The Burst of Mitochondrial Diseases: Neurons and Calcium

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In the last decade there has been a tremendous burst of publications on mitochondrial diseases. In the past 5 years alone more than 5,000 articles have appeared on this subject [Figure 1]. We would like to restrict this review to two aspects: mitochondrial diseases of the nervous system, and their connection to intracellular calcium. Most of the diseases are due to neuronal cell death and are related to mitochondria and calcium dysregulation [1]. In some cases faulty intracellular regulation of calcium is the primary cause of cell death, but in others is secondary to genetic defects. We will focus on five diseases; two of them are fairly common: Parkinson's disease and Alzheimer's disease, while the other three are quite rare: Huntington's disease, Leber's hereditary optic neuropathy, and amyotrophic lateral sclerosis.

Ca2+ ions are of great importance in the normal function of the nervous system. They are involved in a large number of important cellular processes such as transmitter release [2,3], action potential conduction [4], and gene expression [3]. Large increases in the free intracellular calcium concentration ([Ca2+]i) cause cell death (apoptosis) [1]. There is a large number of cellular organelles that control [Ca2+]i (for review see ref. 5). These include, among others, the surface membrane [6,7], endoplasmic reticulum [8], nucleus [8], and secretory vesicles [9]. Of special importance in the context of this review is the regulation of intracellular calcium by the mitochondria. This was suggested more than 30 years ago [10], including their involvement in the function of the nervous system, by one of us [2,11]. However, this notion was not immediately accepted and only in the past decade has the basic importance of the mitochondrion in regulation of the intracellular calcium and neuronal function been shown [12].

Mitochondria are also involved in the generation of ATP [13], in intermediary metabolism, in building and recycling molecular building blocks [6], in protecting the cells from oxidative stress [14], and in production of reactive oxygen species [14]. The ATP molecules, generated by mitochondria, play two roles: ATP is a high energy molecule that participates in a large number of cellular processes. In addition, ATP stored in synaptic vesicles is released upon nerve stimulation and is considered to be one of the important elements of purinergic transmission [15].

Mitochondria may be involved in calcium metabolism in two different ways. First, mitochondria can take up calcium by an energy-dependent process and thus reduce [Ca2+]i. Second, mitochondria can release their calcium content upon appropriate stimulation. These calcium-regulatory processes occur via a specific transporter, the sodium-calcium exchange (Figure 2), and via channels that permit the flow of calcium ions through the mitochondrial membranes. We show in this review that both these functions are involved in a number of mitochondrial diseases of the nervous system. Impaired mitochondrial function causes an increase in [Ca2+]i and quite frequently cell death (for detailed review of the role of mitochondria in apoptosis see ref. 16). Several toxins [17-19] exert their role by affecting the calcium metabolism of the mitochondria. For instance, manganese (Mn2+) shares the unipot-mechanism of mitochondrial Ca2+ influx (Figure 2) and, when in excess, was found to be toxic to neurons of the globus pallidus, leading to a Parkinson-like syndrome [19].

The mitochondrion is an extremely versatile organelle with vital roles for cell function and survival. Any damage can result in cell dysfunction or a degenerative disorder. Identifying the cause of mitochondrial dysfunction may get us closer to understanding the pathogenesis and eventually to a rational treatment.

Figure 1. Graph showing the tremendous increase in recent years of the number of articles published on mitochondria and their involvement in various diseases.
Huntington's disease

One of the diseases where a clear connection has been established between mitochondria, calcium and neurodegeneration is Huntington's disease. This is a dominant autosomal neurodegenerative disorder showing an expansion of a CAG-trinucleotide repeat in the first exon of the HD gene [20]. This expansion induces formation of a mutant HD protein (huntingtin\(_m\)). It has been shown [21] that huntingtin\(_m\) from patients with HD have lower membrane potentials and depolarize at a lower calcium load than huntingtin\(_m\) from controls. These changes were found in liver and in brain mitochondria and were suggested to associate with direct interaction of huntingtin\(_m\) with the mitochondrial membrane [21]. Moreover, incubating normal mitochondria with the fusion protein containing abnormally long polyglutamine repeats, as it appears in huntingtin\(_m\), induces the mitochondrial calcium defect observed in human patients and in transgenic animals with HD [20]. It was shown that huntingtin\(_m\) causes an increased sensitivity of the IP\(_3\)-receptor to IP\(_3\)-mediated Ca\(^{2+}\) signaling in neurons by binding to the neuronal isoform of IP\(_3\)-receptor [22]. Hence, it appears that neuronal cell loss found in HD is the result of huntingtin\(_m\), which causes an increased calcium release from IP\(_3\)-receptor that might be responsible for apoptosis of specific neurons containing relevant IP\(_3\)-receptor isoform.

Leber's hereditary optic neuropathy

While the mutation leading to HD is found in nuclear DNA, there are also neuropathies associated with mutations in mitochondrial DNA. Mitochondria are the only cellular organelles known to have their own DNA. It has been shown that mtDNA undergoes mutations at a rate five to ten times faster than nuclear DNA [23] and mitochondrial ability to repair mutations is very low. One of the mitochondrial mutation diseases is LHON. This condition, described in 1871 by the German ophthalmologist Theodore Leber, is characterized by a bilateral degeneration of the optic nerves with an incidence of 1 in 25,000 [24]. This maternally inherited mitochondrial disease is due to one of three mitochondrial DNA point mutations (G3460A, G11778A, T14484C) that affect different subunits of complex I [25] (Figure 3), which is of key importance for normal mitochondrial function. LHON accounts for about 3% of cases of blindness in young adults. Pathologic studies have shown degeneration of both the ganglion cell layer and the optic nerve without any signs of inflammation [26]. In addition, there is marked degeneration of the lateral geniculate nucleus. Electron microscopy shows swollen ganglion cells containing both swollen mitochondria and Ca\(^{2+}\)-filled double-membrane-bound structures, most probably damaged mitochondria [26]. The molecular mechanism responsible for the increased accumulation of calcium in mitochondria is not yet known. Cells from LHON patients are especially sensitive to oxidative stress. Depletion of Ca\(^{2+}\) from the medium protects these cells from oxidative stress in vitro. Indirect evidence suggested that the defect is in the mitochondrial permeability transition pore (Figure 2). This pore is known to be inhibited by cyclosporin A, and treatment of LHON cells with cyclosporin A significantly rescued them from oxidative damage [27].

Parkinson's disease

PD is a chronic progressive disease and its main symptoms are tremor, rigidity, bradykinesia, and a characteristic disturbance of gait and posture due to selective degeneration of nigro-striatal dopamine neurons [28]. There is strong evidence suggesting the involvement of mitochondria and impaired calcium metabolism in PD. It has been observed that in PD there are dysfunctional mitochondria with reduced activities of complex I and of NADH cytochrome c reductase in neurons from substantia nigra [28]. Complex I is part of the mitochondrial energy-producing apparatus – the electron transport chain. It was found that toxic substances such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine – a byproduct of a synthetic heroin) cause Parkinson-like symptoms [17] (Figure 3). MPTP undergoes oxidation, leading to 1-methyl-4-phenylpyridinium-ion (MPP\(^+\)). MPP\(^+\) causes inhibition of mitochondrial complex I [29] that, in turn, causes excessive generation of ROS, which are known to be toxic to many cells [30]. Calcium ions play an important role in ROS generation [30]. Dysfunctional mitochondria in PD cells fail to regulate Ca\(^{2+}\)\(_m\). Increased calcium levels can stimulate ROS production [30] (Figure 3). MPTP is not the only toxic substance that affects the mitochondria and calcium metabolism. Rotenone [18] and manganese [19] also affect...
Amyotrophic lateral sclerosis
ALS is an age-dependent neurodegenerative disorder. The neurons that suffer the most in this disorder are large neurons in the cerebral cortex, brain stem, and motor neurons in the spinal cord [37], leading to progressive paralysis and death within 3–5 years. Electron microscopy studies have shown bizarre giant mitochondria and intramitochondrial paracrystalline inclusions [38]. Studies have shown that brain tissue of patients with ALS suffer from a damaging effect of ROS [39], which might be due to mutations in the superoxide dismutase gene in familial ALS. The normal function of SOD1 is to eliminate superoxide anion radicals and hydrogen peroxide and to protect the cell from oxidative damage [40]. In sporadic ALS, decreased activity of complexes I and IV was observed (Figure 3). In addition, motor nerve terminals from ALS specimens contain significantly increased intracellular calcium levels, which probably result from a defect in glutamate transport [40]. As previously described, [Ca^{2+}]_i elevation also contributes to ROS production by mitochondria and to increased motor neuron toxicity. To summarize the cascade of damaging events, mutations in SOD1 (or in other defense processes against oxidative damage) cause intracellular oxidative stress that can damage mitochondrial and cellular membranes, thus causing disruption of calcium homeostasis and glutamate transport, hence increasing the sensitivity of motor neurons to excitotoxicity.

Conclusions
In addition to being the cellular powerhouse, mitochondria are important Ca^{2+} regulators. Malfunction of the mitochondria can lead to various neurodegenerative diseases, all showing signs of impaired Ca^{2+} homeostasis. Better understanding of these processes might be the first step towards finding a possible cure for these diseases.

In summary, it seems that in many neurologic disorders – of both genetic and toxic origin – mitochondrial disruption leads to an altered calcium metabolism and thus to cell death. This raises the possibility that various protective agents, such as antioxidants, that improve mitochondrial function efficiency, may be of benefit in high-risk individuals.

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References

27. Wong A, Cortopassi G. mtDNA mutations confer cellular sensitivity to oxidant stress which is partially rescued by calcium depletion and cyclosporin A. Biochem Biophys Res Commun 1997;239(1):139–45.
29. Smith TS, Bennett IP. Mitochondrial toxins in models of neurodegenerative diseases. 1. In vivo brain hydroxyl radical production during systemic MPTP treatment or following microdialysis infusion of methylene blue or azide ions. Brain Res 1997;735(2):183–8.

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