PERSPECTIVES

What's in store for Ca^{2+} oscillations?

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At fertilization, at every heartbeat, and during a myriad of other Ca^{2+}-regulated cellular responses, Ca^{2+} signals are delivered to the cell as Ca^{2+} oscillations. Such digital signals, analogous to action potentials in neurones, are robust and economical, they may protect cells from the toxic effects of sustained increases in cytosolic $[Ca^{2+}]$, and they allow information to be encoded in the frequency, amplitude and duration of the Ca^{2+} spikes (Berridge *et al.* 2000). Certainly there is evidence that cellular events may be tuned to respond optimally to specific frequencies of Ca^{2+} spiking (Berridge *et al.* 2000). The mechanisms underlying Ca^{2+} oscillations are diverse, and while all include elements of negative feedback by Ca^{2+}, they may be driven entirely by events at the plasma membrane, entirely by release of Ca^{2+} from intracellular stores, or by an interplay between intra- and extra-cellular sources of Ca^{2+}. In heart, for example, a slowly depolarizing membrane potential triggers opening of voltage-gated Ca^{2+} channels, Ca^{2+} enters, triggering further Ca^{2+} release from the sarcoplasmic reticulum, and the increase in sarcoplasmic $[Ca^{2+}]$ then both initiates contraction and promotes repolarization. In other cells, Ca^{2+} oscillations are driven entirely by interplay between voltage-gated Ca^{2+} channels and Ca^{2+}-sensitive channels that regulate membrane potential. The mechanisms controlling the Ca^{2+} oscillations evoked by receptors that stimulate IP_3 formation are less clear. In some cells, levels of IP_3 may themselves oscillate and drive periodic release of Ca^{2+} from intracellular stores (Nash *et al.* 2001), but in other cells Ca^{2+} oscillations occur when IP_3 levels are probably stable. The latter are thought to result from episodic release of intracellular Ca^{2+} stores driven by the co-regulation of IP_3 receptors by Ca^{2+} and IP_3. But what role has Ca^{2+} entry to play in such Ca^{2+} oscillations? Ca^{2+} entry is certainly required to sustain these Ca^{2+} oscillations, but does it serve only to replenish stores or does it play a more central role in the oscillatory mechanism, and are all Ca^{2+} entry pathways equally effective in supporting Ca^{2+} oscillations?

Both questions are addressed in a paper (Bird & Putney, 2005) from this issue of *The Journal of Physiology*. Gd^{3+} is often used to inhibit Ca^{2+} channels, indeed at low concentrations ($\sim$1 μM) it can be used selectively to inhibit store-operated Ca^{2+} (SOC) entry, but at higher concentrations it also inhibits plasma membrane Ca^{2+} pumps. The authors cleverly exploit the ability of high concentrations of Gd^{3+} to inhibit both Ca^{2+} entry and extrusion effectively to insulate HEK-293 cells from Ca^{2+} exchanges with the environment. Under these conditions, activation of endogenous muscarinic acetylcholine receptors evokes Ca^{2+} oscillations that are sustained, and in keeping with earlier work, the frequency rather than the amplitude of the individual spikes increases with stimulus intensity. The oscillatory mechanism does not therefore require Ca^{2+} entry, that is required only to replenish Ca^{2+} lost from the stores as cells restore the cytosolic $[Ca^{2+}]$ to its resting level between spikes (Sneyd *et al.* 2004). It is noteworthy that there is no detectable reduction in the overall Ca^{2+} content of the intracellular stores as the Ca^{2+} oscillations fail, suggesting either that only a small subset of stores contributes to Ca^{2+} spiking or that the underlying mechanism depends critically on their complete refilling. The latter would be consistent with various dynamic models of Ca^{2+} oscillations (Sneyd *et al.* 2004).

Under normal conditions, where plasma membrane Ca^{2+} pumps actively buffer cytosolic $[Ca^{2+}]$, Ca^{2+} entry is important, but the route through which Ca^{2+} enters HEK cells to sustain oscillations is contentious. Shuttleworth argues that at the low stimulus intensities that evoke Ca^{2+} oscillations, all Ca^{2+} entry is via an arachidonic acid-activated Ca^{2+} channel (I_{ARC}) and that this is exclusively responsible for sustaining Ca^{2+} oscillations (Shuttleworth, 2004). Bird and Putney conclude that because oscillations are blocked by Gd^{3+} (1μM) and 2-APB, they are sustained entirely by Ca^{2+} entering through the ubiquitously expressed SOC pathway (Bird & Putney, 2005). How can two labs reach such contradictory conclusions? A partial answer comes from the final experiments in the Bird and Putney paper, where they transfect cells with TRPC3 to create an additional non-SOC and Gd^{3+}-insensitive Ca^{2+} entry pathway. In these cells, the new Ca^{2+} entry pathway also supports Ca^{2+} oscillations. There seems not therefore to be any preferential relationship between specific Ca^{2+} entry pathways and their ability to support Ca^{2+} oscillations by fuelling store refilling: SOC, TRPC3 and I_{ARC} are all effective. This is perhaps surprising because the intimate relationship between store depletion and activation of SOC led many to suppose that SOC might preferentially couple to store refilling. The disparity between the two labs thus boils down to understanding why each apportions responsibility for Ca^{2+} entry to a different pathway, rather than to any fundamental difference in the mechanism driving Ca^{2+} oscillations.

References

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