

Appendix A14.17

A Primer on Mitochondrion and the Brain

Prior to discussing the evidence concordant with mitochondrial pathophysiology in bipolar disorder, it may be advantageous to review some of the important features of mitochondria, especially in regards to central nervous system physiology. In addition to the well known function of energy production via oxidative phosphorylation, mitochondria also have important roles in apoptosis and the regulation of intracellular calcium (Ca^{2+}). As we discuss below, increasing evidence suggests that the latter action may be critically important in regulating the release of, and response to, neurotransmitters.

Structurally, mitochondria display tissue-specific variability in size and shape. However, certain characteristics are constantly preserved. The mitochondrion has two membranes: a highly permeable outer membrane, and an ion-tight inner membrane. The space inside the inner membrane, referred to as the mitochondrial matrix, is exceedingly rich in protein, and has tightly regulated ion concentrations. In contrast, the inter-membrane space is essentially in ionic equilibrium with the cytosol.

Disorders of mitochondrial function tend to be most strongly manifest in tissues that have the highest metabolic demand, which are presumably more sensitive to any deficiency in energy availability. Symptoms relating to malfunction of the brain, heart, skeletal muscle, and hormone-secreting tissues are most prominent in mitochondrial disorders (Wallace, 1999). Importantly, since mitochondrial DNA (mtDNA) mutations may accumulate over the lifetime, tissues composed of post-mitotic cells (such as neurons) are particularly sensitive to their deleterious effects.

ATP Production and Oxidative Phosphorylation

The earliest documented function of the mitochondrion is the metabolic production of energy within the cell. In this process, high-energy electrons donated by fuel substrates are passed between the complexes of the electron transport chain (ETC), and the resulting energy release is used to transport hydrogen ions (protons) out of the mitochondrion. This process generates a strong electrochemical gradient across the mitochondrial membrane, which is used to drive the synthesis of ATP from ADP and inorganic phosphate. The maintenance of the mitochondrial membrane potential (MMP) is therefore critical to its function. Indeed, loss of MMP causes the ATP synthase to function in reverse, transporting protons into the mitochondrial matrix at the expense of ATP.

One byproduct of oxidative phosphorylation is the production of reactive oxidative species (ROS), such as hydrogen peroxide, peroxide ions, nitrosyl ions, etc. Because of the unpaired electron present in these compounds, they are capable of reacting a wide variety of biological substrates, including protein thiol groups, membrane lipids, and nucleic acids, leading to damage and mutation. Because of their destructive properties, evolution has selected for mechanisms that protect against ROS. For example, the reduced form of the thiol-containing tripeptide glutathione (GSH) neutralizes ROS, being converted to the oxidized form (GSSG). Interestingly, recent evidence suggests that astrocytes may serve to provide GSH for neighboring neurons, buffering oxidative stress during periods of intense activity (see Dringen and Hirrlinger, 2003 for review).

Calcium Uptake and Neuronal Function

Recent data has evidenced that mitochondria play an important role in sequestering increased intracellular Ca^{2+} by agonist stimulation. Mitochondrial Ca^{2+} uptake from and release into the cytosol has important consequences for neuronal and glial activity, modulating physiological and pathophysiological cytosolic responses (Simpson and Russell, 1998). The mitochondrion and the endoplasmic reticulum (ER) participate cooperatively in the regulation of intracellular Ca^{2+} , with the relative importance of these organelles varying between cell type and subcellular compartment.

The strong negative charge within mitochondria makes Ca^{2+} uptake highly favorable. Because of this property, mitochondria buffer against large rises in intracellular Ca^{2+} . Naturally, a large movement of positively-charged Ca^{2+} into the mitochondrion will exert a depolarizing effect. Should this increase surpass its metabolic capacity to export protons (as well as other cations), the mitochondrion will depolarize, leading to the cessation of ATP synthesis and possibly to apoptosis (discussed later).

Increasing evidence suggests mitochondria may be integrally involved in the general processes of synaptic plasticity. By directly recording from presynaptic mitochondria in the squid giant synapse, researchers determined that transient Ca^{2+} -dependant inner membrane conductance are temporally correlated with post-tetanic potentiation (Jonas et al., 1999). The depolarization of presynaptic mitochondria has been shown to impair neurotransmitter release following tetanic stimulation, through the disruption of intracellular Ca^{2+} buffering (Billups and Forsythe, 2002). Notably, the endoplasmic reticulum was not found to be significantly involved in presynaptic Ca^{2+} buffering. In a more detailed investigation of the relative roles of mitochondrial and ER Ca^{2+} buffering, it was found that the dendritic mitochondrion rapidly

accumulates Ca^{2+} , while the endoplasmic reticulum displays a more delayed increase in Ca^{2+} during high frequency stimulation (Pivovarova et al., 2002).

Several independent additional lines of evidence also favor a role of mitochondria in synaptic plasticity. Genetic deletion of voltage-dependant anion channel (VDAC; porin) 1 and 3 have been shown to cause impairments in learning and memory, as well as synaptic plasticity (Weeber et al., 2002). In further work, these researchers found that cyclosporin A, which inhibits PTP opening, increased the speed at which brain mitochondria take up Ca^{2+} and become depolarized, similar to the porin 1 mutation (Levy et al., 2003). This work suggests the intriguing possibility that PTP opening may occur under physiological circumstances in the nervous system, and contribute to synaptic plasticity.

Other evidence comes from observing mitochondrial adaptations to plasticity. Increased synaptic activity has been shown to induce the expression of mitochondrial-encoded genes, suggesting that the regulation of metabolism is an important component in the long-term regulation of synaptic strength (Williams et al., 1998). This regulation occurred even with stimulations that were under the threshold for long-term potentiation induction, suggesting that a sort of 'metabolic priming' might take place.

The uncoupling protein (UCP) family may have an important role in mitochondrial Ca^{2+} regulation, and by extension, synaptic plasticity and neuronal apoptosis (Mattson and Liu, 2003). The UCPS, originally identified in thermogenic brown adipose tissue, mimic the actions of pharmacological uncouplers by increasing mitochondrial permeability to protons. Interestingly, the UCP family appears to have great importance in the CNS, involved in processes such as neuroprotection (Bechmann et al., 2002;). Finally, the activation of mitochondrial potassium channels is necessary for the phenomenon of ischemic preconditioning, in which neurons or cardiocytes exposed to a brief mild ischemia are less likely to undergo apoptosis upon subsequent ischemia. Pharmacological induction of these channels likewise mimics the effect of preconditioning.

All in all, these findings suggest that mitochondrial Ca^{2+} sequestration has a key role in modulating the tone of synaptic plasticity in a variety of anatomical regions. This buffering appears to 'backload' synaptic transmission, by preventing excessive or asynchronous release during, and preserving or increasing the synaptic response following, high frequency stimulation. Additionally, energy metabolism and its regulation are likely to be highly involved in long-lasting changes in synaptic strength. For further discussion of recent research into the mitochondrion's role in synaptic plasticity, the reader is referred to Mattson & Liu's recent review (Mattson and Liu, 2003).

As mitochondrial Ca^{2+} uptake is thought to be a key regulator of NMDA-mediated intracellular Ca^{2+} surges, failure of the MMP under high Ca^{2+} load may be an essential step in excitotoxicity (Nicholls and Ward, 2000). Upon NMDA receptor activation, channel opening allows a rapid influx of Ca^{2+} into the cytosol, which can lead to the induction of apoptosis. Mitochondria are able to rapidly buffer this Ca^{2+} transient. If, however, mitochondrial Ca^{2+} uptake is blocked, apoptosis is prevented. Notably, this protection occurs despite a significantly larger increase in intracellular Ca^{2+} , indicating that it is the mitochondrial, rather than cytosolic, Ca^{2+} concentration that leads to excitotoxic apoptosis (Stout et al., 1998). ROS production has been observed to increase in response to elevations in mitochondrial Ca^{2+} , possibly via interfering with the electron transport chain (Meynier et al., 2003). However, other work has suggested that Ca^{2+} uptake may activate PTP independently of ROS production (Chalmers and Nicholls, 2003).

In response to a number of stimuli, the mitochondrial membranes may be breached by the PTP. In most cell types, the opening of the PTP is believed to be a catastrophic event, shutting down respiration and potentially inducing apoptosis. However, as mentioned earlier, there is some evidence that transient opening (“flickering”) of the PTP may occur in neurons under physiological conditions (Buckman and Reynolds, 2001).

The opening of the PTP has a number of important consequences. First, mitochondria undergoing permeability transition are immediately depolarized; ATP synthesis immediately stops, or is reversed (i.e., the ATP synthase begins degrading ATP). Secondly, a number of proteins are released from the intermembrane space. Some of these proteins, such as cytochrome c and apoptosis-inducing factor (AIF), lead to the activation in the cytosol of apoptotic proteases (called “caspases”) and other apoptotic processes. It is believed that this release is the first irreversible step of apoptosis, after which the cell is committed to undergo programmed cell death. The permeability transition appears to be an essential step in both apoptotic and necrotic cell death, depending on whether sufficient ATP is still available for apoptosis (Kim et al., 2003).

Intriguingly, mounting evidence suggests that activation of mitochondrial apoptotic cascades may lead to a process of “synaptic apoptosis”, in which apoptotic processes are activated in a highly localized manner. It is thus possible that individual synapses or neurites may selectively undergo atrophy, and provide a mechanism for synapse loss in both physiological and pathophysiological processes. Isolated cortical synaptosomes (pinched-off synapses that retain metabolism and neurotransmitter release) appear to have fully-functional apoptotic signaling pathways, such as MMP depolarization and caspase release (Mattson et al., 1998a; Mattson et al., 1998b). Additionally, apoptotic signaling in the synaptic compartment appears to

have some synapse-specific effects, such as the degradation of AMPA-sensitive glutamate receptors (Glazner et al., 2000).

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