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The effects of Ca^{2+} buffers on cytosolic Ca^{2+} signalling

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Ca^{2+} is one of the most important and universal intracellular signalling agents, controlling a multitude of vitally important cellular processes (Petersen & Verkhratsky 2016). The regulating cytosolic Ca^{2+} signals vary enormously with regard to timing, localization and spatial extent, depending on the specific cell type and the function to be controlled (Petersen & Verkhratsky, 2016). Because of the many different types of Ca^{2+} sensors (Ca^{2+}-binding proteins) found at different locations inside cells, there is often a requirement for Ca^{2+} signals to be strictly localized. A well-known example of this is the control of neurotransmitter release by Ca^{2+} entry through voltage-gated Ca^{2+} channels, generating short-lived local nano-domains of high Ca^{2+} concentration ($[Ca^{2+}]_i$) (Meinrenken *et al.* 2003). A more recent, and very interesting, example is the physiological activation of two Ca^{2+}-dependent transcription isoforms, NFAT1 and NFAT4. This occurs through two coincident, but spatially segregated, intracellular Ca^{2+} signals, namely in the immediate vicinity of Ca^{2+} release-activated Ca^{2+} channels in the plasma membrane and close to inositol trisphosphate receptors in the inner nuclear membrane (Kar *et al.* 2016).

Different spatio-temporal Ca^{2+} signal patterns can occur in the same cell depending on the type, intensity and duration of stimulation (Petersen 1992). In pancreatic acinar cells, both local Ca^{2+} spiking in the region of secretory control and global $[Ca^{2+}]_i$ elevations can be generated, which is important because Ca^{2+} signals control not only secretion but also many other functions, including growth (cell division) (Petersen, 1992). In nerve terminals, functions other than neurotransmitter secretion may depend on the global $[Ca^{2+}]_i$. There has therefore, for a long time, been much interest in measuring the volume-averaged $[Ca^{2+}]_i$ (Meinrenken *et al.* 2003), which can also be particularly helpful for a precise quantitative evaluation of Ca^{2+} handling.

The nature and concentration of intracellular Ca^{2+} buffers play an important role in determining the timing and spreading of Ca^{2+} released into the cytosol by opening of Ca^{2+} channels either in the plasma membrane or in organelle membranes. An early example of experiments showing this phenomenon was the demonstration, in whole-cell patch clamp current recording studies on pancreatic acinar cells, that intracellular addition of a highly mobile low-affinity Ca^{2+} buffer, for example citrate, transformed ACh-evoked local and short-lasting Ca^{2+} spikes into global and much more prolonged Ca^{2+} transients (Petersen, 1992). The timing and spatial extension of physiological Ca^{2+} signals therefore depends not only on the strength and type of stimulation but also on the affinities, mobilities and concentrations of the various intracellular Ca^{2+} buffers, which can vary significantly between different cell types. There is also a practical issue relating to the ability of Ca^{2+} buffers to influence intracellular Ca^{2+} signals. Because all Ca^{2+}-sensitive fluorescent probes are Ca^{2+} buffers, they can distort the $[Ca^{2+}]_i$ signals they are designed to monitor.

It has been challenging to obtain reliable estimates for the parameters that define the dynamics of physiological $[Ca^{2+}]_i$ changes. In this issue of *The Journal of Physiology*, Erwin Neher and his colleagues (Lin *et al.* 2017) now describe the currently most precise quantitative approach to solving this problem by once more taking advantage of the calyx of Held, a giant mammalian glutamatergic nerve terminal which – following the pioneering work of Ian Forsythe (Forsythe, 1994) – has been extensively studied by several groups (Meinrenken *et al.* 2003). Lin *et al.* (2017) describe the dynamic changes of global $[Ca^{2+}]_i$ during single and repetitive voltage-clamp depolarizations and provide quantitative data on Ca^{2+} inflow, Ca^{2+} buffering and Ca^{2+} clearance.

Using low concentrations of the low-affinity Ca^{2+} indicator Fura-6F, in order not to overwhelm the endogenous Ca^{2+} buffers, Lin *et al.* (2017) studied the voltage-clamped nerve terminals with patch pipettes containing solutions with minimal Ca^{2+} buffer concentrations. This allowed them to determine the Ca^{2+} binding properties of the endogenous fixed buffers and also the Ca^{2+} clearance mechanism. With regard to the latter, a comparison was made between the results obtained with Cs^+- or K^+-based pipette solutions. The data from these experiments confirmed the importance of K^+-dependent Na^+–Ca^{2+} exchange for Ca^{2+} extrusion (Schnetkamp, 2004). In other experiments, Lin *et al.* (2017) used pipette solutions with 500 μM of the widely used Ca^{2+} chelator EGTA, determining its Ca^{2+} binding characteristics under realistic intracellular conditions. It turned out that the Ca^{2+} dissociation constant of EGTA is more than 3 times higher than the value previously obtained *in vitro*. This result is of great practical importance as EGTA has been, and no doubt will continue to be, a useful tool as a slow Ca^{2+} chelator buffering global rather than local $[Ca^{2+}]_i$.

Overall, the major importance of the work reported by Lin *et al.* (2017) is that, based on very sensitive experimental protocols, they have been able to generate a consistent set of parameters for modelling $[Ca^{2+}]_i$ transients in a mammalian presynaptic nerve terminal. Estimates of some of the parameters determining $[Ca^{2+}]_i$ dynamics have been reported previously, but they were based on experiments carried out under a variety of different conditions, whereas the new study by Lin *et al.* (2017) has resulted in a comprehensive set of parameters valid for recording conditions generally used for studies of the calyx of Held. The results of the work of Lin *et al.* (2017) that 'one set of parameters accurately describes $[Ca^{2+}]_i$ measurements covering a wide range of amplitudes and obtained using quite different stimulation protocols and ionic conditions' is remarkable and promises that this set will turn out to be of real help as a firm quantitative basis for further studies in this field.

References

Forsythe ID (1994). Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, *in vitro*. *J Physiol* **479**, 381–387.

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Kar P, Mirams GR, Christian HC & Parekh AB (2016). Control of NFAT isoform activation and NFAT-dependent gene expression through two coincident and spatially segregated intracellular Ca^{2+} signals. *Mol Cell* **64**, 746–759.

Lin K-H, Taschenberger H & Neher E (2017). Dynamics of volume-averaged intracellular Ca^{2+} in a rat CNS nerve terminal during single and repetitive voltage-clamp depolarizations. *J Physiol* **595**, 3219–3236.

Meinrenken CJ, Borst JGG & Sakmann B (2003) Local routes revisited: the space and time dependence of the Ca^{2+} signal for phasic transmitter release at the rat calyx of Held. *J Physiol* **547**, 665–689.

Petersen OH (1992). Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. *J Physiol* **448**, 1–51.

Petersen OH & Verkhratsky A (2016). Calcium and ATP control multiple vital functions. *Philos Trans R Soc Lond B Biol Sci* **371**, 20150418.

Schnetkamp PPM (2004). The SLC24 Na^{+}/Ca^{2+}-K^{+} exchanger family: vision and beyond. *Pflügers Arch* **447**, 683–688.

Additional information

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No competing interests declared.

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