Internal Ca\(^{2+}\) In perspective

The list of biological processes requiring Ca\(^{2+}\) is so disparate that there can hardly be any cellular function that is not influenced directly or indirectly by this ubiquitous second messenger.

It is an increase in the free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) within stimulated cells that often underlies such fundamentally important processes as contraction, secretion, metabolism, synthesis and cell growth. A complete understanding of the role that Ca\(^{2+}\) plays in controlling many of these processes will however only follow from experiments in which [Ca\(^{2+}\)]\(_i\) can be continuously monitored as the cell is stimulated. For many years this presented a major obstacle to researchers as there was no reliable, simple method of routinely monitoring [Ca\(^{2+}\)]\(_i\) within stimulated cells. The first mechanism is voltage-sensitive Ca\(^{2+}\) permeability of the plasma membrane itself (or the sarcoplasmic reticulum in the case of muscle cells). This is seen in excitable cells, i.e. cells with action potentials such as neurones, adrenal chromaffin cells and pancreatic B-cells. The second mechanism is voltage-independent and depends on external ligands such as hormones, neurotransmitters and growth factors activating cell surface receptors that are coupled to a transducing mechanism capable of mobilizing internally stored Ca\(^{2+}\). This is seen in excitable cells and non-excitatory cells such as acinar cells, platelets, neutrophils, hepatocytes and mast cells. The biochemistry of this transducing mechanism (the inositol lipid signalling pathway) is currently being unravelled (see recent reviews by Berridge, 1987, 1988a; Downes, 1988; Nahorski, 1988) and will not be discussed in detail here. Suffice to say that in addition to inositol 1,4,5-trisphosphate (Ins\(^{1,4,5}P_3\)), the inositol phosphate released into the cytosol that mobilizes internally stored Ca\(^{2+}\), it appears that other inositol phosphates derived from.

The finding that [Ca\(^{2+}\)]\(_i\) is not constant but oscillates in many cell types after agonist stimulation and that the frequency of these oscillations often depends on the agonist concentration has raised the possibility that a Ca\(^{2+}\) signal can be frequency (in addition to amplitude) encoded. Also, the finding that different stimuli can elicit different spatial distributions of Ca\(^{2+}\) within the same cell raises the possibility that the physiological response triggered may depend on which area of the cell the Ca\(^{2+}\) activates. Oscillations in Ca\(^{2+}\) and their implications, have been described in detail in several recent reviews (Berridge et al. 1988; Berridge & Gallone, 1988; Rink & Jacob, 1989) and will not be discussed here. In this review, I wish to focus on the spatial aspects of the Ca\(^{2+}\) signal by illustrating how this feature of the signalling mechanism has resulted in new ideas of how an elevation in [Ca\(^{2+}\)]\(_i\) can influence the activation of secretory and other cells.

The source of the rise in intracellular Ca\(^{2+}\)

When considering this aspect of the signalling mechanism it is important to bear in mind that there are two main ways of generating Ca\(^{2+}\) signals within cells. The first mechanism depends on having a voltage-sensitive device in the plasma membrane that when activated alters the Ca\(^{2+}\) permeability of the plasma membrane itself (or the sarcoplasmic reticulum in the case of muscle cells). This is seen in excitable cells, i.e. cells with action potentials such as neurones, adrenal chromaffin cells and pancreatic B-cells. The second mechanism is voltage-independent and depends on external ligands such as hormones, neurotransmitters and growth factors activating cell surface receptors that are coupled to a transducing mechanism capable of mobilizing internally stored Ca\(^{2+}\). This is seen in excitable cells and non-excitatory cells such as acinar cells, platelets, neutrophils, hepatocytes and mast cells. The biochemistry of this transducing mechanism (the inositol lipid signalling pathway) is currently being unravelled (see recent reviews by Berridge, 1987, 1988a; Downes, 1988; Nahorski, 1988) and will not be discussed in detail here. Suffice to say that in addition to inositol 1,4,5-trisphosphate (Ins\(^{1,4,5}P_3\)), the inositol phosphate released into the cytosol that mobilizes internally stored Ca\(^{2+}\), it appears that other inositol phosphates derived
from the same plasma membrane inositol lipid pool could also play an integral role in Ca$^{2+}$ homeostasis.

Although described separately, these Ca$^{2+}$ signalling mechanisms are not mutually exclusive. The same excitable cell can have a receptor coupled to Ca$^{2+}$ influx and one coupled to internal Ca$^{2+}$ mobilization. Furthermore, Ca$^{2+}$ influx itself can directly give rise to mobilization of internal Ca$^{2+}$. This can be achieved by a process of either Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) (Lipscombe et al. 1988a; Berridge & Galione, 1988) or Ca$^{2+}$-induced activation of phospholipase C, the enzyme responsible for generating InsP$_3$ (Eberhard & Holz, 1988). There are also examples of the reverse situation, i.e. Ca$^{2+}$ influx following on from mobilization of internal Ca$^{2+}$. In these cases the surface receptors act indirectly through an internal messenger such as Ca$^{2+}$ or an inositol phosphate to open a second messenger-operated channel (SMOC) in the plasma membrane (Meldolesi & Pozzan, 1987; Berridge, 1988a).

The spatial organization of the Ca$^{2+}$ signal

The first direct demonstration of the kinetic compartmentalization of elevated Ca$^{2+}$ within cells was by Rose & Loewenstein (1975). They injected the Ca$^{2+}$-sensitive photoprotein aequorin into the salivary gland of Chironomus and detected the luminescence from an intensified microscopic image. They found that [Ca$^{2+}$], when elevated either by increased local diffusion across the plasma membrane or direct microinjection into the cell, remained very highly constrained in the area of the cell to which it was introduced. This confinement was an active process, since it was not evident after cyanide poisoning. The conclusion was that Ca$^{2+}$ is rapidly sequestered into organelles, and thus away from the aequorin, by active means. This result demonstrated that living cells could sustain local gradients of [Ca$^{2+}$]. In cells exposed to a physiological stimulus, [Ca$^{2+}$], gradients could be achieved if the surface receptors linked to InsP$_3$ generation were not distributed uniformly over the surface of the cell and if the InsP$_3$-sensitive Ca$^{2+}$ store, the endoplasmic reticulum (ER), was localized to one area of the cell. This has been clearly demonstrated in Limulus photoreceptors, also by the use of aequorin (Payne & Fein, 1987). The R-lobe of the photoreceptor is much more sensitive to light than the A-lobe. Both rhodopsin and the InsP$_3$-sensitive ER are localized to the R-lobe with the result that the rise in [Ca$^{2+}$], elicited by a flash of light or microinjection of InsP$_3$ was highly restricted to the R-lobe. Interestingly, the Ca$^{2+}$ response to microinjected InsP$_3$ was further confined around the injection site, a result reminiscent of the earlier Chironomus observations. Another example is the Xenopus oocyte, where both the acetylcholine receptors (Kusano et al. 1982) and other aspects of the signalling pathway (Berridge, 1988b) are confined to the animal pole. It has been suggested (Berridge, 1988b) that the restriction of the Ca$^{2+}$ signal to this area in the activated oocyte may be instrumental in setting up the electric gradient that surrounds these cells and which, in turn, may define the anterior–posterior axis in the developing embryo.

Local Ca$^{2+}$ in secretory cells

Early experimental data supporting a role in exocytotic secretion of kinetically compartmentalized Ca$^{2+}$ was obtained with bovine adrenal chromaffin cells. These cells have a nicotinic receptor linked to Ca$^{2+}$ influx (Burgoyne, 1984) and several receptors (e.g. muscarinic, angiotensin II, bradykinin) linked to inositol lipid hydrolysis and mobilization of internally stored Ca$^{2+}$ (O'Sullivan & Burgoyne, 1989). Nicotinic agonist-stimulated catecholamine secretion requires extracellular Ca$^{2+}$, and direct measurement with fluorescent dyes has shown that [Ca$^{2+}$], is elevated within seconds after stimulation (Cheek & Burgoyne, 1985). Kilpatrick et al. (1982) performed experiments in which $^{45}$Ca$^{2+}$ uptake was continually stimulated by nicotine and then abruptly stopped by addition of curare. The result was that secretion ceased on addition of the curare, despite [Ca$^{2+}$], remaining high. Essentially the same result was seen by Heldman et al. (1984): removal of cholinergic agonists 1 min after stimulation of the cells resulted in immediate cessation of secretion although [Ca$^{2+}$], as monitored by quin2, remained elevated. These results indicated that secretion was better correlated with the rate of Ca$^{2+}$ influx than maximum [Ca$^{2+}$], attained. Ca$^{2+}$ influx rate would be an important determinant of local [Ca$^{2+}$], in the immediate sub-plasmalemmal area. Further evidence that only Ca$^{2+}$ at the plasma membrane may regulate secretion is that muscarinic agonists (Cheek & Burgoyne, 1985) and angiotensin II (O'Sullivan & Burgoyne, 1989) raised [Ca$^{2+}$], by release of internally stored Ca$^{2+}$ but triggered little or no secretion.

Recently we have used fluorescence imaging of fura-2 and video-enhanced microscopy to address directly this issue of compartmentalized Ca$^{2+}$ in chromaffin cells (O'Sullivan et al. 1989; Cheek et al. 1989). This technique has enabled us to study in real-time the spatial organization of the agonist-induced rise in [Ca$^{2+}$], at the level of the single cell. Challenging the cells with nicotine
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resulted in Ca\textsuperscript{2+} being initially localized exclusively to the sub-plasmalemmal area (Fig. 1A,b). The cell then infilled with Ca\textsuperscript{2+} and a more uniform distribution was observed over the cell surface (Fig. 1A,d). This is consistent with Ca\textsuperscript{2+} entering the cell from the external medium. In some cells, such as that shown in Fig. 1A,b,d, a secondary rise in [Ca\textsuperscript{2+}], occurred after the initial influx phase. This was probably a consequence of Ca\textsuperscript{2+} being released from an internal store by either Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release or Ca\textsuperscript{2+} activation of phospholipase C, as mentioned earlier. As a result, the maximum [Ca\textsuperscript{2+}], was recorded in only one pole of the cell (see asterisk in Fig. 1A,d). Using a technique involving fluorescent imaging of co-cultured cells, we confirmed that this pattern of Ca\textsuperscript{2+} resulted in a strong secretory response by detecting the ATP that was co-released with the catecholamine from the stimulated cell (Cheek et al. unpublished). The pattern of Ca\textsuperscript{2+} elicited by ineffective secretagogues such as the InsP\textsubscript{3}-mobilizing muscarinic agonist muscarine was markedly different from that described above. Often, the rise in [Ca\textsuperscript{2+}]\textsubscript{i} originated only in one discrete area of the cell (Fig. 1A,a), and even at the peak of the response, when peak [Ca\textsuperscript{2+}], was comparable with that due to nicotine, the Ca\textsuperscript{2+} was still generally confined to the area from which it originated (Fig. 1A,c). This is consistent with the finding that the InsP\textsubscript{3}-sensitive ER is often specifically localized to one pole in these cells (O'Sullivan et al. 1989). This same pattern of Ca\textsuperscript{2+} was observed in another cell responding to the alternative InsP\textsubscript{3}-mobilizing agonist angiotensin II (Fig. 1B), and was shown not to trigger secretion from this cell (Cheek et al. unpublished). In response to a subsequent challenge with nicotine, however, the same cell became infilled with Ca\textsuperscript{2+} (Fig. 1B), and secretion was triggered (Cheek et al. unpublished). These results not only directly demonstrate that different classes of agonists are capable of giving rise to different localizations of internal Ca\textsuperscript{2+} within the same cell, but also suggest that this could be a significant determinant as to which physiological response is stimulated. In exocytosis from bovine chromaffin cells it appears that the optimal signal for triggering release requires Ca\textsuperscript{2+} activation of the entire sub-plasmalemmal area. Release of internally stored Ca\textsuperscript{2+} may be incapable of activating such sub-plasmalemmal events as disruption of the cortical cytoskeleton, translocation of protein kinase C to the plasma membrane and recruitment of cytosolic proteins to the granule membrane, all of which are thought to precede fusion, and may instead trigger metabolic processes such as the biosynthesis of catecholamine (Cheek et al. 1989a; unpublished). Localization of the rise in [Ca\textsuperscript{2+}], to an inappropriate area of the cell may also explain why the antigen-induced oscillations in [Ca\textsuperscript{2+}], seen in mast cells (Neher & Almers, 1986) did not necessarily trigger exocytosis since, in another study, this rise in [Ca\textsuperscript{2+}], has been localized not to the sub-plasmalemmal region, but to the nuclear area (Ryan et al. 1988).

There may also be a role for compartmentalized Ca\textsuperscript{2+} in the promotion of non-exocytotic fluid secretion. A recent study (Foskett et al. 1989) has indicated that the InsP\textsubscript{3}-sensitive Ca\textsuperscript{2+} store is localized at or near the basolateral membrane in rat parotid acinar cells and, hence, that it is this area of the cell to which the Ca\textsuperscript{2+} rise in response to cholinergic agonists is initially confined. The significance of this is that the basolateral membrane contains the Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} channels whose activation by Ca\textsuperscript{2+} is an important prerequisite for Cl\textsuperscript{−} uptake and subsequent fluid secretion in response to these agonists (Petersen, 1986).

**Localized Ca\textsuperscript{2+} in neuronal cells**

Sympathetic neurones share a common embryonic origin, the neural crest, with adrenal chromaffin cells. With reference to this point, it is interesting that a role for localized Ca\textsuperscript{2+} is also implicated in transmitter release from these neurones. A recent fluorescent imaging study using cultured frog sympathetic neurones loaded with fura-2 (Lipscombe et al. 1988b) has shown that, in response to depolarization, [Ca\textsuperscript{2+}], was elevated in the cell body and in the growth cones (the area responsible for neurotransmitter release), whereas release of internally stored Ca\textsuperscript{2+}, in response to caffeine, resulted in a large rise in [Ca\textsuperscript{2+}], only in the cell body (see also Tsien, 1988). This suggests that, although release of internal Ca\textsuperscript{2+} may play an important role in regulating Ca\textsuperscript{2+}-dependent events in the cell body, it probably has little influence over events such as neurotransmitter release that occur in the processes and growth cones. This notion is supported by the finding that depolarization with high K\textsuperscript{+}, but not release of internal Ca\textsuperscript{2+} due to muscarinic receptor activation, was able to trigger neurotransmitter release from cultured chick sympathetic neurones (Bhave et al. 1988).

Neuronal cells (and possibly other excitable cells) can have a further adaptation designed to give rise to intracellular Ca\textsuperscript{2+} gradients: their voltage-sensitive Ca\textsuperscript{2+} channels can be clustered into one small area of the plasma membrane. Evidence, also from fluorescent imaging of fura-2 (Smith et al. 1988), has suggested that Ca\textsuperscript{2+} influx into the presynaptic membrane of the squid giant synapse is at least 10-fold higher in the areas that contain the active zones than in regions devoid of active zones. These active zones are the specialist sites of synaptic transmitter release at nerve terminals that release fast-acting neurotransmitters such as glutamate, GABA and acetylcholine. They contain the synaptic vesicles, cytoskeletal elements, and other structures (some extracellular) responsible for the recycling of vesicular membranes and the alignment of the release site to the receptor-rich postsynaptic region (Kelly, 1988). As pointed out by Smith & Augustine (1988), the localization of the Ca\textsuperscript{2+} channels to these areas means not only that very large (>100μM) and rapid Ca\textsuperscript{2+} transients can be generated following action potentials, but also that this occurs in precisely the correct area of the neurone to be able to trigger fast (0.1-1 ms following Ca\textsuperscript{2+} influx) and synchronous neurotransmitter release. The non-uniform localization of Ca\textsuperscript{2+}-binding proteins and Ca\textsuperscript{2+}-sequestering organelles within the presynaptic terminal would

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also be expected to contribute to the maintenance of such an intracellular [Ca\(^{2+}\)]\(_i\) gradient (Blaustein, 1988).

The unifying factor linking these examples is that they demonstrate how the sub-cellular localization of various aspects of the Ca\(^{2+}\) signalling mechanism can result in a stimulus-induced rise in [Ca\(^{2+}\)]\(_i\), being targeted to a specific area of a cell. There are however examples of localized Ca\(^{2+}\) signals within cells where the underlying mechanism is less clear. Probably the most dramatic of these is the change in [Ca\(^{2+}\)]\(_i\), that occurs during egg fertilization. In many species, a conducting wave of Ca\(^{2+}\) release starts from the point of sperm-egg fusion and spreads to the opposing pole at a rate of \(-10\, \mu m\, s^{-1}\) (Jaffe, 1983). This Ca\(^{2+}\) transient probably triggers the subsequent wave of cortical granule exocytosis (Busa & Nuccitelli, 1985). A role for inositol lipids is strongly implicated since fertilization results in InsP\(_3\) production (Ciapa & Whitaker, 1986), and microinjection of InsP\(_3\) gives rise to a wave of cortical granule exocytosis (Whitaker & Irvine, 1984). Although a number of suggestions have been proposed (see Berridge, 1988a), the mechanism by which the Ca\(^{2+}\) signal is conducted through the egg, and how the Ca\(^{2+}\) is kept in the form of a propagating band that remains confined to the cell periphery in some cases (Gilkey et al., 1978), or can oscillate at 1- to 3-min intervals in others (Miyazaki et al., 1986), still remains to be elucidated.

Concluding remarks

Recent technological advances that permit the detailed study of intracellular Ca\(^{2+}\) signals have revealed that there is a remarkable spatial organization of the Ca\(^{2+}\) signal within stimulated cells.

A stimulus-induced rise in [Ca\(^{2+}\)]\(_i\), can be targeted to a specific area of the cell (see Fig. 1). This can be achieved by the non-uniform distribution of surface receptors linked to the generation of InsP\(_3\), the restriction of the InsP\(_3\)-sensitive Ca\(^{2+}\) store to one area of the cell, or by cell surface Ca\(^{2+}\) channels being clustered into one small area of the plasma membrane. The targeting of the Ca\(^{2+}\) signal in this way not only represents the most effective method of triggering a Ca\(^{2+}\)-dependent cellular response, but also provides a means whereby different Ca\(^{2+}\)-dependent processes within the same cell can be selectively activated by stimuli which evoke different patterns of Ca\(^{2+}\).

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References


