical Ca^{2+} spikes are distinguished by a heightened sensitivity to Ins(1,4,5)P_3 (Fogarty et al., 2000).

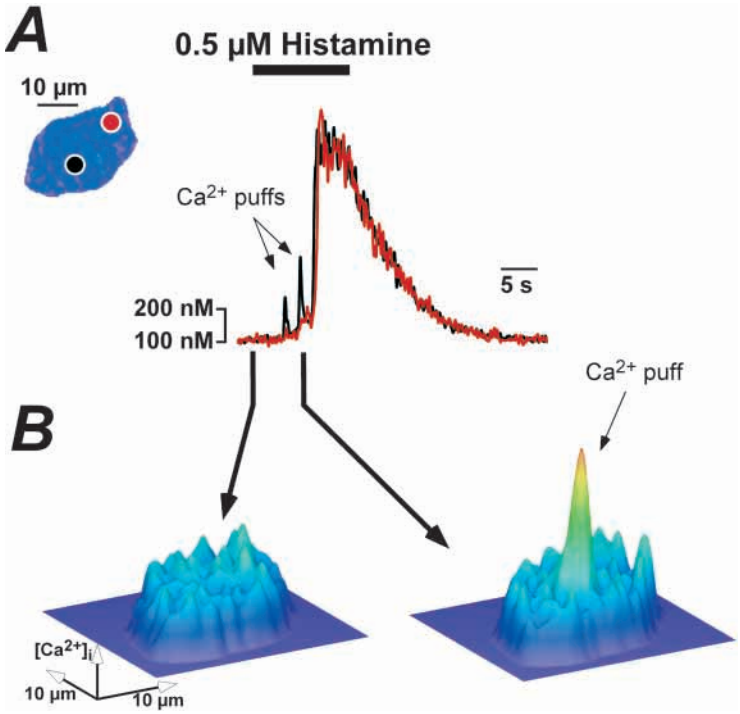

Fig. 1. Ca^{2+} puffs in a HeLa cell. The black and red traces in A show the onset of a Ca^{2+} wave in a single histamine-stimulated HeLa cell. The Ca^{2+} puffs are visible prior to the Ca^{2+} wave. The spatial profile of a Ca^{2+} puff is indicated by the surface plot in B. Ca^{2+} concentration is coded by the height and colour of the surface. The black and red traces in A were obtained by averaging the Ca^{2+} concentration over the regions marked by the correspondingly coloured circles on the inset cell image. Modified figure reproduced, with permission, from Thomas et al., 2000.

Like Ca^{2+} puffs, such apical Ca^{2+} spikes probably arise from the coordinated release of Ca^{2+} from multiple Ca^{2+}-release channels (Kidd et al., 1999). However, their slow rise time (>1 second; Kidd et al., 1999) and large spatial spread (~10 μm; Kidd et al., 1999) distinguishes them from the Ca^{2+} puffs in HeLa cells and *Xenopus* oocytes. Recent evidence indicates that the apical spikes arise from a stimulus-dependent hierarchical activation of different types of Ca^{2+}-release channel (Cancela et al., 1999; Cancela et al., 2000).

When cells are stimulated to low levels, the Ca^{2+} spikes stay restricted to the apical pole of the acinar cells, where they can activate ion channels and trigger limited secretion. Greater stimulation causes the Ca^{2+} spikes to trigger Ca^{2+} waves that propagate towards the basal pole. The restriction of the Ca^{2+} signal to the apical pole appears to be due in part to a 'firewall' of mitochondria that buffer Ca^{2+} as it diffuses from the apical pole and prevent the activation of ryanodine receptors (RyRs) in the basal pole (Tinel et al,. 2000; Straub et al., 2000).

Local signalling via Ca^{2+} entry

In addition to local Ca^{2+} signals arising through release from internal stores, Ca^{2+} entry can give rise to local signals with specific functions in electrically non-excitable cells. Cooper and colleagues have shown that both Ca^{2+}-activated and Ca^{2+}-inhibited isozymes of adenylyl cyclase are more sensitive to influx through store-operated Ca^{2+} channels (SOCC) than to Ca^{2+} release (Fagan et al., 2000a; Fagan et al., 2000b). Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOCCs; Fagan et al., 2000b) or a novel arachidonic acid-activated Ca^{2+} channel (Shuttleworth and Thompson, 1999) does not modulate adenylyl cyclase activity to the same degree as SOCC entry. These data point to a close localisation of SOCC channels and adenylyl cyclases. One way in which this coupling might be achieved is through the co-localisation of SOCCs and target enzymes in specific cellular structures such as caveolae. These 'flask-shaped' invaginations of the plasma membrane have been proposed to contain Ca^{2+}-entry channels and Ins(1,4,5)P_3Rs (reviewed by Isshiki and Anderson, 1999). In addition to regulating adenylyl cyclase, localisation of Ca^{2+} entry to caveolae could underlie the specific requirement for Ca^{2+} entry for activation of endothelial nitric oxide synthase (Paltauf-Doburzynska et al., 1998; Lin et al., 2000).

Mitochondria sense local Ca^{2+} signals

Sequestration of Ca^{2+} signals by mitochondria (by both electrically excitable and non-excitable cells) serves at least two essential purposes: buffering of cytosolic Ca^{2+} loads and consequent activation of citric acid enzymes (for example, see Robb-Gaspers et al., 1998; reviewed by Duchen, 1999). Mitochondria sequester Ca^{2+} through a low-affinity, high-speed uniporter powered by the mitochondrial membrane potential. The in vitro affinity of the uniporter for Ca^{2+} is ~10 μM, which is lower than the global Ca^{2+} concentrations observed in vivo during physiological responses. However, mitochondria have been shown to sense and modulate both Ca^{2+} entry and Ca^{2+} release (for example, see Hoth et al., 1997; Sheppard et al., 1997; Landolfi et al., 1998; Hajnoczky et al., 1999; Collins et al., 2000a). To explain the paradox that mitochondria accumulate Ca^{2+} during physiological responses despite the insensitivity of the Ca^{2+} uniporter, it has been suggested that mitochondria are located in close proximity to sites of either Ca^{2+} release or Ca^{2+} entry (for example, see Rizzuto et al., 1998; Csordás et al., 1999). In these locations, mitochondria can respond to Ca^{2+} changes within the microdomains around the mouth of Ca^{2+} channels.

It is therefore generally thought that microdomains of cytosolic Ca^{2+} may be the most efficient way of increasing mitochondrial matrix Ca^{2+} concentration. However, several studies have shown that modest cytosolic Ca^{2+} increases that do not form microdomains can also substantially increase mitochondrial Ca^{2+} levels (for example, see Collins et al., 2000b; Colegrove et al., 2000; Collins et al., 2001). Furthermore, mitochondrial Ca^{2+} uptake shows a steep dependence on the cytosolic Ca^{2+} concentration and might therefore facilitate its own sequestration (for example, see Colegrove et al., 2000; Collins et al., 2001; reviewed by Gunter et al., 1994).

Excitable cells

Cardiac and skeletal muscle

In heart and skeletal muscle, release of Ca^{2+} from the sarcoplasmic reticulum (SR) by RyRs is the key event linking membrane depolarisation and mechanical activity during

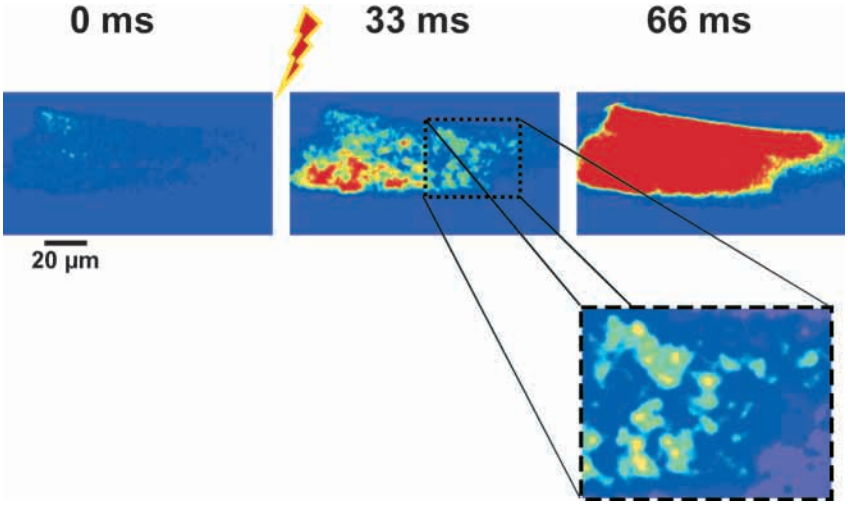

Fig. 2. Ca^{2+} sparks underlie global Ca^{2+} signals in cardiac myocytes. The figure shows a sequence of images of the same ventricular cardiomyocyte taken at 33 millisecond intervals. The left-hand image shows the cell with a low resting Ca^{2+} concentration prior to stimulation. The right-hand image shows that 66 milliseconds after electrical stimulation (marked with 'lightening zap' symbol) the cell shows a homogenous Ca^{2+} increase. On the rising phase (middle image), the Ca^{2+} increase can be seen as many focal Ca^{2+} increases (Ca^{2+} sparks).

excitation-contraction coupling. RyRs occur in clusters that give rise to localised Ca^{2+}-release events denoted 'Ca^{2+} sparks'. These events are analogous to the Ca^{2+} puffs described above, although they are usually faster in onset and decline, and have a more restricted spread (~1-3 μm). Spatio-temporal recruitment of Ca^{2+} sparks underlies the global Ca^{2+} signals that subsequently activate myocyte contraction (Fig. 2; reviewed by Cannell and Soeller, 1998; Niggli, 1999). The failure to recruit Ca^{2+} sparks appropriately can lead to defective excitation-contraction coupling in cardiac cells (Gomez et al., 1997).

In addition to forming the global Ca^{2+} transient underlying contraction, Ca^{2+} sparks can also cause depolarisation of cardiac cells and thereby enhance or corrupt the rhythm of the heart. An example of this is in primary pacemaker cells, which maintain the normal heartbeat. The electrical activity of cardiac pacemaking cells in the sino-atrial node depends on the interplay of several different sarcolemmal ion channels. In addition, Ca^{2+} release from the SR can also affect the frequency of generating action potentials (Rigg and Terrar, 1996). Hüser and colleagues observed that, following recovery from a previous action potential, there is a gradual increase in cytosolic Ca^{2+} concentration until the next depolarisation (Hüser et al., 2000). Imaging this slow Ca^{2+} rise revealed that it is due to the summation of infrequent subsarcolemmal Ca^{2+} sparks. Essentially, low-voltage-activated T-type Ca^{2+} channels provide a trigger Ca^{2+}-influx current that evokes Ca^{2+} spark activity. In turn, the progressive increase in cytoplasmic Ca^{2+} caused by the Ca^{2+} sparks promotes electrogenic forward-mode Na^+/Ca^{2+} exchange, and the resultant inward current drives the cell towards the threshold for depolarisation (Fig. 3).

A similar situation occurs in atrial myocytes stimulated with hormones that activate phospholipase C (PLC). Incubation of electrically-paced atrial myocytes with hormones such as endothelin 1 or phenylephrine causes the appearance of spontaneous subsarcolemmal Ca^{2+} sparks (Lipp et al., 1999a; Lipp et al., 1999b), which are probably due to the activation of Ins(1,4,5)P_3Rs that co-localise with RyRs in these cells (Lipp et al., 2000). If the Ca^{2+} spark frequency is sufficient, a spontaneous action potential can be evoked.

These two examples of Ca^{2+} sparks driving myocyte depolarisation are entirely analogous. In both situations, a small trigger Ca^{2+} current (either from T-type Ca^{2+} channels or Ins(1,4,5)P_3Rs) activates Ca^{2+} sparks that lead to enhancement of Na^+/Ca^{2+} exchange (Fig. 3). Because of the strategic firing of subsarcolemmal Ca^{2+} spark sites, only a few such events may be necessary to drive a cell to the threshold for depolarisation (Hüser et al., 2000). The ability of a few Ca^{2+} sparks to enhance cardiac automaticity – that is, to increase the frequency of spontaneous action potentials – has potentially serious implications for the generation of cardiac arrhythmias and sudden heart failure.

The generation of spontaneous action potentials, and therefore a global Ca^{2+} response, by the Ca^{2+} sparks is analogous to the situation whereby Ca^{2+} puffs trigger regenerative Ca^{2+} waves in non-excitable cells (see above). Although the mechanisms involved are distinct, in both cases the infrequent firing of a limited number of elementary Ca^{2+}-release sites leads to larger regenerative Ca^{2+} signals.

Smooth muscle – STOCs, STICs and STOICs

Another cell type in which local Ca^{2+} signals have a clear role is smooth muscle. Paradoxically, Ca^{2+} can activate smooth muscle contraction, but it can also inhibit contraction by causing hyperpolarisation of the sarcolemma (reviewed by Jaggar et al., 2000). The different effects of Ca^{2+} in smooth muscle are essentially determined by the spatial properties of the Ca^{2+} signal; global responses induce vasocontraction by activating Ca^{2+}/calmodulin-dependent enzymes, whereas subsarcolemmal Ca^{2+} sparks promote vasorelaxation by activating Ca^{2+}-dependent plasma membrane ion channels (Nelson et al., 1995).

It is well established that the Ca^{2+} sparks in smooth muscle can activate K^+ and Cl^- conductances, and thus give rise to brief currents known as STOCs (Spontaneous Transient Outward Currents; K^+ current), STICs (Spontaneous Transient Inward Current; Cl^- current) and STOICs (mixed K^+ and Cl^- currents). STOCs have been measured in a wide variety of smooth muscle cell types and serve to hyperpolarise the cell membrane by ~20 mV. They primarily arise through activation of large-conductance Ca^{2+}-activated K^+ channels (BK channels). These Ca^{2+}-activated channels have a low sensitivity to cytosolic Ca^{2+}, requiring concentrations of >1 μM for significant activity (although their actual sensitivity also depends on membrane

potential). It has been proposed that the BK channels are closely apposed to Ca^{2+} spark sites and sense rapid step-like Ca^{2+} changes during RyR activation (Fig. 4; ZhuGe et al., 2000). Walsh and co-workers have estimated that STOCs reflect the activation of a cluster of ~15 BK channels on the sarcolemma, although the actual BK-channel:RyR stoichiometry may be variable (ZhuGe et al., 2000).

The global Ca^{2+} signals that activate smooth muscle cell contraction are largely due to activation of L-type VOCCs. Unlike cardiac muscle, in which Ca^{2+} influx also occurs through L-type VOCCs, there is little amplification of the entry signal by Ca^{2+}-induced Ca^{2+} release (CICR) in most smooth muscle cell types (Jaggar et al., 2000), which means that few subplasmalemmal Ca^{2+} spark sites are recruited. Local and global Ca^{2+} signalling in smooth muscle cells is therefore segregated in terms of both source and function.

The dramatic effect of a few Ca^{2+} sparks on the automaticity of atrial cardiomyocytes (see above) is mimicked in smooth muscle cells, in which the membrane potential can be regulated by STOCs occurring at low frequencies (~1 Hz; Nelson et al., 1995). Pharmacological inhibition of either BK channels or RyRs leads to cellular depolarisation and vasoconstriction. The modulation of vascular tone by vasoactive messengers might be largely due to changes in Ca^{2+} spark frequency (Jaggar et al., 2000). In some smooth muscle cell types, only one or a few 'frequent-discharge sites' are responsible for generating the Ca^{2+} sparks that activate STOCs (Gordienko et al., 2001). In addition to showing repetitive Ca^{2+} sparks, these sites also activate Ca^{2+} waves and may be analogous to the pacemaking Ca^{2+} puff sites in non-excitable cells (see above).

The BK channels are closely apposed to Ca^{2+} sparks sites to ensure that they can sense the narrow plume of Ca^{2+} around the mouth of the RyRs. In addition, the Ca^{2+}-activated K^+ channels express subunits that 'tune' their Ca^{2+} sensitivity and enable them to respond to Ca^{2+} sparks. BK channels comprise channel-forming α and auxilliary β subunits. Murine smooth muscle cells lacking the β subunit exhibit reduced sensitivity of BK channels such that they barely respond to the on-going Ca^{2+} sparks (Brenner et al., 2000; Pluger et al., 2000). In addition, the β-subunit-deficient animals display an elevated mean arterial blood pressure and cardiac hypertrophy.

Ca^{2+}-activated Cl^- channels are not as widely expressed in smooth muscle types, compared with BK channels. The STICs produced by the Ca^{2+}-activated Cl^- channels have an essentially opposite effect to that of STOCs in that they serve to depolarise smooth muscle cells (Fig. 4; Jaggar et al., 2000). The STIC channels are approximately one order of magnitude more sensitive to cytosolic Ca^{2+} than are STOC channels. This means that their activity more closely follows the decay of the Ca^{2+} spark than a STOC. In cells expressing both Ca^{2+}-activated K^+ and Cl^- channels, mixed events denoted 'STOICs' have been recorded (ZhuGe et al., 1998). The ambient membrane potential reciprocally controls the flux through the Ca^{2+}-activated K^+ and Cl^- channels. This suggests that such STOICs might function to stabilise membrane potential (ZhuGe et al., 1998).

Most smooth muscle cells express both RyRs and Ins(1,4,5)P_3Rs. The relative contributions of these two channels to Ca^{2+} release is not entirely clear. In ureteric smooth muscle, the elementary events are 'Ca^{2+} puffs' derived solely from Ins(1,4,5)P_3Rs (Boittin et al., 2000). In contrast, the Ins(1,4,5)P_3Rs in pulmonary artery cells do not appear to form clusters that can produce significant elementary events (Janiak et al., 2001). Instead, Ins(1,4,5)P_3Rs appear to reside within a cluster of RyRs, so that activation of Ins(1,4,5)P_3Rs gives rise to an event whose characteristics are more similar to those of Ca^{2+} sparks (Boittin et al., 1998). A similar crosstalk between Ins(1,4,5)P_3Rs and RyRs to give 'mixed' elementary events has also been described in neuronal (Koizumi et al., 1999) and cardiac (Lipp et al., 2000) cells.

The Ca^{2+} puffs observed during purinergic stimulation of colonic myocytes stimulate STOCs, which suggests that Ins(1,4,5)P_3Rs and RyRs are functionally equivalent in generating local subplasmalemmal Ca^{2+} signals that regulate smooth muscle tone (Bayguinov et al., 2000). However, in contrast to purinergic stimulation of colonic myocytes, purinergic stimulation of cerebral artery smooth muscle cells causes vasoconstriction. In both colonic myocytes and cerebral artery smooth muscle cells, the purinergic agonists are coupled via P_{2Y} receptors to PLC. The difference between the responses appears to be the relative actions of Ins(1,4,5)P_3 and protein kinase C (PKC), the downstream effectors of PLC. In the colonic myocytes, Ins(1,4,5)P_3 dominates and increases the frequency of Ca^{2+} puffs and associated STOCs, thus causing relaxation (Bayguinov et al., 2000). In cerebral artery smooth muscle cells, PKC-dependent desensitisation of RyRs causes a drop in the frequencies of Ca^{2+} sparks and STOCs, leading to contraction (Jaggar and Nelson, 2000). The effect of PLC on smooth muscle activity is therefore cell type dependent. Even more confusing are the observations that within the same smooth muscle cell type different PLC-linked agonists can cause contraction or relaxation because of their differential regulation of STOCs (Bayguinov et al., 2001).

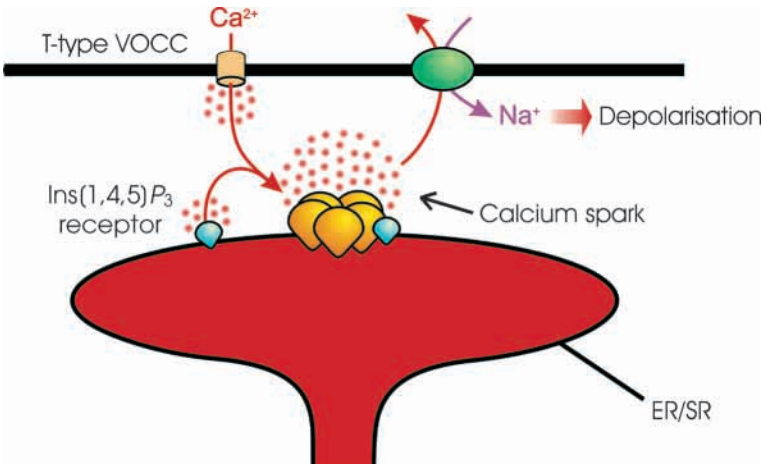

Fig. 3. Triggering Ca^{2+} sparks by T-type VOCCs or Ins(1,4,5)P_3Rs. This figure illustrates the activation of spontaneous Ca^{2+} sparks by T-type VOCCs in primary cardiac pacemaker cells and Ins(1,4,5)P_3Rs in atrial cells. RyRs and Ins(1,4,5)P_3Rs are coloured orange and blue, respectively. The Na^+/Ca^{2+} exchanger is green.

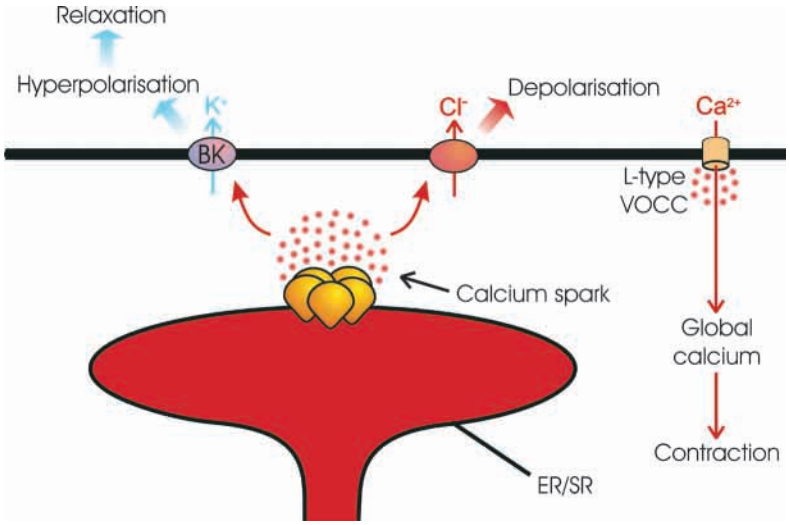

Fig. 4. Activation of STOCs and STICs by Ca^{2+} sparks in smooth muscle. The Ca^{2+} spark originating from RyRs can activate BK channels, Cl^- channels or both (producing a STOIC). Ca^{2+} entry via L-type VOCCs does not recruit such Ca^{2+} spark sites but leads to global Ca^{2+} increases that trigger contraction.

Neurons

Local Ca^{2+} signalling in dendritic spines

Subcellular Ca^{2+} signalling is the forte of neuronal cells. Because of their often intricate morphologies and the selective distribution of Ca^{2+} channels, neurons produce a dazzling array of local Ca^{2+} signals. Synaptic input can produce a hierarchy of Ca^{2+} signals. These can range from single spines or portions of the dendritic tree, up to responses that invade the soma (reviewed by Denk et al., 1996).

Ca^{2+} signals within spines are akin to elementary events in other cell types in that they are spatially constrained, short-lived responses that can trigger further propagation of a Ca^{2+} wave, depending on the underlying excitability of the cell (reviewed by Berridge, 1998). Spine morphology and number can be dramatically affected by Ca^{2+} signals that occur within their tiny volumes (reviewed by Segal, 2001).

Ca^{2+} signalling within spines underlies changes in synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD). Paradoxically, Ca^{2+} is implicated in both stimulating and depressing the transmission of nervous signals. It seems that subtle changes in the amplitude, spatial or temporal arrangement of Ca^{2+} signals account for the different actions of Ca^{2+} in synaptic plasticity (reviewed by Yuste et al., 2000). In particular, the need for coincident signals can dictate the resulting change in synaptic coupling. LTD in cerebellar Purkinje fibres, for example, requires Ca^{2+} release from Ins(1,4,5)P_3R within the spines in conjunction with a large Ca^{2+} signal emanating from the activation of VOCCs. Knockout mice that lack type 1 Ins(1,4,5)P_3Rs in their Purkinje cells do not display LTD in response to a standard stimulation protocol (Inoue et al., 1998). Furthermore, Purkinje neurons lacking the motor protein myosin Va, which express Ins(1,4,5)P_3Rs in dendritic shafts but not in spines, also fail to show LTD (Miyata et al., 2000). Although the Ins(1,4,5)P_3Rs in the dendrites are <1 μm distant from those in the spines, they are not functionally equivalent. A complex interplay between local Ca^{2+} signals appears to underlie the polarity and spread of synaptic modification in CA1 hippocampal neurons (Nishiyama et al., 2000). A relay of Ca^{2+} between Ca^{2+}-influx channels, RyRs and Ins(1,4,5)P_3Rs within the same microdomain determines whether LTD or LTP is observed following synaptic stimulation (but see Fujii et al., 2000).

The production of Ins(1,4,5)P_3 in spines is due to activation of metabotropic glutamate receptors, which are generally located on the periphery of these structures (reviewed by Blackstone and Sheng, 1999). The glutamate receptors are physically linked with Ins(1,4,5)P_3Rs through members of the Homer protein family (Tu et al., 1998). By virtue of their coiled-coil domains, these proteins can multimerise and link other proteins bearing a proline-rich motif (found in Ins(1,4,5)P_3Rs, RyRs and other signalling proteins). One member of the Homer family, Homer 1a, does not posses a coiled-coil domain, and can disrupt the coupling between glutamate receptors and Ins(1,4,5)P_3Rs, which results in reduced Ca^{2+}-release signals (Tu et al., 1998). Interestingly, *Homer 1a* is an immediate early gene that is rapidly transcribed after synaptic activity. The induction of this protein therefore provides an activity-dependent mechanism for regulating local Ca^{2+} release.

Local Ca^{2+} signals underlying repolarisation and hyperpolarisation of neurons

As in smooth muscle cells, neurons express Ca^{2+}-activated K^+ channels that repolarise or hyperpolarise the plasma membrane following an action potential. Events analogous to STOCs have been recorded in neuronal cell types. These spontaneous miniature outward currents (SMOCs) are probably also generated by Ca^{2+} sparks firing in close proximity to the plasma membrane (Berridge, 1998). Elementary Ca^{2+} signals analogous to Ca^{2+} sparks and Ca^{2+} puffs have also been observed in the neurites of PC12 cells and hippocampal neurons (Koizumi et al., 1999)

A significant function of the Ca^{2+}-activated K^+ channels in neuronal cells is to promote repolarisation of the cell after an action potential and to regulate the frequency of firing action potentials. Recent studies have illustrated complex relationships between VOCCs, Ca^{2+}-release channels and Ca^{2+}-activated K^+ channels in different types of neurons (for a review on the subcellular distribution of VOCCs in neurons, see Caterall, 1998). In the soma of hippocampal pyramidal neurons, N-type VOCCs give a coincident activation of BK channels (Marrion and Tavalin, 1998). The communication between N-type VOCCs and BK channels is not inhibited by the rapid Ca^{2+} buffer BAPTA, which indicates that these two types of channel are probably within 30 nm of each other. In contrast, L-type VOCCs are associated with small-conductance Ca^{2+}-activated K^+ channels (SK channels) and are estimated to be ~50-150 nm apart (Marrion and Tavalin, 1998). The close coupling of BK channels with N-type VOCCs allows them to be rapidly activated and cause cellular repolarisation. The spatial linkage between SK channels and L-type VOCCs is

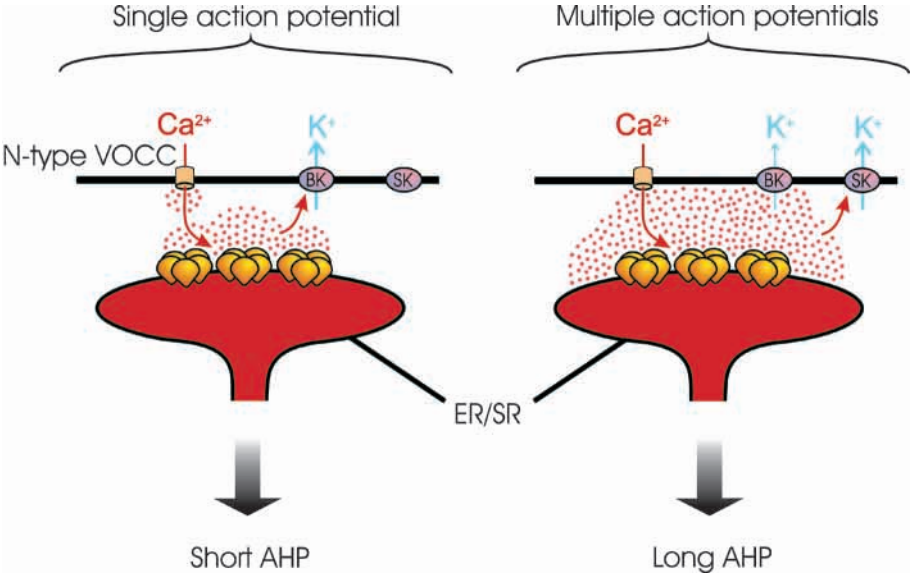

Fig. 5. Modulation of the after hyperpolarisation (AHP) by Ca^{2+} release. The figure illustrates the consequences of single or multiple action potentials on the activation of BK and SK channels in bullfrog sympathetic neurons. See text for details. Modified figure reproduced, with permission, from Akita and Kuba, 2000.

more intruiging. The SK channels are responsible for the 'slow after hyperpolarisation' (sAHP) – a period in which the cell remains hyperpolarised and action potential firing is inhibited. Although the close proximity of L-type VOCCs and SK channels allows communication between them, the AHP develops relatively slowly, and usually when the cell membrane has a negative potential (Berridge, 1998). In this case, the L-type Ca^{2+} channels must change their behaviour to be activated at negative membrane potentials, or the SK channels need to rely on local Ca^{2+} release from intracellular stores to generate the sAHP.

The involvement of intracellular Ca^{2+} release in the generation of AHPs seems clear (Berridge, 1998), but there can be an intricate interplay in the microdomains connecting the Ca^{2+} channels and K^+ channels that modulate the membrane potential. In bullfrog sympathetic neurons, N-type VOCCs open during a single action potential and lead to the subsequent activation of BK channels that are responsible for repolarisation (Akita and Kuba, 2000). However, it appears that the Ca^{2+} signal from the VOCCs to the BK channels is relayed via RyRs, since inhibitors of CICR depress the rate of repolarisation and the extent of the subsequent AHP (Fig. 5). The situation is more complex when multiple action potentials are evoked, in that the AHP becomes less dependent on BK channels, and instead SK channels are recruited following RyR activation (Akita and Kuba, 2000). Akita and Kuba estimated that BK channels, RyRs and N-type VOCCs are within a ~100 nm domain and that the SK channels are outside this region. The switch from BK to SK channels following multiple action potentials probably reflects the progressive diffusion of Ca^{2+} from the N-type VOCCs and RyRs beyond the 100 nm domain (for a detailed discussion see Marchant and Parker, 2000). The SK channels can respond to a more diffuse Ca^{2+} signal, since they have a high sensitivity to Ca^{2+}, compared with BK channels (Hirschberg et al., 1999).

The regulation of Ca^{2+}-sensitive ion channels by local Ca^{2+}-release signals in neurons and smooth muscle depends on the close apposition of Ca^{2+}-release channels to their targets on the plasma membrane. In these cell types, projections of the ER/SR bring the Ca^{2+}-release channels within 10-20 nm of the cell boundary (Berridge, 1998; Jaggar et al., 2000). These structures are akin to the diadic junctions of cardiac muscle, in which RyRs are brought to within ~15 nm of sarcolemma (Cannell and Soeller, 1998). However, a significant difference between cardiac and neuronal/smooth-muscle cells is the degree of regenerativity of Ca^{2+} signals following activation of VOCCs. In cardiac myocytes, a large fraction of global Ca^{2+} signals is due to Ca^{2+} release (i.e. recruitment of Ca^{2+} sparks). By contrast, in neuronal and smooth muscle cells, the degree of regenerative Ca^{2+} release is variable, and when it does occur it can lag behind the Ca^{2+} influx by hundreds of milliseconds (Berridge, 1998; Jaggar et al., 2000). These data suggest that the coupling between L-type VOCCs and Ca^{2+}-release channels in cardiac cells is much tighter than in neuronal and smooth muscle cells. Alternatively, loose coupling might introduce plasticity into the response. In both neuronal and smooth muscle cells, the Ca^{2+} stores can retain a 'memory' of previous stimulation, and Ca^{2+} release can be modulated by a variety of different factors (for example, see Andreasen and Lambert, 1995; Berridge, 1998; Jaggar et al., 2000).

The effect of T-type VOCCs on excitation-contraction coupling in guinea pig cardiac myocytes functionally resembles the loose coupling observed between VOCCs and Ca^{2+}-release channels in neurons and smooth muscle. For the same Ca^{2+} current, T-type VOCCs activate much less CICR than does L-type VOCCs (Sipido et al., 1998), which suggests that they are not localised within the diadic junctions. However, blockade of the T-type VOCCs does cause a progressive decrease of the amplitude of action-potential-induced Ca^{2+} transients (Sipido et al., 1998). It is plausible that these channels are expressed outside the diadic junctions and are not needed for excitation-contraction coupling but function to refill depleted Ca^{2+} stores.

Local Ca^{2+} signalling and neurotransmitter release

Local Ca^{2+} signals control the release of neurotransmitter in the active zones of pre-synaptic nerve terminals and neuroendocrine cells (Neher, 1998). In rat cortical synaptosomes, release of small synaptic vesicles (SSVs) containing amino acids (glutamate and GABA) and large dense-core vesicles (LDCVs) containing neuropeptide (cholecystokinin) can be activated by N-, P- and Q-type VOCCs. However, these channels are not equivalent in their efficacy of releasing SSVs and LDCVs: P-type VOCCs preferentially release SSVs, whereas LDCVs are more sensitive to Ca^{2+} influx through Q-type channels (Leenders et al., 1999). Such selectivity between VOCCs and exocytotic

vesicles is reminiscent of the linkage between VOCCs and BK/SK channels described earlier. The SSVs are released from the active zones of pre-synaptic nerve terminals, where they can be pre-docked and ready for fusion. In these locations, they are ideally placed to sense the high-amplitude transient Ca^{2+} microdomains upon activation of VOCCs (for example, see DiGregorio et al., 1999). LDCVs, in contrast, are more distant from the active zone, and their exocytosis requires prolonged depolarisation (Leenders et al., 1999). The significance of the relative distances of these vesicle types from the active zone is that, for the same Ca^{2+} signal, there will be a different secretory output. A single action potential, for example, will release SSVs but have little effect on LDCVs.

Ca^{2+} release from intracellular stores can also trigger exocytosis of neurotransmitters from pre-synaptic junctions (Emptage et al., 2001). Release of Ca^{2+} from stores in presynaptic boutons contributes to the enhancement of neurotransmitter release with repetitive stimulation (Emptage et al., 2001). In addition, spontaneous Ca^{2+}-release events, akin to Ca^{2+} sparks and puffs, were recorded in synaptic boutons and appear to be responsible for unevoked synaptic signals. The continual release of neurotransmitter by such spontaneous events could cause long-term changes in strength of synaptic communication (Emptage et al., 2001).

Since the profile of a local Ca^{2+} signal falls dramatically with distance from the source (Neher, 1998), activation of exocytosis by release of Ca^{2+} from internal stores critically relies on the close apposition of elements of the ER/SR and the secretory machinery (reviewed by Tse and Tse, 1999).

Local Ca^{2+} signalling controls neuronal growth

Many neuronal cells display spontaneous Ca^{2+} signals necessary for growth, migration and differentiation (for example, see Komuro and Rakic, 1996; Owens and Kriegstein, 1998; Gomez and Spitzer, 2000; reviewed by Spitzer et al., 2000). In particular, it is well established that Ca^{2+} regulates the motility and directionality of neuronal growth cones. However, recent studies have shown that it is not always easy to predict the consequence of a rise in Ca^{2+} levels on growth cone behaviour. Large rises can cause growth cone collapse, whereas more modest signals can either slow or promote neurite outgrowth (Spitzer et al., 2000). Using localised release of caged Ca^{2+}, Zheng showed that growth cones turn into or away from a focal Ca^{2+} source (Zheng, 2000). Local gradients of Ca^{2+} might also underlie the turning responses of growth cones to netrin 1, a molecule involved in axonal pathfinding in vivo (Hong et al., 2000). Interestingly, such local gradients must be set up by both Ca^{2+} influx and Ca^{2+} release, since abolition of turning responses occurs only if both of these Ca^{2+} sources are removed. Furthermore, whether a Ca^{2+} increase evoked attraction or repulsion depended on the basal level of Ca^{2+} (Zheng, 2000) and the activity of protein kinase A (Hong et al., 2000). The ability of such local Ca^{2+} signals to activate growth cone turning may be due to restricted rearrangement of the cytoskeleton on one side of a growth cone, perhaps by activation of the Ca^{2+}-dependent phosphatase calcineurin (Lautermilch and Spitzer, 2000).

Just how the local Ca^{2+} signals are generated by growth cones is unclear. One suggestion is that they are due to activation of VOCCs at resting membrane potential by the action of arachidonic acid (Archer et al., 1999). Exogenous application of arachidonic acid can stimulate axon outgrowth that is blocked by L-type VOCC channel antagonists. Interestingly, in dorsal root ganglia, the Ca^{2+} signal resulting from arachidonic-acid-mediated VOCC activation cannot be visualised by imaging, but is blocked by BAPTA, which suggests that it is localised to a diameter of tens of nanometres (Archer et al., 1999).

The finger-like filopodia that project from growth cones can also relay local Ca^{2+} signals to regulate the direction of neurite outgrowth. Spitzer and colleagues have shown that the filopodia of *Xenopus* spinal neurons can discriminate between different types of substrate by generating local Ca^{2+} signals of varying frequency that are relayed back to the growth cone (Gomez et al., 2001). The neurons turn away from substrates that give higher frequencies of Ca^{2+} transients. The impression is that the filopodia act as scouts that sense the environment in front of the growth cone and direct the axon to preferential substrates.

Ca^{2+} effectors mediate local and global Ca^{2+} signals

The plasticity of Ca^{2+} as an intracellular messenger is almost matched by the versatility of some of its effectors. The ubiquitous Ca^{2+}-binding protein calmodulin (CaM), for example, mediates many of the effects of Ca^{2+}, and it can similarly work over different temporal and spatial scales (for example, see Török et al., 1998; reviewed by Chin and Means, 2000; Toutenhoofd and Strehler, 2000). Although a large portion of intracellular CaM is tethered, it can redistribute around cells in a Ca^{2+}-dependent manner. In particular, CaM has been shown to move by facilitated diffusion into the nucleus following activation of post-synaptic Ca^{2+} channels (Deisseroth et al., 1998) or global Ca^{2+} waves (Craske et al., 1999). Furthermore, the extent of CaM translocation is determined by the nature of the cellular Ca^{2+} signal (Teruel et al., 2000). The example of nuclear CaM translocation in neurons demonstrates how local Ca^{2+} signals can have more distant longer-term consequences.

CaM has two pairs of ‘EF hand’ motifs that can bind one Ca^{2+} ion each. The binding of Ca^{2+} to the different EF hands may mediate distinct effects. In the case of L- and P/Q-type VOCCs, for example, CaM can mediate both facilitation and inhibition of the Ca^{2+} current by binding to a single site on the VOCC (Zuhlke et al., 1999; DeMaria et al., 2001). Although this implicates CaM as an essential component in short-term regulation of Ca^{2+} channels, it seems paradoxical that CaM can have opposing effects at a single binding site on its target. Interestingly, in the case of P/Q-type VOCCs, it appears that binding of Ca^{2+} to the N-terminal EF hand pair is responsible for inactivation, whereas the C-terminal EF hand pair underlies facilitation (DeMaria et al., 2001). The empirical observation that facilitation precedes inactivation (DeMaria et al., 2001) suggests that the two pairs of EF hands impart their effects in a temporally regulated manner, perhaps by causing different parts of CaM to interact with the Ca^{2+} channel. Since the different EF hands display distinct affinities for Ca^{2+}, it is plausible they could sense different components of a Ca^{2+} signal (i.e. local versus global). Therefore, just as with Ca^{2+} itself, CaM can have distinct functions over distances of nanometres up to whole cells.

In addition to its Ca^{2+}-regulated activities, CaM is known to bind to Ca^{2+} entry and Ca^{2+} release channels in its Ca^{2+}-free

form (apoCaM) (DeMaria et al., 2001; Rodney et al., 2001). Such binding of apoCaM may be a means of tethering a Ca^{2+} sensor for rapid detection of signalling events (DeMaria et al., 2001), or apoCaM may have regulatory effects distinct from those of Ca^{2+}-bound CaM (for example, see Cardy and Taylor, 1998).

Conclusion

The many functions of local Ca^{2+} signals illustrate the versatility of Ca^{2+} as an intracellular messenger. No doubt, future studies will provide more examples of Ca^{2+} acting at the microscopic and nanoscopic levels within cells. The panoply of local Ca^{2+} signals and functions arises through the utilisation of a 'Ca^{2+} signalling toolkit' (Berridge et al., 1998; Berridge et al., 2000), whereby different cells express particular combinations of proteins that determine the nature and output of the Ca^{2+} signal. In addition, it is becoming apparent that many of the proteins involved in producing and sensing Ca^{2+} signals are organised into multiprotein complexes, which provide further means of shaping local events. These complexes include receptors that produce Ins(1,4,5)P_3 (see Muallem and Wilkie, 1999), intracellular Ca^{2+}-release channels (see Mackrill, 1999) and Ca^{2+}-entry channels (see Blackstone and Sheng, 1999). Furthermore, many effectors of Ca^{2+} signals are scaffolded to their Ca^{2+} source (for example, see Isshiki and Anderson, 1999). Although local Ca^{2+} signals can have immediate effects within the vicinity of the channels from which they originate, the effects of such signals on cardiac pacemaking, growth cone directionality, neuronal membrane potential (see above) and gene transcription (reviewed by Bito et al., 1997) illustrate that brief local Ca^{2+} signals can have long-term consequences.

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