Journal of Neuroscience Research 70:357–360 (2002)

Perspective

Role of Mitochondrial Dysfunction in Alzheimer's Disease

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Abnormalities in mitochondrial function relate to the spectrum of pathological changes seen in Alzheimer's disease. Here we review the causes and consequences of mitochondrial disturbances in Alzheimer's disease as well as how this information might impact on therapeutic approaches to this disease.

Key words: Alzheimer's disease; mitochondria; oxidative stress

The association between mitochondrial abnormalities and disease has been known for about forty years, with the description of a patient with hypermetabolism and a skeletal muscle biopsy demonstrating large numbers of abnormal mitochondria, a disorder now termed mitochondrial myopathy. The first mitochondrial diseases to be understood at the molecular level include Leber's hereditary optic neuropathy (LHON) and a spontaneously occurring group of neuromuscular diseases, now classified as chronic progressive external ophthalmoplegia and the Kearns-Sayre syndrome, occurring as a result of mitochondrial DNA deletions (Holt et al., 1988; Wallace et al., 1988). Other mitochondrial diseases have been linked to nuclear genes whose inactivation either inhibit mitochondrial bioenergetics or disrupt mitochondrial/mtDNA biogenesis (Wallace, 1999).

Blass and Gibson (1999) were among the first who prompted the notion that defective energy metabolism in Alzheimer's disease (AD) was a fundamental component of the disease. Many such studies focused on two mitochondrial enzymes, pyruvate dehydrogenase and α ketoglutarate dehydrogenase, the rate-limiting steps of the Krebs cycle (Blass and Brown, 2000). Presently, a large number of studies implicate metabolic defects in AD, such that a reduced rate of brain metabolism is one of the best documented abnormalities in AD (Blass, 2000). There is substantial data from positron emission tomography (PET) that consistently shows reduced cerebral metabolism in temporoparietal cortices in AD (Minoshima et al., 1997). An increased oxidative utilization in comparison with glucose utilization in AD patients is also well documented (Hoyer, 1993; Fukuyama et al., 1994). Importantly, such cerebral metabolic rate abnormalities precede any evidence for functional impairment by neuropsychological testing or of brain atrophy by neuroimaging (Blass, 2000). It should also be noted that metabolic derangements, comparable to those seen in AD (e.g., hypoxic hypoxia, hypoglycemia, vitamin deficiencies) are sufficient, by themselves to induce neuropsychological deficits similar to those in AD (Gibson et al., 1981, Blass and Gibson, 1999). The process by which these deficits become irreversible in AD remains an important unanswered question.

The most consistent defect in mitochondrial electron transport enzymes in AD has been a deficiency in cytochrome c oxidase. Initially reported in AD platelets (Parker et al., 1989), reduced cytochrome c oxidase activity in post-mortem brain tissue from patients with AD, particularly in neurofibrillary tangle-bearing neurons, is also described (Kish et al., 1992; Mutisya et al., 1994). Preliminary studies from our laboratory have demonstrated perikaryal accumulation of cytochrome c oxidase protein, immunolocalized to the vacuolar component of lipofuscin by immunoelectron microscopy in the face of

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Received 2 April 2002; Accepted 3 June 2002

reduced numbers of intact mitochondria. We therefore suspect that enhanced degradation of mitochondria occurs in AD, leaving behind lysosomal detritus containing nonfunctioning mitochondrial proteins. Our preliminary finding of diminished numbers of microtubules in AD, with nitration of microtubules and alterations in tau, are also consistent with altered mitochondria trafficking and enhanced degradation in AD.

Cybrid technology, which involves the transfer of mitochondria from living patients to cell lines deficient in mitochondria, has facilitated the study of energy metabolism in AD (King and Attardi, 1989). The cell lines are initially depleted of mitochondrial DNA by exposing them to low concentrations of ethidium bromide, which preferentially inhibits DNA replication. The exposed cells lose their mitochondrial DNA and assume an anaerobic phenotype. Studies using this technique have demonstrated that the deficits in cytochrome c oxidase in AD platelets could be transferred to Rho^0 cells, which retain the cytochrome oxidase deficit (Davis et al., 1997; Swerdlow et al., 1997). Additionally, the resulting cybrid cells showed markedly increased free radical production, impaired intracellular calcium buffering, elevated basal cytosolic calcium concentration, and enhanced sensitivity to inositol 1,4,5-trisphosphate-mediated calcium release.

These and other findings have raised the issue of whether genetic alterations, inherited or acquired, underlie the disordered energy metabolism. Although a number of inherited and acquired mitochondrial diseases resulting from mutation are well known, the studies in AD have been hampered by the presence of ancient mitochondrial DNA fragments incorporated into the nuclear genome, or nuclear pseudogenes (Hirano et al., 1997; Wallace et al., 1997). Other putative mutations could not be reproduced, and specific mitochondrial DNA polymorphisms that were suggested to segregate with AD remain controversial (Zsurka et al., 1998). One individual with a mutation in the gene for amyloid precursor protein, however, also had a G→C mutation at position 5705 of the mitochondrial genome, in the gene encoding $tRNA^{Asn}$ (Hutchin et al., 1997); two novel polymorphisms have been identified in Japanese patients with AD (Tanno et al., 1998). At present, no uniform genetic basis for the mitochondrial abnormalities observed in AD is apparent. Several other neurodegenerative diseases, however, are worth noting in this regard. Friedreich's ataxia is an autosomal recessive disease characterized by ataxia, diabetes mellitus, and cardiomyopathy, due to GAA trinucleotide expansion on chromosome 9, resulting in reduced protein, frataxin, and subsequent insufficiency in oxidative phosphorylation, loss of mitochondrial DNA, and hypersensitivity to oxidative stress. Frataxin is a mitochondrial protein involved in iron transport, deficiency of which leads to iron accumulation and free radical generation via the Fenton reaction. Similarly, Wilson's disease is caused by mutations in copper P-ATPase, leading to increased intracellular copper and oxidative damage. One of two copper P-ATPase isoforms localizes to the mitochondria. Hereditary spastic paraplegia, dystonia, and Huntington's disease are other inherited neurodegenerative diseases in which mutations in proteins related to mitochondrial function have been implicated or identified (Beal, 2000). Thus, mitochondrial genetic defects are a driving factor behind at least a subset of neurodegenerative disease.

As the controversial role of apoptosis in AD is unraveled, it should also be noted that mitochondria likely play an important role in apoptosis. There is compelling evidence that mitochondria contain a molecular switch for the initiation of apoptosis in the form of a nonspecific mitochondrial inner membrane channel, the mitochondrial permeability transition pore (Wallace, 1999). Opening of the pore causes collapse of the electrochemical gradient, and activation of apoptosis-inducing factor and caspases, leading to apoptosis. Opening of the pore can be affected by excessive calcium uptake, reactive oxygen species, or a decline in energetic capacity. Thus, a marked reduction in mitochondrial energy production and a chronic increase in oxidative stress could theoretically activate the mitochondrial permeability transition pore and initiate apoptosis, potentially contributing to neurodegeneration in AD.

Oxidative phosphorylation is a major source of endogenous toxic free radicals, including H_2O_2, OH^-, and O_2^-, which are products of normal cellular respiration (Wallace, 1999). With inhibition of electron transport, electrons accumulate in early stages of the electron transport chain, namely Complex I and coenzyme Q, where they can be donated directly to molecular oxygen to give superoxide radical O_2^-. Superoxide anion is detoxified by the mitochondrial Mn superoxide dismutase to given H_2O_2. H_2O_2 is converted to water by glutathione peroxidase. Additionally, H_2O_2 can be converted to toxic hydroxyl radicals in the presence of reduced transition metals via the Fenton reaction, a process that we have specifically localized to neurofibrillary pathology in AD as well as Parkinson's disease (Smith et al., 1997a, Castellani et al., 2000). Multiple classes of macromolecules are altered by free radicals, including protein, lipid, and nucleic acid. Additionally, the lack of histones in mitochondrial DNA and diminished capacity for DNA repair apparently renders mitochondrial DNA susceptible to oxidative stress. Preliminary evidence in our laboratory demonstrates possible lipid peroxidation affecting lipoic acid, a critical component of the active site of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase complex, possibly having a profound effect on energy metabolism.

Combined and synergistic effects of insufficient oxidative phosphorylation, altered calcium homeostasis, and oxidative stress have thus been demonstrated. We have studied in detail the cellular substrates of the oxidative stress component, including the immunolocalization of redox-active transition metals, metal binding proteins, advanced glycation and lipid peroxidation end products, DNA alterations, protein nitration, and oxidative stress response induction (reviewed in Smith et al., 2000). In our studies, we have characterized the biochemical compo-

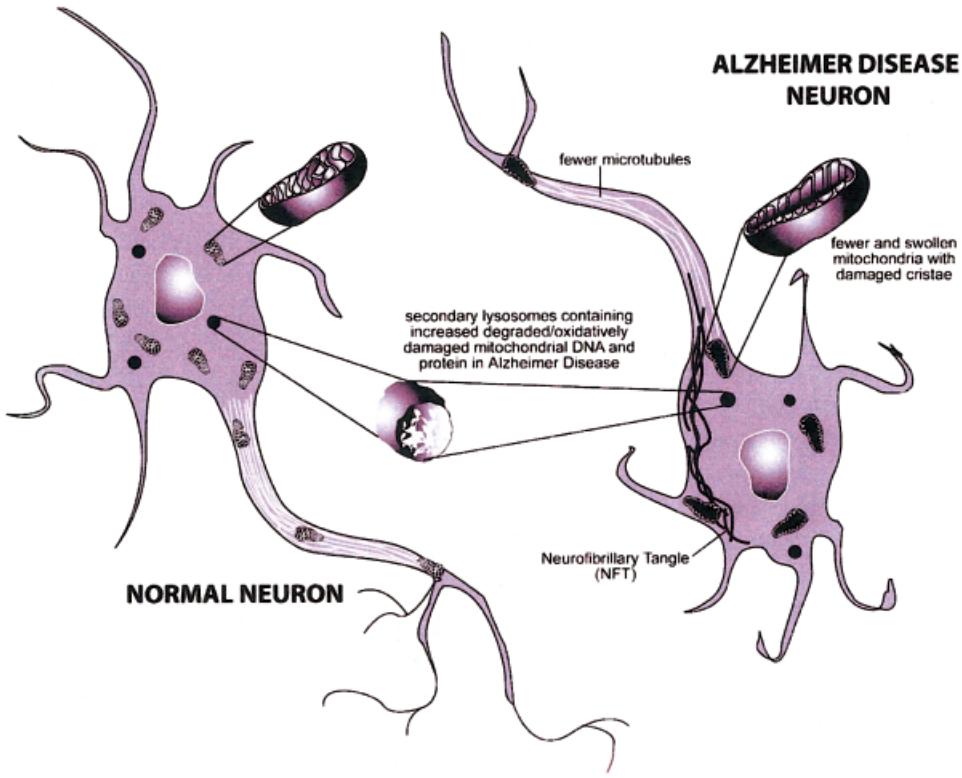

Fig. 1. Mitochondrial abnormalities in Alzheimer's disease likely contribute to many of the salient features of disease including secondary lysosomes, dendritic arborization and microtubule loss, neurofibrillary tangles and oxidative stress.

nents of pathologic lesions in AD, and detailed a dynamic process involving oxidative stress, macromolecular changes resulting from oxidative stress, and consequent pathology (e.g., Smith et al., 1997b). We further noted that the biochemical changes identified in our studies occur early in the disease process, likely preceding recognizable pathological changes and neurological deficits. Nevertheless, the precise origin of free radicals, or processes enabling their capacity to irreversibly damage cells, is not entirely worked out.

In light of our previous work and the increasing evidence for disturbed energy metabolism in AD, we hypothesized that defective mitochondrial metabolism sets up a cascade of pathological events that initiates AD. Therefore, to further examine the role of mitochondria in AD, we used in situ hybridization to mitochondrial DNA (mtDNA), immunocytochemistry of cytochrome oxidase, and morphometry of electron micrographs of biopsy specimens to determine whether there were mitochondrial abnormalities in AD (Hirai et al., 2001). Additionally, their relationship to oxidative damage marked by 8-hydroxyguanosine and nitrotyrosine modifications. The former is a well known DNA modification that directly results from oxidative stress, whereas the latter is a more recently characterized adduct of peroxynitrite, produced from the reaction of superoxide and nitric oxide, with tyrosine. We found that the same neurons showing increased oxidative damage in AD have a striking and significant increase in mtDNA and cytochrome oxidase. Surprisingly, much of the mtDNA and cytochrome oxidase is found in the neuronal cytoplasm and in the case of mtDNA, the vacuoles associated with lipofuscin, whereas morphometric analysis showed that mitochondria are significantly *reduced* in AD, consistent with perturbed mitochondrial turnover. In a preliminary study (data not published), we also noticed an overall reduction in microtubules in AD compared to controls. Therefore, the abnormal mitochondrial turnover, as indicated by increased perikaryal mtDNA and mitochondrial protein accumulation in the face of reduced numbers of mitochondria, may be due to defective microtubule metabolism resulting in deficient mitochondrial transport. This may, in turn, set up a pathological cascade of events in the perikaryal mitochondria, an important component of which is oxidative stress. It is also noteworthy that studies such as ours documenting the cellular and subcellular localization of mitochondrial pathology are conspicuously lacking. This is not a trivial issue when one considers the complexity of not only mitochondrial genetics and biosynthesis, but also the importance of energy metabolism in the brain per se, and the unique feature mitochondrial transport in dendrites and axons mediated by cytoskeletal elements including microtubules (Fig. 1).

In summary, mitochondrial defects are now described in a wide spectrum of human conditions, including degenerative and metabolic diseases, aging, and cancer (Wallace, 1999). The evidence that supports a critical role of mitochondria in neurodegenerative diseases and AD in

particular, is compelling. Studies from our laboratory have further elucidated the role of mitochondria in AD by showing that neurons in AD brains accumulate mitochondrial debris in their perikaryon, which results from oxidative damage to mtDNA and mitochondria proteins and may be related a priori to deficient or defective microtubule metabolism. Further studies examining the importance of mitochondrial pathophysiology in aging and AD may provide important insight into neurodegenerative disease pathogenesis and may indeed provide a target for specific therapies.

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