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Calcium mobilization from mitochondria in synaptic transmitter release

Rosario Rizzuto

Department of Experimental and Diagnostic Medicine and Interdisciplinary Center for the Study of Inflammation, University of Ferrara, 44100 Ferrara, Italy

Mitochondria can rapidly accumulate and release Ca^{2+} upon cell stimulation. A paper by Yang and coworkers in this issue reports an unusual form of synaptic potentiation, dependent on Ca^{2+} release from mitochondria through the Na^+/Ca^{2+} exchanger and triggered by Na^+ entry through voltage-gated channels (Yang et al., 2003).

Potentiation of active synapses represents a general neuronal mechanism for recording past activity and optimizing new responses of the organism to the environment. The plasticity of neuronal connections is thus a key feature of widely diverse classes of neurons, ranging from cortical and hippocampal neurons, where the phenomenon of long-term potentiation underlies the basic principles of cellular memory, to motoneurons, where high frequency stimulation results in an sustained enhancement of neurotransmitter release (a process known as posttetanic potentiation). Yang et al. (2003) focus on the latter process and calls into action a quite unexpected player, the mitochondria. The authors studied the enhanced transmitter release that follows the tetanic stimulation of *Xenopus* motoneurons and observed that it is maintained in Ca^{2+}-free, EGTA-containing medium, i.e., an experimental condition in which no Ca^{2+} influx can occur from the extracellular space. They thus ruled out a role for voltage-gated Ca^{2+} channels of the plasma membrane and pointed to a role of intracellular Ca^{2+} stores. Unexpectedly, this store proved not to be the ER, as the pharmacological modulation of neither the inositol 1,4,5 trisphosphate–sensitive (IP3R) nor the ryanodine-sensitive (RyR) ER channel affected the post-tetanic transmitter potentiation. The authors thus proceeded to investigate the possibility that mitochondria act as a Ca^{2+} reservoir that is mobilized by the Na^+ influx triggered by tetanic stimulation. The increase of intracellular Na^+ concentration could, in principle, activate the Na^+/Ca^{2+} exchanger of mitochondria, the prevailing route for Ca^{2+} efflux from the organelle in excitable cells. Experimental evidence obtained by the authors indicates that this is indeed the case, and thus introduces a new dynamic player in synaptic Ca^{2+} signaling.

Mitochondria appear to have come a long way in Ca^{2+}-mediated cell signaling (Rizzuto et al., 2000). Indeed, in the 1960's and 1970's mitochondria were considered crucial organelles in intracellular Ca^{2+} homeostasis, acting as a major internal reservoir of this ion. The electrical gradient established through proton translocation by the respiratory chain complexes provides the driving force for Ca^{2+} accumulation across the ion-impermeable inner mitochondrial membrane. A membrane potential of 180–200 mV in respiring mitochondria maintains a constant, large driving force for Ca^{2+} uptake (thermodynamic equilibrium would be attained only if Ca^{2+} in the matrix reached concentrations 10^6 higher than in the cytoplasm, i.e., ~1 M). Biochemical work also characterized the fundamental properties of Ca^{2+} transport (whereas molecular definition is still lacking in our days). Uptake occurs through an electrogenic route, the "uniporter," presumably a gated Ca^{2+} channel that is inhibited by La^{3+} and Ruthenium red. Most efflux occurs through two exchangers: a Na^+/Ca^{2+} exchanger (mNCX, mainly active in mitochondria from muscle and neurons) and a ubiquitous H^+/Ca^{2+} exchanger (the prevalent route in nonexcitable cells). Although the molecular identity of the carrier is unknown, a number of cell-permeant inhibitors are available, the most useful being the compound CGP37157 employed in this study, which shows a good specificity for the mitochondrial mNCX, over the voltage-gated Ca^{2+} channels of the plasma membrane (Cox and Matlib, 1993). mNCX currently represents the easiest pharmacological target for affecting mitochondrial Ca^{2+} homeostasis (a common alternative choice is the inhibition of respiration or the collapse of the proton gradient with ionophores, but these procedures severely affect a variety of basic mitochondrial functions, including ATP production and often organelle structure). Finally, much interest has been raised recently by a channel of very large conductance known as permeability transition pore (PTP), the opening of which is triggered by a variety of drugs and cellular stress conditions. Although it is unlikely that this route plays a role in mitochondrial Ca^{2+} uptake or

Address correspondence to Dr. Rosario Rizzuto Dept. of Experimental and Diagnostic Medicine, University of Ferrara, Via Borsari, 46 44100 Ferrara, Italy. Tel.: 39-0532-291-361. Fax: 39-0532-247-278. email: rzr@dns.unife.it

Abbreviations used in this paper: PKC, protein kinase C; PTP, permeability transition pore.

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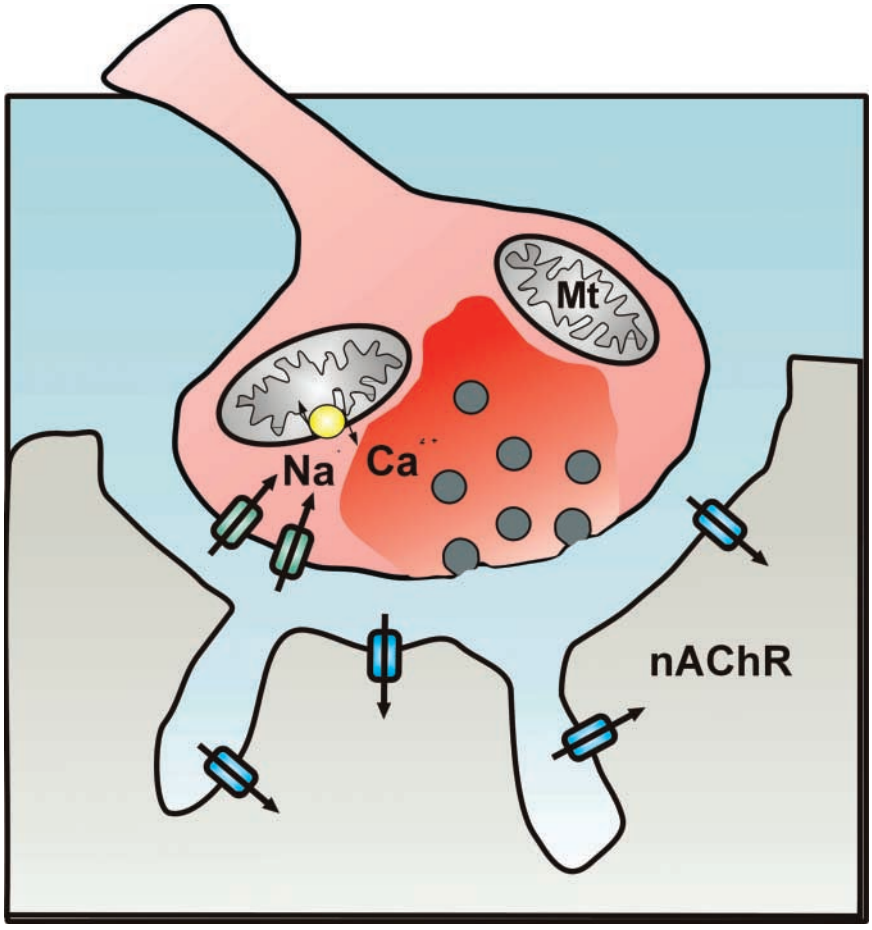

Figure 1. **Schematic outline of the proposed role for mitochondria (Mt) in synaptic release at the neuromuscular junction.** Na^+ influx through voltage gated Na^+ channels (green) increases Na^+ concentration in the presynaptic terminal, in turn triggering mitochondrial Ca^{2+} release through the mitochondrial Na^+/Ca^{2+} exchanger (yellow). Ca^{2+} release triggers the fusion of presynaptic vesicles with the plasma membrane and the release of neurotransmitter. The released neurotransmitter binds to and opens acetylcholine receptors (blue) on the postsynaptic terminal.

release occurring in physiological conditions, the facilitatory role of Ca^{2+} in PTP opening and its putative role in mitochondria-dependent apoptosis make it an interesting molecular complex that needs to be considered in organelle Ca^{2+} signaling.

Despite this sophisticated machinery dedicated to Ca^{2+} homeostasis, in the 1980's the role of mitochondria in calcium signaling declined into oblivion. In those years, it became clear that the endo/sarcoplasmic reticulum was the source of rapidly released Ca^{2+} upon agonist stimulation (Streb et al., 1983) and that the bulk cytosolic Ca^{2+} concentration, in both resting and stimulated cells, was too low to allow significant accumulation through the low-affinity uniporter of the inner mitochondrial membrane. Thus, the role of mitochondria was thought to be restricted to conditions of calcium overload, e.g., those that can occur in neurons in excitotoxicity.

The situation was reversed when tools became available for selectively monitoring Ca^{2+} concentration within the mitochondria: the targeted chimeras of the photoprotein aequorin, the positively charged fluorescent dyes accumulating in the mitochondria and, more recently, the recombinant fluorescent indicators obtained by molecularly engineering GFP (for review see Rudolf et al., 2003). These probes showed that, in all cell types, a rapid rise in mitochondrial Ca^{2+} concentration follows that induced in the cytoplasm by the opening of ER/SR or plasma membrane channels. The key to this efficiency in vivo was shown to be the ability of mitochondria to be in close contact with the Ca^{2+} channels and thus sense local domains of high Ca^{2+} concentration, sufficient to cause accumulation through the uniporter (Rizzuto et al., 1998).

Thus, mitochondria returned to the calcium signaling stage and their prompt responses were shown to be involved in the control of widely different cell functions: the stimulation of dehydrogenases located in the matrix (Hajnoczky et al., 1995), with consequent enhancement of ATP production (Jouaville et al., 1999), the gross alteration of mitochondrial structure in some apoptotic pathways (Pinton et al., 2001), the spatial limitation of Ca^{2+} increases to defined cell portions, e.g., restricting Ca^{2+} increases to the apical pole of pancreatic acinar cells through the firewall activity of densely packed mitochondria (Tinel et al., 1999), the clearance of large Ca^{2+} loads in adrenal chromaffin cells (Herrington et al., 1996), and the modulation of Ca^{2+} release through channels that are positively or negatively regulated by Ca^{2+} itself (e.g., the IP3-sensitive channel of the ER or the CRAC channel of the plasma membrane) (Hoth et al., 1997; Hajnoczky et al., 1999).

What is the situation in neurons? Much interesting work has been produced in the past years, showing that neurons are no exception to the mitochondrial renaissance. Studies performed in different types of neurons with different experimental approaches such as measurement of free Ca^{2+} with dyes (Billups and Forsythe, 2002; David et al., 1998) or of total calcium by x-ray microanalysis or electron spectro-

scopic imaging (Pivovarova et al., 1999; Pezzati et al., 2001), demonstrated rapid Ca^{2+} uptake into mitochondria upon opening of voltage-gated channels or ionotropic glutamate receptors. The uptake is reversible, with efflux occurring rapidly through the mNCX, and depends on close proximity to the plasma membrane and the capacity to sense the microdomains generated by the opening of Ca^{2+} channels (Pivovarova et al., 1999). Ca^{2+} uptake stimulates organelle metabolism and/or the activation of ROS production and other deleterious effects in the mitochondria (Carriedo et al., 2000), whereas in the cytoplasm mitochondria act as a fixed buffer shaping the pattern of Ca^{2+} increase by rapidly taking up Ca^{2+} entering from the plasma membrane channels (Werth and Thayer, 1994), and then releasing it through the mNCX (Hoyt et al., 1998).

According to Yang et al. (2003), however, mitochondria can also play a completely different role. In their view, mitochondria are partially loaded with Ca^{2+} even before a train of synaptic impulses. Na^+ influx triggered by the opening of voltage-gated Na^+ channels then induces the release of Ca^{2+} through the mNCX (Fig. 1). This mitochondria-dependent $[Ca^{2+}]$ rise is in turn responsible for the potentiation of neurotransmitter release from the motoneuron terminal. In other words, mitochondria act as a rapidly mobilisable Ca^{2+} pool activated not by a second messenger or by Ca^{2+} itself (as for the ER pool), but by the ionic change responsible for the membrane depolarization. Moreover, the authors show that mitochondrial Ca^{2+} release (and the ensuing posttetanic potentiation of transmitter release) is dependent on protein kinase C (PKC) activity, as it is blocked by a number of PKC inhibitors. This latter observation is in agreement with recent unpublished results from our laboratory showing that some PKC isoforms specifically modify the mitochondrial Ca^{2+} responses of HeLa cells. This result suggests that mitochondria may modify their participation in calcium signaling by integrating through time the activity of different signaling pathways, essentially making mitochondria a tunable Ca^{2+} buffer.

Obviously, much remains to be understood: in particular, the Ca^{2+} content of mitochondria before cell stimulation was thought to be low and the release rate through the mNCX was not expected to be able to generate the high $[Ca^{2+}]$ needed to trigger secretion. These very interesting results must thus be confirmed in different neuronal systems and experimental setups and include the possibility of using the available probes to directly monitor the kinetics of mitochondrial Ca^{2+} change. If this proves to be a general mechanism for the potentiation of transmitter release, mitochondria will emerge as unique, versatile players in neuronal Ca^{2+} homeostasis, acting both as sources and sinks for Ca^{2+} in different cellular domains and exerting an important regulatory role. In this case, many fascinating questions are ready to be addressed: do other signaling pathways besides PKCs converge on mitochondrial Ca^{2+} homeostasis; how long does this mitochondrial sensitization last; and is the mitochondrial distribution to active synapses controlled, and how? But one can already conclude that mitochondria have finished their long march to reach the spotlight of neuronal calcium signaling.

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