

## Commentary

# Contrasting Contributions of Endoplasmic Reticulum and Mitochondria to Ca<sup>2+</sup> Handling in Neurons

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This issue of *The Journal* includes an important pair of articles (Albrecht et al., 2001; Hongpaisan et al., 2001) that provide a comprehensive analysis of ER contributions to the handling of depolarization-induced Ca<sup>2+</sup> loads in bullfrog sympathetic ganglion neurons. The articles include measurements of ER total [Ca] made within individual cisternae identified in cryosections from rapidly frozen cells, using energy-dispersive X-ray microanalysis. Use of the perforated patch recording technique (which avoids the cytosolic dialysis produced by whole-cell recording) allowed an impressive degree of stability in the voltage-clamped currents and fura-2-based measurements of cytosolic [Ca<sup>2+</sup>] (see Fig. 1 of Albrecht et al. [2001]).

Measurements of ER total [Ca] are more direct than the conventional approach of inferring changes in ER [Ca] by measuring how various ER-modifying drugs influence cytosolic [Ca<sup>2+</sup>] transients. In fact, one of the fundamental contributions of these articles is verification that these pharmacological manipulations actually do (in most cases) produce the predicted changes in basal and stimulated total [Ca] within morphologically identified ER. For example, the relatively high resting value of ER [Ca] (12.8 mmol/kg dry weight, or ~3.6 mmol/liter hydrated) is shown to be reduced to 5.5 mmol/kg dry weight when ER Ca<sup>2+</sup> uptake is inhibited with thapsigargin, and to 4.7 mmol/kg dry weight when ER Ca<sup>2+</sup> release channels are opened with a combination of 1 μM ryanodine and 10 mM caffeine.

The articles make another fundamental contribution, namely the documentation of changes in ER total [Ca] in response to the size of the Ca<sup>2+</sup> load. Albrecht et al. (2001) present evidence that small Ca<sup>2+</sup> loads (produced, e.g., by 2 min in 30 mM K<sup>+</sup>, average cytosolic [Ca<sup>2+</sup>] ≤ 350 nM) are accompanied by both uptake and release of Ca<sup>2+</sup> from ER. Since average total ER [Ca] increases from 12.8 to 17.0 mmol/kg dry weight, it is clear that uptake predominates, and that under this condition ER acts as a Ca<sup>2+</sup> sink. The authors call this “Mode 1” behavior (Fig. 1). Hongpaisan et al. (2001) present evidence that moderate Ca<sup>2+</sup> loads (e.g., 2 min in 50 mM K<sup>+</sup>, average cytosolic [Ca<sup>2+</sup>] = 600–800 nM) are also accompanied by both uptake and

release; but release does not become regenerative, and there is no significant change in average ER [Ca]. In this situation, termed “Mode 2,” the ER might appear to be just “spinning its wheels”; but, the articles provide evidence for spatial gradients in ER behavior, as discussed below. With higher Ca<sup>2+</sup> loads (e.g., 2 min in 50 mM K<sup>+</sup> with mitochondrial Ca<sup>2+</sup> uptake inhibited, producing peak cytosolic [Ca<sup>2+</sup>] exceeding 1,000 nM), there was a net loss of Ca from the ER, with total ER [Ca] falling from 12.8 to 6.5 mmol/kg dry weight. In this situation, termed “Mode 3,” ER acts as a Ca<sup>2+</sup> source, releasing Ca<sup>2+</sup> in a regenerative manner. All of these Ca<sup>2+</sup> loads were physiological, in the sense that the “excess” Ca<sup>2+</sup> could be extruded completely by the cells. These articles, and the discussion below, do not deal with pathologically high Ca<sup>2+</sup> loads such as that depicted in diagram 4 of Fig. 1.

The idea that the net direction of Ca<sup>2+</sup> flux across ER varies with stimulus intensity and cytosolic [Ca<sup>2+</sup>] is not new; but the systematic variation of the Ca<sup>2+</sup> load applied to a single neuronal type combined with the linked measurements of total ER [Ca] unite what can be a confusing, fractionated story into a more integrated whole. Albrecht et al. (2001) elaborate a quantitative model that emphasizes graded activation of a single population of Ca<sup>2+</sup>- and ryanodine-sensitive Ca<sup>2+</sup> channels in the ER membrane. Their model reproduces many of the measured effects of ryanodine, caffeine, and thapsigargin on cytosolic [Ca<sup>2+</sup>] transients and total ER [Ca]. The model applies mainly to Mode 1 conditions, where mitochondrial Ca<sup>2+</sup> uptake (which is omitted from the model) is least prominent; but the authors suggest that this model can also account for many of the phenomena measured with the higher Ca<sup>2+</sup> loads that evoke Mode 2 and Mode 3 behaviors.

This picture of spatially averaged ER behavior in response to varying Ca<sup>2+</sup> loads is complemented by the demonstration of a spatial gradient of ER [Ca] in neurons subjected to a moderate Ca<sup>2+</sup> load. ER segments nearest the plasma membrane (and, therefore, presumably exposed to the greatest elevation of cytosolic [Ca<sup>2+</sup>]) experienced a net Ca loss (Mode 3), whereas ER segments farther from the plasma mem-

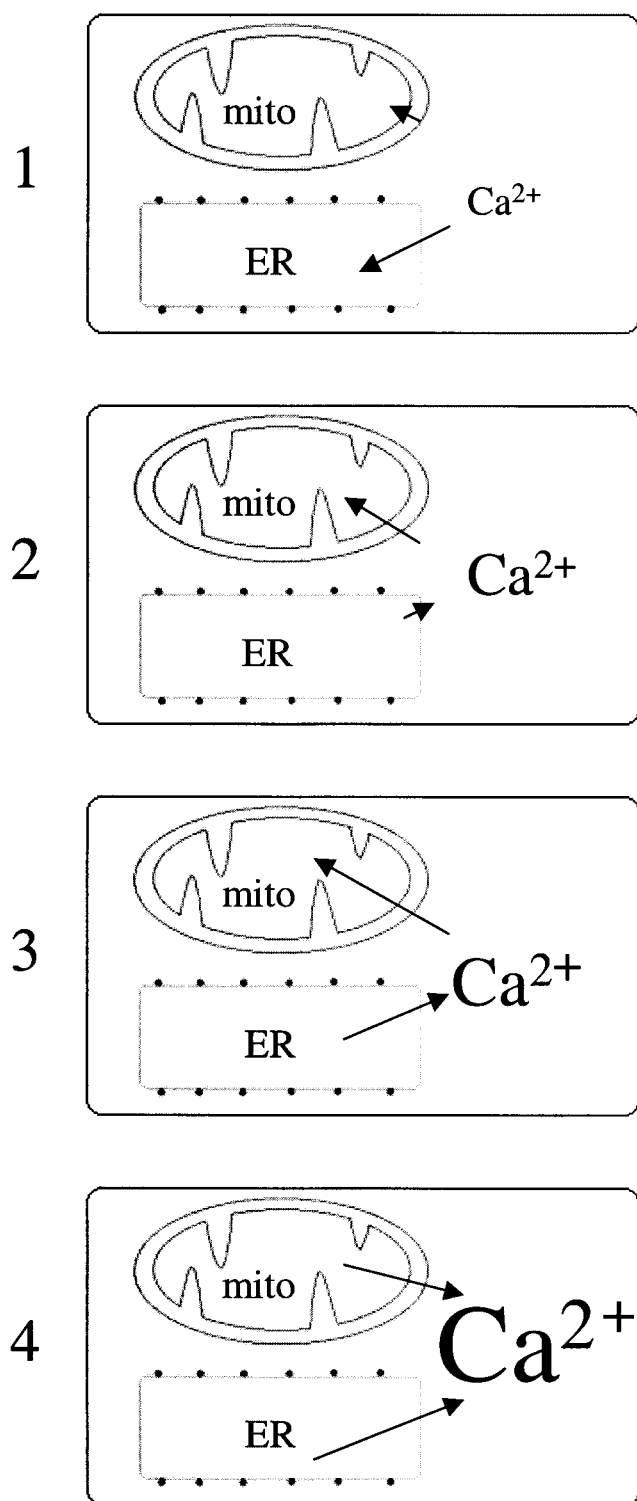


FIGURE 1. Net movements of  $\text{Ca}^{2+}$  across ER and mitochondrial (mito) membranes vary as a function of the average increase in cytosolic  $[\text{Ca}^{2+}]$ . Diagrams labeled 1, 2, and 3 depict the direction of the net average flux of  $\text{Ca}^{2+}$  across ER in states labeled modes 1, 2, and 3, respectively, by Albrecht et al. (2001) and Hongpaisan et al. (2001). In Mode 2, there is either no net average  $\text{Ca}^{2+}$  flux across ER (Hongpaisan et al., 2001) or a slight net efflux insufficient to evoke regenerative release (Albrecht et al., 2001). The cytosolic  $[\text{Ca}^{2+}]$  in modes 1–3 would produce net mitochondrial  $\text{Ca}^{2+}$  uptake as well. Diagram 4 depicts a pathologically high  $\text{Ca}^{2+}$  load suffi-

cient to open the mitochondrial permeability transition pore. These diagrams assume that the cell was in a resting state (high  $[\text{Ca}]$  in ER lumen, low  $[\text{Ca}]$  in mitochondrial matrix) before the elevation of cytosolic  $[\text{Ca}^{2+}]$ . Albrecht et al. (2001) and Hongpaisan et al. (2001) present evidence that the net ER  $\text{Ca}^{2+}$  movements represent the sum of simultaneously occurring influx and efflux, and that spatial gradients in cytosolic  $[\text{Ca}^{2+}]$  during depolarizing stimuli may cause ER and mitochondria near the plasma membrane to behave differently from their counterparts in the cell interior.

brane showed no net change in total  $[\text{Ca}]$  (Mode 2, Fig. 5 A of Hongpaisan et al., 2001). The behavior of ER segments near the plasma membrane reinforces previous evidence (Hua et al., 1993) that regenerative  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR, Mode 3 behavior) can occur even in the absence of drugs that alter ER or mitochondrial  $\text{Ca}^{2+}$  handling.

In Hongpaisan et al. (2001), measurements of ER total  $[\text{Ca}]$  are complemented by measurements of total  $[\text{Ca}]$  within identified mitochondria, based on values from Pivovarova et al. (1999). These measurements present an interesting opportunity to compare the  $\text{Ca}^{2+}$  handling behavior of ER and mitochondria. The picture that emerges presents many contrasts. Hongpaisan et al. (2001) report that, under resting physiological conditions, the total  $[\text{Ca}]$  in ER is almost four times greater than total  $[\text{Ca}]$  in cytosol (12.8 vs. 3.3 mmol/kg dry weight). In contrast, total  $[\text{Ca}]$  within the mitochondrial matrix of resting neurons was undetectably low (Pivovarova et al., 1999). ER takes up  $\text{Ca}^{2+}$  by active transport (SERCA pumps) and releases it passively via channels sensitive to cytosolic  $[\text{Ca}^{2+}]$ , ryanodine, and caffeine. The uniporter, which transports  $\text{Ca}^{2+}$  across in the inner mitochondrial membrane, is also sensitive to cytosolic  $[\text{Ca}^{2+}]$ , but in this organelle, the net direction of passive  $\text{Ca}^{2+}$  movement is inward, powered by the sizeable membrane potential ( $-150$  to  $-200$  mV) created by the extrusion of protons via the electron transport chain (for review see Gunter and Pfeiffer, 1990). Thus,  $\text{Ca}^{2+}$  efflux from mitochondria is the energy-requiring step, mediated via secondary active transport systems (e.g., mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchanger). In bullfrog sympathetic neurons, the net direction of the  $\text{Ca}^{2+}$  transport in mitochondria was always inward during high  $[\text{K}^+]$  stimuli, in contrast to the transition from net uptake to net release described above for ER.

The lumina of both organelles have powerful  $\text{Ca}^{2+}$  buffering abilities, but measurements of total  $[\text{Ca}]$  in ER and mitochondria suggest an interesting difference in the modes of  $\text{Ca}^{2+}$  storage. Hongpaisan et al. (2001) found that measurements of total  $[\text{Ca}]$  within the lumen of ER formed a normal distribution under both resting and drug-depleted conditions. This result is consistent with their suggestion that the majority of ER stores in sympathetic neurons are ryanodine-sensitive,

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and with the idea that Ca (presumably combined with buffers such as calsequestrin) is uniformly distributed within a given ER profile. In contrast, Pivovarova et al. (1999) found marked variability in values of total [Ca] measured in three nonoverlapping regions within the matrix of an individual mitochondrial profile after neuronal depolarization. The measurements of mitochondrial matrix total [Ca] presented in Hongpaisan et al. (2001) used a novel analytical approach (electron energy loss spectrum imaging) that spatially averaged [Ca] over the matrix and, thus, overcame the quantification challenge posed by the intramitochondrial [Ca] heterogeneity. A model proposed by David (1999) suggests that this microheterogeneity is due to punctate, reversible formation of a Ca salt, consistent with his finding that during repetitive stimulation of lizard motor nerve terminals, mitochondrial matrix free [Ca<sup>2+</sup>] increased to a plateau level (estimated to be ~1 μM) that was maintained even though mitochondria appeared to continue taking up Ca<sup>2+</sup>. His data, as well as those presented for hormone-stimulated pituitary gonadotropes by Kaftan et al. (2000), are consistent with the hypothesis that mitochondria are dynamic buffers, whose buffering power increases with increasing Ca<sup>2+</sup> uptake. Note, however, that some studies that measured matrix [Ca<sup>2+</sup>] using mitochondrially targeted aequorins (rather than fluorescent indicator dyes) have reported stimulation-induced elevations of matrix [Ca<sup>2+</sup>] reaching millimolar levels (e.g., Montero et al.'s [2000] study in bovine adrenal chromaffin cells).

ER and mitochondrial Ca<sup>2+</sup> uptakes have different effects on peak cytosolic [Ca<sup>2+</sup>] produced during stimulation. The relationship between peak cytosolic [Ca<sup>2+</sup>] and the size of the Ca<sup>2+</sup> load is supralinear under ER-dominant conditions (Hua et al., 1993), but sublinear for mitochondrial-dominant conditions (David et al., 1998). Albrecht et al. (2001) showed that drugs that inhibit Ca<sup>2+</sup> uptake into ER (e.g., thapsigargin) altered the time course of small, depolarization-induced cytosolic [Ca<sup>2+</sup>] transients, but had relatively little effect on peak or final cytosolic [Ca<sup>2+</sup>]. These observations, taken together with the high resting values of ER total [Ca], are consistent with the idea that the main Ca<sup>2+</sup>-related task of ER is not to limit the increase in cytosolic [Ca<sup>2+</sup>] during a sustained Ca<sup>2+</sup> challenge, but rather to contribute to intracellular signaling by acting as an "active" filter that dampens the effect of small [Ca<sup>2+</sup>] transients but amplifies the effect of [Ca<sup>2+</sup>] transients that exceed a certain threshold. In contrast, depolarizing mitochondria with protonophores (which removes the electrical gradient driving Ca<sup>2+</sup> uptake) produced a large increase in the peak amplitude of cytosolic [Ca<sup>2+</sup>] transients in cells subjected to moderate-to-large Ca<sup>2+</sup> loads (Werth and Thayer, 1994; Stuenkel, 1994; White and Reynolds, 1995; Babcock et al., 1997; Sidky and Baimbridge, 1997; David et al., 1998, David and Barrett,

2000; Hongpaisan et al., 2001), consistent with the idea that a major Ca<sup>2+</sup>-related task of mitochondria is to limit the peak amplitude of stimulation-induced cytosolic [Ca<sup>2+</sup>] transients. The passive nature of mitochondrial Ca<sup>2+</sup> uptake, combined with their large uptake capacity, may make these organelles ideally suited for rapid sequestration of large Ca<sup>2+</sup> loads.

For both ER and mitochondria, stimulation-induced elevations in Ca content decayed slowly (>15 min). On a practical note, this finding suggests that investigators need to use long intertrial intervals if they want to ensure that mitochondrial and ER Ca contents return to resting levels between successive experimental manipulations. A possible functional significance of these long decay times is that stored organellar Ca may contribute to cellular memory mechanisms (Tang and Zucker, 1997). As pointed out by Hongpaisan et al. (2001), this type of ER "memory" may be prolonged by mitochondrial Ca<sup>2+</sup> extrusion, which (after stimulation) produces a prolonged tail of elevated cytosolic [Ca<sup>2+</sup>] within the Mode 1 concentration range (200–300 nM) most conducive to ER Ca<sup>2+</sup> accumulation (Fig. 1). Additional evidence for mitochondrial influence on ER Ca<sup>2+</sup> handling is that disabling mitochondrial Ca<sup>2+</sup> uptake with a protonophore (FCCP) changed averaged ER behavior from Mode 2 (little or no net Ca loss) to Mode 3 (significant Ca loss).

Hongpaisan et al. (2001) found an interesting reciprocal relationship between the net Ca uptakes of mitochondria and ER and their distance from the plasma membrane. As noted above, during a moderate Ca<sup>2+</sup> load ER profiles near the plasma membrane lost Ca, whereas mitochondria near the membrane gained Ca. The authors mention an interesting question raised by this apparent reciprocity: is the Ca uptake by mitochondria near the plasma membrane influenced by (or might it even require) the loss of Ca from nearby ER, or is this apparent reciprocity due simply to the different but independent responses of these two organelles to the spatial gradient of cytosolic [Ca<sup>2+</sup>] that bathes them both? Isolated mitochondria require substantial levels (micromolars) of bath [Ca<sup>2+</sup>] for sustained Ca<sup>2+</sup> uptake (Gunter and Pfeiffer, 1990), and evidence has been presented that in some preparations the uptake of Ca<sup>2+</sup> by mitochondria takes place mainly from highly localized domains of high [Ca<sup>2+</sup>] found only in the vicinity of open plasma membrane or ER Ca<sup>2+</sup> channels (Rizzuto et al., 1999; Szalai et al., 2000). However, a higher affinity, saturable rapid uptake mode has been described in isolated mitochondria (Gunter et al., 1998), and others have found evidence for substantial Ca<sup>2+</sup> uptake by mitochondria in neurons and other secretory cells at lower cytosolic [Ca<sup>2+</sup>] (a few hundred nanomolars) with no required proximity to such channels (Babcock et al., 1997; David et al., 1998; Colegrove et al., 2000). The ability to measure total [Ca] within

ER and mitochondria in the same rapidly frozen cells offers the possibility of testing these ideas by determining whether or not the increase in mitochondrial total [Ca] in response to a particular stimulus is substantially reduced by drugs that block ER Ca<sup>2+</sup> release.

Another worthwhile effort would be to measure free [Ca<sup>2+</sup>] and total [Ca] within both ER and mitochondria subjected to the same stimulation patterns. Such measurements would provide insight into the buffering power within the matrix of both organelles. The reversible salt formation model of David (1999) predicts that during sustained stimulation mitochondrial total [Ca] should continue to increase even after matrix free [Ca<sup>2+</sup>] attains a plateau level. Matched measurements of ER free [Ca<sup>2+</sup>] and total [Ca] would help answer the question of whether activation of CICR in ER depends mainly on the level of cytosolic [Ca<sup>2+</sup>] (as in the model of Albrecht et al., 2001) or is also influenced by free [Ca<sup>2+</sup>] or total [Ca] accumulated within the ER lumen. Similar matched measurements in mitochondria might indicate how opening of the permeability transition pore (which could be viewed as a mitochondrial correlate of CICR) varies with changes in cytosolic and matrix [Ca<sup>2+</sup>] and total matrix [Ca].

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