

Commentary

Mitochondrial Memory Banks

Calcium Stores Keep a Record of Neuronal Stimulation

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Many of the central questions in neurobiology today can be reduced to a single form: How does brief stimulation of a neuron lead to a more prolonged change in its cellular properties? For example, a train of action potentials lasting only a few seconds can alter the firing pattern of a neuron, or its ability to release neurotransmitter, for periods of many minutes or hours and can even lead to long-term changes in gene expression that affect the properties of the cells for many days. A slightly briefer stimulus, or one in which the same number of action potentials are applied at a lower frequency, may fail to evoke these events. Ongoing research suggests that mitochondria may be key organelles that participate early in such decision making.

The work of cell biologists in the last few years has provided an explosion in our knowledge of how mitochondria participate in the life-or-death decision of whether a cell will undergo apoptosis or survive in response to specific biological signals (Kroemer et al., 1995; Kluck et al., 1997; Yang et al., 1997). An appreciation of how these organelles contribute to the normal physiological responses of vertebrate neurons has, however, been slower to develop. Mitochondria, of course, participate in the production of ATP that is required for normal cellular functions and play a role in the buffering of cytosolic calcium (Nicholls and Ferguson, 1997; Babcock and Hille, 1998). These roles have, however, frequently been relegated to the domain of "housekeeping" functions.

A more recently recognized role for mitochondria in nerve terminals is in the phenomenon of posttetanic potentiation. When a presynaptic axon is stimulated repetitively for several seconds, usually at rates of ~ 10 – 50 Hz, its ability to release neurotransmitter is subsequently changed in complex ways (Fisher et al., 1997; Zucker, 1999). In many cases, a single presynaptic action potential triggered within 20–60 s of the end of the stimulus train evokes significantly more neurotransmitter release than an action potential applied before the tetanus. Stronger stimulation can extend the duration of potentiated responses to many minutes. Such posttetanic po-

tentiation depends on a persistent elevation of cytoplasmic calcium levels in the presynaptic terminal after the stimulus train (Swandulla et al., 1991). This lingering tail of elevated calcium levels has also been termed "residual calcium." Experiments by Tang and Zucker (1997) using the crayfish neuromuscular junction have provided clear evidence that mitochondria in the presynaptic terminals are required for the prolonged elevation of calcium levels. One key experiment used the protonophore CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), which impairs the function of mitochondria by dissipating the negative membrane potential across the inner mitochondrial membrane, thereby preventing calcium accumulation into the matrix of the mitochondria. The residual tail of calcium, and the accompanying potentiation of synaptic responses, were completely eliminated by this drug. Similar results were obtained using ruthenium red, an inhibitor of calcium entry into mitochondria, and by an inhibitor of mitochondrial calcium efflux. The findings of these authors therefore indicated that, during the tetanus, calcium is rapidly taken up from the cytoplasm into the mitochondria within the presynaptic terminal. After the end of the tetanus, calcium is then slowly released back into the cytoplasm over a period of several minutes.

Colegrove et al. (2000a,b) have now provided a full quantitative analysis of the uptake and subsequent release of calcium from mitochondria in intact sympathetic neurons, and have developed a kinetic model, which accounts for the prolonged plateau of calcium elevation that follows stimulation of these cells. The use of pharmacological agents allowed them to separate the net flux of calcium into and out of mitochondria into its uptake and release components at various times during and after a depolarizing stimulus. The contribution of intracellular organelles other than mitochondria was eliminated by using cells treated with thapsigargin, an inhibitor of calcium accumulation into endoplasmic reticulum stores. The neurons were stimulated using either elevated extracellular potassium ion concentrations or voltage-clamp pulses, rather than repetitive

trains of action potentials, to trigger calcium entry into the cells through plasma membrane channels. As expected, it was found that the development of a prolonged plateau of residual calcium depended on the strength and duration of the stimulus. Brief or weak depolarizations produced elevations of cytoplasmic calcium that decayed rapidly towards basal levels after the end of the stimulus, largely as a result of extrusion through plasma membrane pumps. In contrast, stronger stimuli, analogous to tetanic stimulation, produced prolonged plateaus of cytoplasmic calcium that depended on the duration of stimulation and could last for as much as 5–10 min after the end of the stimulus.

The occurrence of prolonged plateaus of calcium after stimulation, as well as their quantitative properties, can be attributed to the action of two mitochondrial processes that occur across the inner mitochondrial membrane: the calcium uniporter and the sodium/calcium exchanger. Calcium entry from the cytoplasm into the mitochondrial matrix occurs rapidly during stimulation, as a result of the calcium movement down its electrochemical gradient through the uniporter. The subsequent release of calcium back into the cytoplasm occurs through the sodium/calcium exchanger and can be inhibited by CGP 37157, a specific inhibitor of the exchanger, or by intracellular solutions lacking sodium ions. Because of the high activity of the uniporter during the stimulus, and because the exchanger appears to be inhibited by high levels of cytoplasmic calcium, the exchanger makes little contribution to the overall calcium transient during stimulation. After the stimulus, however, both processes (as well as plasma membrane pumps) contribute to setting the level of calcium within the cell. In particular, it is the slow rate of calcium extrusion through the exchanger that causes the development of the prolonged plateau after stimulation (provided that the stimulus has been strong enough to cause a significant accumulation of calcium into the mitochondrial matrix).

One unexpected finding of the quantitative analysis of calcium fluxes in the intact sympathetic neurons is that substantial fluxes of calcium into and out of the matrix occur even at low levels of cytoplasmic calcium, such as those achieved with weaker stimuli that do not trigger residual plateaus in calcium levels. During such weak stimulation, the rates of influx and release are comparable and do not therefore significantly influence calcium levels in the cytoplasm. Such weak stimulation may, however, alter calcium levels within the mitochondrial matrix, and thereby alter biochemical events such as the rate of production of ATP (Robb-Gaspers et al., 1998). This finding raises an interesting comparison between the activity of mitochondria in neurons and nonexcitable cells. It has been shown that, in nonexcitable cells, the ability of mitochondria to

take up calcium at low levels of cytoplasmic calcium concentrations results from their specialized physical association with the endoplasmic reticulum (Rizzuto et al., 1998). As a result of this close association, when the cells are exposed to stimuli that trigger the release of calcium from the endoplasmic reticulum, mitochondria are exposed to a level of calcium that is substantially higher than that in bulk cytoplasm. It is not yet known if a similar functional interaction between endoplasmic reticulum and mitochondria occurs at the somata of neurons, and, as stated above, the experiments of Colegrove et al. (2000a,b) on bullfrog sympathetic neurons were carried out under conditions in which endoplasmic reticulum stores were rendered inoperative. The synaptic terminals of many neurons, however, appear to be relatively deficient in endoplasmic reticular membranes (Jonas et al., 1999), suggesting that mitochondria at these locations probably interact with cytoplasmic calcium directly.

The relative simplicity of the uniporter/exchanger model and its success in predicting the time course of calcium transients under a relatively wide range of stimulus conditions raises a number of questions. For example, what is the role of other mitochondrial processes that have been suggested to control the flux of calcium and other ions across mitochondrial membranes? Studies with isolated mitochondria have found that depolarization or elevations of matrix calcium can trigger a large increase in the permeability of the inner mitochondrial membrane. This occurs through the activation by calcium of a large relatively nonselective conductance channel known as the mitochondrial permeability transition pore (mPTP; Zoratti and Szabo, 1995; Bernardi and Petronilli, 1996). While it has been considered that activation of the mPTP channel may be an early step in the initiation of apoptosis, experiments have suggested that mPTP, acting in a low-conductance mode, also may become activated during normal physiological signaling in nonexcitable cells and heart cells (Altschuld et al., 1992; Ichas et al., 1997).

In addition to the uniporter and mPTP, a variety of other ion channels have been described in both inner and outer mitochondrial membranes (Sorgato and Moran, 1993). Historically, the outer mitochondrial membrane has often been considered to be a leaky sieve, through which ions and smaller metabolites can pass freely. Nevertheless, more recent experiments have suggested that the passage of ATP and other metabolites between the intermembrane space and the cytoplasm may be gated by the activity of VDAC (voltage-dependent anion channel), a relatively nonselective channel of the outer membrane (Rostovtseva and Colombini, 1996; Hodge and Colombini, 1997). Moreover, patch-clamp studies of mitochondria within intact presynaptic terminals of the squid giant synapse have found that

a large and prolonged increase in the conductance of mitochondrial membranes occurs shortly after the onset of a tetanizing train of action potentials (Jonas et al., 1999). The increase in conductance lasts for tens of seconds after the stimulus, and its time course generally matches that expected for residual calcium in the terminal. It is not yet clear if this conductance change represents ion channel activity in the outer or inner mitochondrial membranes, or a combination of both. One possible explanation of the conductance increase, however, is that the efflux of calcium ions through the calcium/sodium exchanger in the inner membrane is coupled to ion channel activity in the outer membrane, allowing the passage of both calcium and metabolites into the cytoplasm after tetanic stimulation.

It is not known whether the activation of mPTP, or of other conductances in inner or outer mitochondrial membranes, can be triggered by stimulation of bullfrog sympathetic neurons. The modeling studies of Colegrove et al. (2000b) certainly suggest that such additional processes are not required to simulate the pattern of calcium transients that result from the stimulus conditions that were used in these experiments. It is likely, however, that mitochondria in different cell types are more specialized than is generally recognized. For example, at some synapses, mitochondria appear not to take up calcium ions under normal conditions (Zenisek and Matthews, 2000). Mitochondria in cells such as gonadotropes, which secrete only infrequently, have calcium responses that differ substantially from those of classical neurons (Kaftan et al., 1999). In developing neurons, patterns of stimulation influence not only the subsequent release of neurotransmitter, but determine whether specific synaptic connections will survive or whether a neuron undergoes apoptosis, events that are very likely to involve changes in mitochondrial function. Moreover, in many adult neurons, repeated bursts of synaptic stimulation separated by several minutes can produce long-term cellular effects that differ from those of a single tetanic stimulus (Reymann et al., 1985; Huang et al., 1994; Mauelshagen et al., 1996). It will be interesting to determine to what extent mitochondrial ion channels and transporters are adapted to function in each of these types of neuronal responses.

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