DOI: 10.1017/S1431927603440221
Microsc Microanal 9(Suppl 2), 2003

Correlated Measurements Of Free And Total Intracellular Calcium

S.B. Andrews,* N.B. Pivovarova,* J. Hongpaisan,* C.A. Brantner* and R.D. Leapman**

*Laboratory of Neurobiology, NINDS, and ** Division of Bioengineering and Physical Science, ORS, NIH, Bethesda, MD 20892.

Transient changes in the intracellular distribution of calcium ions act as a trigger for a large number of important physiological functions [1,2]. The nature of intracellular Ca changes is complex, however, because Ca is dynamically distributed between several compartments, the most important being the cytosol, mitochondria and the endoplasmic reticulum (ER). Within each compartment, Ca is further partitioned between a large pool bound to buffers (mainly proteins) and a much smaller pool of "free Ca^{2+}." Although small, concentration changes in the latter pools, especially cytosolic free Ca^{2+} ($[Ca^{2+}]_i$), are especially critical for signal transduction. To obtain a general picture of the interactions and spatio-temporal concentration fluctuations that underlie Ca^{2+} signaling, correlated measurements of both free and bound Ca pools are highly advantageous [3]. Optical imaging of fluorescent indicators in living cells is the method of choice for measuring $[Ca^{2+}]_i$, while analytical electron microscopy—either electron probe x-ray microanalysis (EPMA) or electron energy loss spectroscopy (EELS)—is the well established approach for determining total Ca (essentially equivalent to bound Ca) within specific subcellular compartments. When these techniques are used in tandem, the combined information provides unique insights into the regulation of Ca^{2+} signaling. To illustrate the kinds of information available with this approach, we present here results from recent work aimed at elucidating the role of mitochondrial and ER Ca^{2+} transport in the modulation of Ca^{2+} signaling in neurons.

Our first example explores the role of mitochondrial Ca^{2+} uptake in blunting the impact of depolarization-induced Ca^{2+} entry on the signaling Ca^{2+} pool, i.e., on $[Ca^{2+}]_i$ [4,5]. Optical measurements (fura-2) show that sympathetic neurons respond to depolarization with a rise in $[Ca^{2+}]_i$ that has several prominent phases (Fig. 1, upper panel). Following a sharp initial rise, $[Ca^{2+}]_i$ is clamped at a steady elevated concentration (~750 nM for a depolarization to ~0 mV) for the duration of the stimuli (<2 min), even though Ca^{2+} entry continues; upon repolarization (which closes membrane channels and stops Ca^{2+} influx), $[Ca^{2+}]_i$ recovers only slowly, exhibiting a plateau phase ($[Ca^{2+}]_i$ ~400 nM) of several minutes duration (arrow). The explanation for this complex behavior is revealed by parallel EMPA measurements of mitochondrial total calcium ($[Ca]_{MT}$) (Fig. 1, lower panel), which show that: 1) continuous Ca^{2+} uptake by mitochondria during sustained stimulation targets entering Ca^{2+} directly into mitochondria, thereby accounting for the blunted rise and clamped phase of $[Ca^{2+}]_i$; and 2) subsequent release of the accumulated mitochondrial Ca load, at a rate determined by the responsible transporters and of a length determined by the size of the load, underlies the delayed $[Ca^{2+}]_i$ recovery.

During the recovery phase, ER Ca^{2+} transport also comes into play [6]. EPMA data on total ER Ca concentrations ($[Ca]_{ER}$), indicate that $[Ca]_{ER}$, which changes little during depolarization, rises during $[Ca^{2+}]_i$ recovery, as Ca that was formerly part of the mitochondrial Ca load is transferred into the ER. This observation illustrates one face of the dual nature of ER Ca^{2+} handling, namely, its role as a Ca sink. Perhaps more commonly, the ER is viewed as a Ca source, responding to agonists like inositol trisphosphate (IP_3) or Ca^{2+} itself with Ca^{2+} release from preloaded stores [2]. This face of ER function can also be revealed by correlated free and total Ca analyses. Thus, EMPA shows that in resting neurons, the highest Ca concentration is found in the ER, as would be predicted for a Ca store (Fig. 2) [6]. Following treatments that discharge this store, for example by inhibiting the uptake pump (thapsigargin) or by opening the ER Ca^{2+} release channel (caffeine/ryanodine), $[Ca]_{ER}$ falls dramatically (Fig. 2), in conjunction with a cytosolic $[Ca^{2+}]_i$ transient. It is interesting to note that the Ca content of "fully discharged" ER is not zero.

These results are but two examples of the advantages of combining optical imaging of free Ca^{2+} with organelle-level microanalytical measurements of total Ca. In our view, this approach has general applicability in the field of Ca^{2+} regulation. For example, it has recently been used to dissect temporal aspects of Ca^{2+} signaling in synaptically activated hippocampal neurons [7]. These studies also reveal that the propagation of Ca^{2+} signals has a strong spatial component, driving home the need for spatially resolved measurements in future studies. This in turn point to a future goal for analytical microscopy—the continued development of high-resolution imaging approaches, such as EELS spectrum imaging [8-10].

References

[1] T. Pozzan et al., *Physiol. Rev.* 74 (1994) 595.
[2] M.J. Berridge, *Neuron* 21 (1998) 13.
[3] L.D. Pozzo-Miller et al., *Microsc. Res. Tech.* 46 (1999) 295.
[4] D.D. Friel & R.W. Tsien, *J. Neurosci.* 14 (1994) 4007.
[5] N. Pivovarova et al., *J. Neurosci.* 19 (1999) 6372.
[6] J. Hongpaisan et al., *J. Gen. Physiol.* 118 (2001) 101.
[7] N. Pivovarova et al., *J. Neurosci.* 22 (2002) 10653.
[8] J.L. Lavergne et al., *Microsc. Microanal. Microstruct.* 3 (1992) 517.
[9] J.A. Hunt et al., *Microsc. Microanal.* 5 (Suppl. 2) (1999) 616.
[10] Supported by the NIH Intramural Research Program.

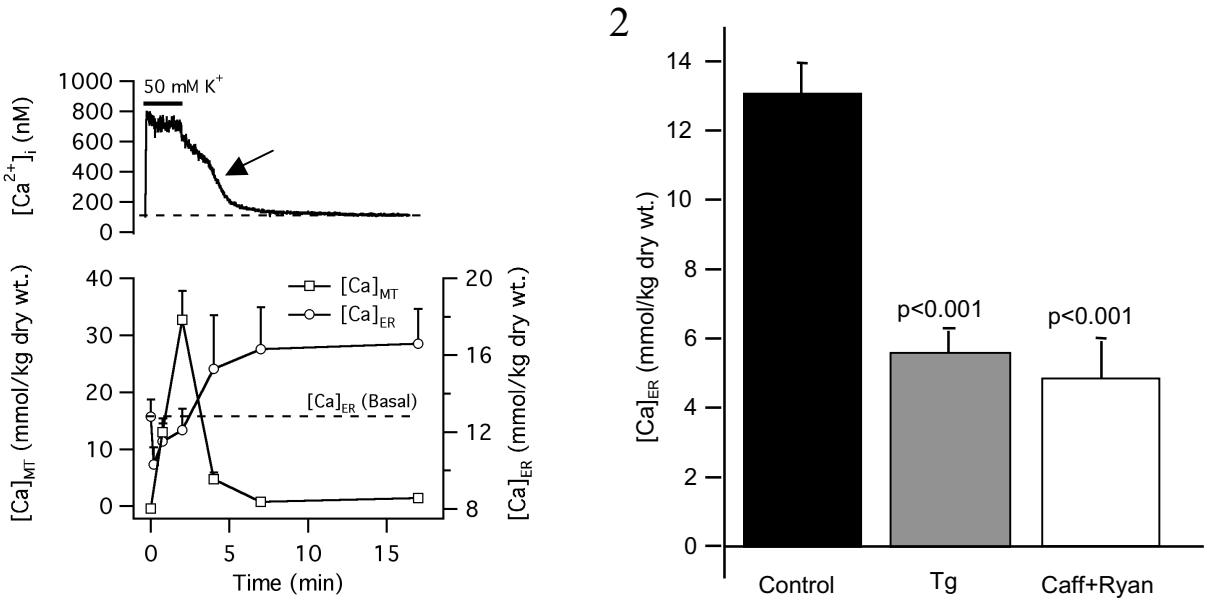

FIG. 1. Comparison between the time courses of $[Ca^{2+}]_i$ (top), $[Ca]_{ER}$ (circles) and $[Ca]_{MT}$ (squares) during a 120 s exposure to 50 mM K^+. Top trace is a representative measurement from a single fura-2 loaded sympathetic neuron, while data in lower panel are collected EPMA results from multiple neurons that were stimulated and rapidly frozen at the indicated times.

FIG. 2. The total Ca content of the ER in resting, untreated sympathetic neurons is high (left bar). Inhibition of the ER Ca^{2+} pump by 1 µM thapsigargin (Tg) leads to a large reduction in $[Ca]_{ER}$. Similarly, treatments that open the ryanodine receptor (the ER Ca^{2+} channel; 1 µM ryanodine plus 10 mM caffeine) deplete $[Ca]_{ER}$ just as effectively. Both drugs induce a transient release of Ca^{2+} into the cytosol (not shown).

https://doi.org/10.1017/S1431927603440221 Published online by Cambridge University Press