

PERSPECTIVES

The effects of Ca²⁺ buffers on cytosolic Ca²⁺ signalling

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Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ sensors (Ca²⁺-binding proteins) found at different locations inside cells, there is often a requirement for Ca²⁺ signals to be strictly localized. A well-known example of this is the control of neurotransmitter release by Ca²⁺ entry through voltage-gated Ca²⁺ channels, generating short-lived local nano-domains of high Ca²⁺ concentration ([Ca²⁺]_i) (Meinrenken *et al.* 2003). A more recent, and very interesting, example is the physiological activation of two Ca²⁺-dependent transcription isoforms, NFAT1 and NFAT4. This occurs through two coincident, but spatially segregated, intracellular Ca²⁺ signals, namely in the immediate vicinity of Ca²⁺ release-activated Ca²⁺ channels in the plasma membrane and close to inositol trisphosphate receptors in the inner nuclear membrane (Kar *et al.* 2016).

Different spatio-temporal Ca²⁺ 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 Ca²⁺ spiking in the region of secretory control and global [Ca²⁺]_i elevations can be generated, which is important because Ca²⁺ 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 [Ca²⁺]_i. There has therefore, for a long time, been much interest in measuring

the volume-averaged [Ca²⁺]_i (Meinrenken *et al.* 2003), which can also be particularly helpful for a precise quantitative evaluation of Ca²⁺ handling.

The nature and concentration of intracellular Ca²⁺ buffers play an important role in determining the timing and spreading of Ca²⁺ released into the cytosol by opening of Ca²⁺ 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 Ca²⁺ buffer, for example citrate, transformed ACh-evoked local and short-lasting Ca²⁺ spikes into global and much more prolonged Ca²⁺ transients (Petersen, 1992). The timing and spatial extension of physiological Ca²⁺ signals therefore depends not only on the strength and type of stimulation but also on the affinities, mobilities and concentrations of the various intracellular Ca²⁺ buffers, which can vary significantly between different cell types. There is also a practical issue relating to the ability of Ca²⁺ buffers to influence intracellular Ca²⁺ signals. Because all Ca²⁺-sensitive fluorescent probes are Ca²⁺ buffers, they can distort the [Ca²⁺]_i signals they are designed to monitor.

It has been challenging to obtain reliable estimates for the parameters that define the dynamics of physiological [Ca²⁺]_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 [Ca²⁺]_i during single and repetitive voltage-clamp depolarizations and provide quantitative data on Ca²⁺ inflow, Ca²⁺ buffering and Ca²⁺ clearance.

Using low concentrations of the low-affinity Ca²⁺ indicator Fura-6F, in order not to overwhelm the endogenous Ca²⁺ buffers, Lin *et al.* (2017) studied the voltage-clamped nerve terminals with

patch pipettes containing solutions with minimal Ca²⁺ buffer concentrations. This allowed them to determine the Ca²⁺ binding properties of the endogenous fixed buffers and also the Ca²⁺ 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⁺–Ca²⁺ exchange for Ca²⁺ extrusion (Schnetkamp, 2004). In other experiments, Lin *et al.* (2017) used pipette solutions with 500 μM of the widely used Ca²⁺ chelator EGTA, determining its Ca²⁺ binding characteristics under realistic intracellular conditions. It turned out that the Ca²⁺ 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 Ca²⁺ chelator buffering global rather than local [Ca²⁺]_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 [Ca²⁺]_i transients in a mammalian presynaptic nerve terminal. Estimates of some of the parameters determining [Ca²⁺]_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 [Ca²⁺]_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.

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Additional information

Competing interests

No competing interests declared.

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