Commentary

Functional Interactions in Ca^{2+} Signaling over Different Time and Distance Scales

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Cytosolic Ca^{2+} plays a central role in the regulation of numerous aspects of cellular activity in virtually all cell types. Much of this versatility arises through the diverse mechanisms by which Ca^{2+} signals are generated and transmitted to act over very different time and distance scales. For example, Ca^{2+} can act in a very fast and highly localized manner, as in the triggering of neurotransmitter release within microseconds of Ca^{2+} entry through voltage-gated channels closely apposed to active release zones (Neher, 1998); or it can evoke slower responses involving global Ca^{2+} elevations throughout the cell, as in the generation of Ca^{2+}-dependent Cl^- currents by Ca^{2+} waves that take many seconds to sweep across *Xenopus* oocytes (Parker and Yao, 1994). A common starting point in considering these differences is that a Ca^{2+} in the cytosol can move only by passive diffusion, a process that is further modified by the presence of endogenous mobile and immobile Ca^{2+} buffers, so that precipitous Ca^{2+} gradients (microdomains) exist around an open Ca^{2+} channel. The concentration near the channel mouth may be 100 μM or more, whereas concentrations as close as 1 or 2 μm fall below 1 μM (Naraghi and Neher, 1997; Rios and Stern 1997; Neher, 1998). Therefore, Ca^{2+} has only a restricted "range of action," in the order of 5 μm (Allbritton et al., 1992). Furthermore, the mean distance a Ca^{2+} diffuses decreases as a square root function of time. Whereas the Ca^{2+} concentration at the mouth of a Ca^{2+} channel will track almost instantly the opening and closing of the pore, the kinetics are slowed by ~0.1 ms at a distance of 100 nm, 10 ms at 1 μm, and by 1s at 10 μm (assuming an apparent diffusion coefficient of 17 $\mu m^2 s^{-1}$ for Ca^{2+} in cytosol). The characteristics and specificity of Ca^{2+} signaling systems can, therefore, be tuned by using sensors with differing affinities located at different distances from a Ca^{2+} source. A Ca^{2+}-activated effector with low (tens of micromolars) affinity located close to a Ca^{2+} channel will signal rapidly, whereas an effector with higher affinity situated further away will respond more slowly, but will integrate Ca^{2+} signals across a wider area.

On the basis of passive diffusion alone, one would expect the action of Ca^{2+} entering the cell to be restricted to a subplasmalemmal shell a few micrometers thick. However, cells possess further mechanisms that allow these local signals to be amplified and propagated globally throughout the cell interior. Both of the major families of Ca^{2+} channel that mediate Ca^{2+} release from intracellular Ca^{2+} stores (inositol trisphosphate receptors [IP_3R] and ryanodine receptors [RyR]) display a property of Ca^{2+}-induced Ca^{2+} release (CICR), so that long-range signaling is possible through the generation of actively propagating Ca^{2+} waves that travel at velocities of a few tens of micrometers/second by successive cycles of Ca^{2+} release, diffusion, and CICR (Berridge, 1997). Here also, the spatial organization of Ca^{2+} signaling plays a crucial role. Ca^{2+} release channels are not usually distributed homogeneously, but are clustered at functional release sites spaced a few micrometers apart, so that Ca^{2+} waves propagate in a saltatory manner, jumping from site to site. Microdomains of Ca^{2+} exist within the wavefront and, in some circumstances, individual release sites can be activated in isolation to produce local, "elementary" Ca^{2+} signals (Parker et al., 1996; Berridge, 1997). On a more macroscopic scale, subcellular variations in the properties and density of release sites, as well as in the distribution of other Ca^{2+} handling systems such as mitochondria, dictate the spatial organization of global Ca^{2+} signals; i.e., where Ca^{2+} waves arise in the cell, what direction they travel in, and whether they propagate robustly or become abortive and fizzle out.

Progress in understanding the complex spatiotemporal organization of Ca^{2+} signaling has been greatly facilitated by the development of efficient fluorescent Ca^{2+} indicators together with confocal imaging systems with improved temporal (<1 ms) and spatial (~0.5 μm) resolution (Pawley, 1995). Nevertheless, the resolution of these techniques is insufficient to visualize directly what is happening within submicron regions around Ca^{2+} channels, and can report only the spreading tails of local Ca^{2+} gradients. Instead, information about the cru-

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cial microdomains must be inferred either through modeling or, more directly, from the activity of the endogenous Ca^{2+} sensors themselves. Examples of all these approaches are provided by two recent papers (Akita and Kuba, 2000; Straub et al., 2000), which illustrate the involvement of intracellular Ca^{2+} release channels in Ca^{2+} signaling across spatial and temporal scales that encompass >2 orders of magnitude.

Short (<1 μm) Range Ca^{2+} Signals Shaping Neuronal Excitability

Akita and Kuba (2000) investigate the role of intracellular CICR in regulating membrane excitability in bullfrog sympathetic ganglion cells. Using linescanning confocal microscopy to image near-membrane Ca^{2+} changes, they correlate the role of intracellular Ca^{2+} release during physiological depolarizations with the activation of Ca^{2+}-dependent K^+ currents measured using the whole-cell patch-clamp technique. Their results provide important insight into the spatial organization of two ion channels that act as Ca^{2+} sources in neurons (N-type Ca^{2+} channels and RyRs), with two types of Ca^{2+}-activated K^+ channels (small conductance [SK] and high conductance K^+ channels [BK]) that play discrete roles in regulating neuronal excitability (for reviews see Sah, 1996; Berridge, 1998). The spatial relationship of these four types of ion channel permits regulation of neuronal K^+ conductance over two very different time scales: (a) a fast (<1 ms) conductance activated during the falling phase of individual action potentials, which shapes the action potential duration; and (b) a slower (~100 ms) conductance responsible for modulating the time course of the delayed afterhyperpolarization (AHP).

To set the stage, Akita and Kuba (2000) used linescan confocal imaging to show that Ca^{2+} influx during action potentials evoked a strong Ca^{2+} wave that rapidly propagated into the neuron for ~2 μm before failing. This local Ca^{2+} signal colocalized with the distribution of RyRs as determined by fluorescence imaging, and was abolished after blocking RyRs with ryanodine, indicating that it arose because Ca^{2+} entry across the plasma membrane was amplified by CICR through RyRs. Previous attempts to visualize the involvement of CICR during action potentials in these cells had failed, and the present success owes much to enhancements in time and spatial resolution of confocal linescan imaging, together with the use of a low indicator concentration to minimize exogenous Ca^{2+} buffering. The localization of RyR-mediated Ca^{2+} liberation to a narrow subplasmalemmal band suggests a potential role for CICR in Ca^{2+} signaling at the cell membrane, though not for global transmission of signals into the interior of the neuron. Three aspects of their work are particularly noteworthy with respect to the theme of Ca^{2+} signaling over different spatial and temporal domains.

First, they demonstrate that CICR, triggered by Ca^{2+} influx through N-type Ca^{2+} channels, regulates membrane excitability within the time course of a single action potential by modulating the rate of repolarization. Although CICR modulation of various phases of the AHP (over time scales of seconds) has been demonstrated in many different types of neurons (Sah and McLachlan, 1991; Yoshizaki et al., 1995; Moore et al., 1998; Cordoba-Rodriguez et al., 1999), the role of CICR in activating the BK channels that shape action potential repolarization on a submillisecond time scale is novel. BK channels typically require $[Ca^{2+}]_i$ >5 μM for activation (McManus, 1991), such that their close localization (~10–30 nm) to Ca^{2+} entry channels (Prakriya et al., 1996; Marrion and Tavalin, 1998) ensures their rapid activation and closure when the cell is depolarized. The finding by Akita and Kuba (2000) that approximately half of the BK channels contributing to action potential repolarization are activated indirectly by CICR, rather than directly by Ca^{2+} entry, highlights the role of RyRs as a third partner within a fast, intimate Ca^{2+} signaling domain. This functional triad responds within <0.6 ms of the peak of an action potential, implying a maximal separation of <100 nm between the RyRs and the BK or Ca^{2+} entry channels.

Second, the results of Akita and Kuba (2000) illustrate how the spatial organization of Ca^{2+}-sensitive proteins can dictate the temporal sequence of ion channel activation. The microdomains of Ca^{2+} resulting from Ca^{2+} entry amplified through CICR, initially activate the closely localized BK channels, and then activate the more distant SK channels that modulate the kinetics of the AHP. Evidence supporting this conclusion derives from the observation that with an increasing number of action potentials in a train, SK channel activity increases but BK channel activity declines (Akita and Kuba, 2000). The increase in SK channel activation indicates that these channels respond to the spatially averaged summation of submembrane $[Ca^{2+}]$ that accumulates during many action potentials. In contrast, the activity of the closely coupled BK channels directly depends on the extent of local CICR within the submembrane Ca^{2+} microdomain, which declines during repetitive stimulation. Coupling between Ca^{2+} channels and SK channels is, therefore, more "loose" than the coupling to BK channels. Indeed, the time course of closing of SK channels during the decay of the AHP more closely resembles the decay of the fluorescence Ca^{2+} signal. Although, even in this case, the more rapid kinetics of the current indicate that the SK channels sense local Ca^{2+} microdomains that are below the resolution of confocal microscopy. BK channels, with their low affinity for Ca^{2+} (McManus, 1991), rapidly activate

at the high local [Ca^{2+}] attained within the core of the microdomain, and rapidly close as the central peak of the microdomain collapses after closure of the N-type Ca^{2+} channels. SK channels, excluded from this most intimate coupling, are nonetheless capable of responding to the more distant "tail" of the same microdomains, owing to their higher sensitivity to Ca^{2+} (K_d 100–400 nM; Sah, 1996). A similar picture of sequential activation of Ca^{2+}-dependent K^+ channels is seen in many other nerve cells, where action potentials are followed by a multiphasic AHP, comprising a fast (50–200 ms) component mediated by BK channels, followed by a slower (several seconds) component mediated by SK channels (Sah, 1996; Berridge, 1998). This organization also closely resembles the triadic organization of RyRs, voltage-operated Ca^{2+} channels, and Ca^{2+}-activated K^+ currents in certain types of smooth muscle (for review see Jaggar et al., 1998; Imaizumi et al., 1999). However, the results of Akita and Kuba (2000), further emphasize the wide dispersion of temporal signaling that can be attained by appropriate spatial localization of discrete Ca^{2+}-sensitive channels.

Third, one might wonder why a CICR mechanism need be introduced as an intermediate step between Ca^{2+} entry and stimulation of Ca^{2+}-activated K^+ channels in the plasma membrane. Larger Ca^{2+} signals could be achieved simply through an increased density of voltage-gated Ca^{2+} channels. Instead, a more intriguing possibility is that localized CICR adds spice to an otherwise stereotyped relationship between Ca^{2+} entry channels and Ca^{2+}-activated K^+ channels, by providing a variable degree of gain within the system. In the spatial domain, enhancement of CICR would extend the range of action of Ca^{2+} to recruit more K^+ channels; and plenty of headroom appears to be available as, for example, only 10–20% of the BK channel population is activated during normal action potential activity in chromaffin cells (Prakriya et al., 1996). In the temporal domain, changes in the extent and duration of CICR would alter the kinetics of the AHP, and thereby modulate neuronal discharge frequency, spiking pattern, and the wider coordination of neuronal activity (Cordoba-Rodriguez et al., 1999).

One way of regulating the degree of gain provided by CICR is through the spatial proximity of Ca^{2+} channels and RyRs (Rios and Stern, 1997). Thus, in neurons with especially "tight" coupling, Ca^{2+} entry during a single action potential is sufficient to trigger CICR (Yoshizaki et al., 1995; Cohen et al., 1997; Sandler and Barbara, 1999; Akita and Kuba, 2000), whereas in neurons where the separation is greater, repeated action potentials may be required to trigger CICR. Furthermore, dynamic changes in the sensitivity of CICR within a given cell are likely to be regulated by factors including the bulk cytosolic [Ca^{2+}], the proximity of local Ca^{2+} uptake and export mechanisms, and the state of loading of intracellular Ca^{2+} stores (Berridge, 1998). For example, Akita and Kuba (2000) demonstrate that as stores become depleted during repetitive activity, BK channel activity declines, and the duration of the action potential broadens. Finally, many neurotransmitters modulate the kinetics of, or block, the slow AHP (Sah, 1996), effects that may result from regulation of CICR by intracellular messengers such as cAMP, inositol trisphosphate (IP_3), cyclic ADP-ribose, and NAADP (Chavis et al., 1996; Sah, 1996; Berridge, 1998).

Long-range (>1 μm) Global Ca^{2+} Signaling

The paper by Straub et al. (2000) also concerns CICR, but deals with its involvement over a much greater distance scale, to coordinate the propagation of Ca^{2+} signals globally throughout a cell. Specifically, the authors examine how local, agonist-evoked $[Ca^{2+}]_i$ elevations in pancreatic acinar cells become transformed into global Ca^{2+} waves that propagate from the apical to basal pole of these polarized cells to regulate secretion (Kasai and Augustine, 1990).

The initial stimulus is provided by IP_3-dependent Ca^{2+} release in an apical "trigger zone" (Kasai and Augustine, 1990), which contains a high density of IP_3R and displays an intrinsically higher sensitivity to IP_3 (Lee et al., 1997; Fogarty et al., 2000a). Whereas low agonist concentrations evoke Ca^{2+} signals that remain localized within this zone and can be resolved as discrete elementary events (Fogarty et al., 2000b), higher agonist concentrations evoke global Ca^{2+} waves that propagate beyond this region to encompass the entire cell. This transition from local to global signals is highly dependent upon the activity of a belt of mitochondria surrounding the apical pole (Tinel et al., 1999, Straub et al., 2000), as well as a cooperative interaction between IP_3Rs and RyRs in surmounting the local buffering capacity of these mitochondria (Straub et al., 2000). When mitochondrial function is inhibited, local Ca^{2+} spikes become transformed into global Ca^{2+} signals (Tinel et al., 1999; Straub et al., 2000); but, this happens only when active RyR are available to amplify the initial IP_3-evoked Ca^{2+} release in the apical pole (Straub et al. 2000). Blockade of RyR with ryanodine diminishes and shortens Ca^{2+} signals evoked by photoreleased Ca^{2+}, and limits IP_3-mediated signals to a region only slightly larger than the trigger zone, even when mitochondrial Ca^{2+} import mechanisms are inactive (Straub et al. 2000). The ability of mitochondria to modulate wave propagation most likely arises from both their macroscopic distribution around the trigger zone, and from their intimate microscopic relationship (<60 nm contacts) with the endoplasmic reticulum, which allow them to buffer and hence modulate, the

Ca^{2+} microdomains surrounding individual Ca^{2+} release sites (Rutter and Rizzuto, 2000).

The data of Straub et al. (2000) underscore the theme that the spatiotemporal properties of cellular Ca^{2+} transients are defined by the interaction of different types of Ca^{2+} channels at both nanometer and micrometer distance scales. Ca^{2+} released through RyRs can sensitize IP_3Rs, and reciprocally, Ca^{2+} released after IP_3R activation can directly activate CICR through RyRs, as elegantly demonstrated in vascular smooth muscle (Boittin et al., 1999). Such intracellular cross-talk is well exemplified within the pancreatic acinar cell, where local interaction between three distinct intracellular messenger systems (IP_3, cyclic ADP-ribose, and NAADP) combines to shape the spatiotemporal characteristics of intracellular Ca^{2+} signals in response to different cell-surface agonists (Cancela et al., 2000).

Conclusions

A recurring theme running through this work is that whole-cell responses mediated by Ca^{2+} signaling derive from the summated performance of individual cameos interacting within and between local Ca^{2+} microdomains. Ca^{2+} signaling depends not only on the macroscopic subcellular Ca^{2+} gradients that can be directly visualized by current imaging techniques, but also on the Ca^{2+} microdomains that play out beneath our resolution. The microdomains permit rapid, targeted signaling across very short distances and, by means of Ca^{2+} diffusion and CICR, allow long distance communication, albeit at much slower speeds. Such spatiotemporal issues have been investigated most extensively for Ca^{2+}, where we obtain at least fuzzy images of what is going on within localized regions of the cell; but, the principles apply equally to physiological gradients of other diffusible messengers, such as cAMP (Rich et al., 2000), for which imaging techniques are still in their infancy. Future studies, combining optical, electrophysiological, and molecular techniques, will help identify how such specialized membrane microdomains regulate cellular function and should direct our attention towards the cellular mechanisms of protein targeting and molecular coupling that orchestrate the correct assembly of signaling components with nanometer precision.

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