Mitoenergetic failure in Alzheimer disease

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ALZHEIMER DISEASE (AD) is a neurodegenerative disorder characterized primarily by a progressive deterioration of cognitive function with memory loss. The most vulnerable regions of brain in AD include the basal forebrain, amygdaloid body, hippocampus, entorhinal cortex, neocortex, and certain brain stem nuclei (10, 231). Most cases are sporadic with no known genetic linkage, but ~5% of AD cases are caused by known genetic abnormalities. The pathogenic pathways leading to neurodegeneration in AD include accumulation of aberrant or misfolded proteins, ubiquitin-proteasome system dysfunction, excitotoxic reactions, oxidative and nitrosative stress, mitochondrial injury, synaptic failure, altered metal homeostasis, dysfunction of axonal and dendritic transport, and misoperation of chaperones (46, 130). The presence of extracellular amyloid-β (Aβ) peptide-containing neuritic plaques and intracellular neurofibrillary tangles and the loss of synapses in defined regions of the brain are the hallmarks associated with both familial and sporadic AD postmortem pathology. Aβ deposition in the plaques is composed of a 39- to 42-amino acid peptide (Aβ), which is the proteolytic product of the amyloid precursor protein (APP) (182). Although the underlying cause for the selective neuronal loss remains unclear, experimental approaches indicate that fibrillar or oligomeric Aβ accumulation in vivo may initiate and/or contribute to the process of neurodegeneration observed in the brain (66, 272). Neurodegeneration could result from Aβ acting to inhibit mitochondrial function as part of a cascade of mitoenergetic failure (90, 155).

The major obstacle in managing the disease and designing rational therapeutic targets is our incomplete understanding of the pathogenesis of the disease. Several hypotheses, mainly focusing on these hallmarks, have been proposed in an attempt to explain the pathogenesis of AD, including theories involving amyloid deposition, tau phosphorylation, oxidative stress, metal ion dysregulation, and inflammation. Unfortunately, despite strong evidence that these aspects are associated with AD and almost certainly play a role in the disease process, no single one of these theories is sufficient to explain the spectrum of abnormalities found in the disease. Therefore, it is worthwhile to look for a fundamental initiator of the pathological cascade. Dysregulation of mitochondrial metabolism together with oxidative abnormalities may be such an initiator for the major known pathological events (276). The recent short-term improvement of AD patients treated with memantine implies a need for better control of responses to glutamate toxicity (148), including calcium dysregulation and oxidative stress. Oxidative stress and therapeutic interventions have been extensively reviewed (121, 204). Given the known differences in energy metabolism between the normal and AD brain (22, 207), we discuss the need for further investigation of the neural mechanisms through which brain cell functions influence en-
energy balance during cellular stress. As such, targeting both oxidative stress and mitochondrial energy metabolism may be therapeutically efficacious for the treatment of AD. Therefore, in this review, we focus on the role of reduction in mitochondrial activity and energy availability for neuronal function, depletion of energy metabolizing substrates in AD, and potential therapeutic targets. We also comment on the role of dysregulation in calcium homeostasis and oxidative stress in mediating the effects of mitochondrial respiratory chain and DNA damage for AD pathogenesis.

NORMAL BRAIN ENERGY METABOLISM

Cellular bioenergetic homeostasis requires production and delivery of energy-rich phosphoryls and nicotinamide adenine dinucleotide (NAD\(^{+}\)) oxidizing power (83, 223). Facilitation of intracellular energetic communication requires contributions of cytoplasmic streaming and positioning of mitochondria and their movement in response to changes in energy utilization, along with formation of enzymatic complexes (118, 140). Mitochondria contribute \(\sim 90\%\) of the required energy for cellular functions (264) by two linked metabolic processes: the citric acid cycle (anaerobic) and the electron transport chain (ETC; aerobic). Under aerobic conditions, the majority of ATP used to maintain cellular homeostasis results from oxidation of the glycolytic product pyruvate. ATP-generating systems, including glucose uptake, glycolysis, NADH, and fatty acid oxidation (260), are induced with a rise in AMP-to-ATP ratio by activation of AMP-activated protein kinase (54, 110). However, to fulfill all cellular energetic needs, such arrangements are insufficient (83). Thus other homeostatic mechanisms contribute to efficient intracellular energetic communication in maintaining the ATP-generating and ATP-consuming processes. Such systems include intracellular enzymatic networks, catalyzed by creatine kinase, adenylate kinase, carbonic anhydrase, and glycolytic enzymes (82, 223, 265). The creatine kinase-creatine phosphate and adenylate kinase systems facilitate processes in the brain that would otherwise depend entirely on the diffusion of ATP and ADP (for review, see Ref. 3). Creatine kinase is a major phosphotransfer system in cells specifically located at places of energy metabolism (266) and acts in concert with other enzymatic systems to facilitate intracellular energetic communication (84, 127, 190) and enhancement of cytoplasmic high-energy phosphates (39). Because of its location at the adenine nucleotide transporter on the mitochondrial membrane, intramitochondrial ATP is actually released from mitochondria by interconversion to phosphocreatine (228). The importance of phosphocreatine to the heart and its role in the protection from heart attacks (222) probably also apply to the brain and brain attacks (strokes). Both mitochondrial and cytosolic creatine kinase isozymes are active in brain cells with high and variable ATP metabolic rates (119). The importance of phosphocreatine to energetic homeostasis is indicated by promotion of hypoxic seizures (120) and glutamate and Aβ toxicity (30) in its absence.

The central focus underlying brain energy metabolism is predominantly, if not exclusively, placed on neuronal energy metabolism. However, other brain cells such as glial and vascular endothelial cells play an active role in the flux of energy substrates to neurons (156). Brain cells generate most of the energy in the form of ATP through the mitochondrial

ETC coupled to oxidative phosphorylation from the oxidation of NADH, a substrate for complex I, and FADH\(_2\), a complex II substrate (Fig. 1) with atomic oxygen to generate water and ATP. NADH and FADH\(_2\) serve as electron carriers by transferring electrons derived from glycolysis and Krebs cycle into the ETC through NADH:ubiquinone oxidoreductase (complex I) and succinate:ubiquinone oxidoreductase (complex II), respectively. Ubiquinone:cytochrome c-oxidoreductase (complex III) couples the oxidation of ubiquinol to cytochrome c reduction (217), whereas cytochrome c:oxygen oxidoreductase (complex IV) catalyzes the oxidation of cytochrome c, which ultimately reduces molecular oxygen to water (52, 116). During redox cycling, released potential energy drives the extrusion of protons across the mitochondrial inner membrane at complexes I, III, and IV. This electrochemical gradient produced as a result of proton translocation powers ATP synthesis by the activity of ATPase (153). It is possible to measure in vivo \(O_2\) consumption and ATP synthesis rates, which lead to assessment of the mitochondrial ATP-to-\(O_2\) or P-to-O ratio (162). The P-to-O ratio represents the efficiency of coupling between mitochondrial phosphorylation and oxygen consumption.

The brain has low levels of stored glycogen and is highly dependent on oxidative metabolism. Glucose is the obligatory energy substrate for the brain (88), but fatty acids are used by brain as well (86). However, fatty acid oxidation in brain is limited by the low activity of mitochondrial 3-ketoacyl-CoA thiolase (271). Although glucose is the primary energy substrate, brain cells metabolize ketones during fasting reductions of blood glucose (48, 102). Ketone bodies, consisting of acetoacetate, and \(\beta\)-hydroxybutyrate, derived from fat metabolism in the liver, are transported into the brain through the blood-brain barrier via monocarboxylic transporters (202, 134). The basal rate of glucose utilization in astrocytes is higher than in neurons (156). A metabolic compartmentation exists whereby glucose taken up by astrocytes is metabolized to lactate glycolytically, which is then released in the extracellular space to be utilized by neurons. Lactate, which is characteristic found in the brain, can sustain synaptic activity by serving as an energy source through pyruvate by lactate dehydrogenase (20). Pyruvate is another important energy substrate abundantly distributed in brain synapses (191). Because of limited permeability across the blood-brain barrier, both lactate and pyruvate cannot adequately substitute for plasma glucose to maintain normal brain function (200); however, they are useful metabolic substrates for neurons if formed inside brain parenchyma (123, 253).

Mitochondrial electron transport is not always perfect. Even under ideal conditions, some electrons “leak” from the ETC and interact with oxygen to produce superoxide anion and other reactive oxygen nitrogen species. Leakage of electrons can increase significantly with mitochondrial dysfunction during aging. The close proximity of mitochondrial DNA (mtDNA) to the flux of reactive oxygen nitrogen species and the relative lack of mtDNA protection and repair mechanisms lead to free radical-mediated mutations and deletions (135).

DECLINES IN SYNAPTIC ACTIVITY AND ENERGY DEMAND

Mitochondria are positioned within axons, dendrites, and synaptic terminals to provide ATP, oxidizing power (NAD\(^{+}\)),
and calcium buffering for these compartments (174). Because of greater metabolic demands, the mitochondria of these compartments are subjected to relatively greater oxidative and calcium burdens than other regions of neurons. Thus synaptic compartments, particularly dendritic spines, are regions of neurons that may be exposed to the highest levels of oxidative and metabolic stress. Synapses are the sites where the neurodegenerative process occurs early in AD (67). The outer part of the dentate gyrus has a reduced synaptic density in AD (141), and synapses are completely lost within the dense amyloid core of a classic senile plaque (141). Degeneration of synapses correlates strongly with cognitive decline (75). This occurs mainly because glutamate receptors and calcium channels are concentrated in synaptic compartments, and membrane depolarization and calcium influx caused by activation of these ion channels result in oxidative stress and a high energy (ATP) demand (Fig. 1). The emerging data provide considerable evidence to suggest that synapses are primary sites of calcium deregulation in AD. In addition, the apoptotic process has been shown to be activated in vulnerable neuronal populations in AD and can also be activated locally in synaptic compartments after exposure to Aβ (172). In several different animal and cell culture models of AD, overactivation of glutamate receptors, which are concentrated on postsynaptic spines of neuronal dendrites, plays an important role in the neuronal death process (17, 105). Studies on transgenic mice expressing AD-linked

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**Fig. 1.** Synaptic function and energy demand. At the axon terminal, vesicles containing glutamate fuse with the presynaptic membrane by exocytosis and release glutamate into the synaptic cleft. Stimulation of glutamatergic neurons by glutamate produces a postsynaptic potential in the dendritic spine of the neuron through the influx of Ca²⁺ and Na⁺. The 2 main types of glutamate receptors, ionotropic (further classified as NMDA, AMPA, and kainite) and metabotropic (MTG-R), in the postsynaptic neuron bind glutamate in the synapse. The ion flux activates the Na⁺-K⁺-ATPase and the Ca²⁺-ATPase, leading to increased energy (ATP) demand. This increased energy demand, together with the Ca²⁺ influx, stimulates oxidative phosphorylation, which forms ATP by consumption of NADH and also produces toxic reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (·OH), nitric oxide (NO), and peroxynitrite (ONOO⁻). NADH is produced by glycolysis and the mitochondrial (mt) TCA cycle dehydrogenases. Activation of biochemical cascades during amyloid beta (Aβ) and glutamate excitotoxicity results in ionic disturbance and energy depletion in synaptic terminals and dendrites. Aβ secreted into the synaptic cleft via sequential cleavage of the amyloid precursor protein (APP) promotes the endocytosis of NMDA receptors (NMDA-R), reducing their density at the synapse (245). This will impair glutamatergic transmission. CK, creatine kinase; cyt c, cytochrome c; IP3, inositol trisphosphate; UQ, ubiquinone.
APP and/or PS1 mutations have provided considerable support for the importance of perturbation of calcium homeostasis in AD (137). Synaptic loss associated with downstream processes may elicit neurodegeneration through reduction in metabolic activity, regional cerebral blood flow (150), and activation of microglia (125). Neuronal-astrocytic interactions are also impaired during AD (115, 157).

Does synaptic loss occur because of excess release of glutamate or increased susceptibility to glutamate levels released by young neurons? The clinical success of memantine, a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, could support either of these possibilities. Our study (29) of aging rat neurons support the increased susceptibility hypothesis as an age-related contributor to the problem. The basic question is where does synaptic pathology start? Distal dendrites are first to be affected before the appearance of neurofibrillary tangles (28). Synaptophysin as a presynaptic marker is lost early in the AD frontal cortex (166).

Synaptic plasticity is likely dependent on the capacity of neurons to meet energy demands to maintain ion homeostasis (261). Emerging evidence indicates that events associated with energy balance can impact synaptic and cognitive function (149). Brain-derived neurotrophic factor (BDNF) has been found to facilitate synaptic plasticity (183) through modulation of molecules such as synapsin I and cAMP-response element-binding protein, which have been implicated in synaptic function underlying learning and memory (270). Given the involvement of BDNF in energy balance and synaptic plasticity, a central question is whether alterations in energy balance can impact aspects of synaptic plasticity modulated by BDNF. A role of uncoupling protein 2 (UCP2), a member of the super family of UCPs located in the mitochondrial inner membrane and abundantly expressed in the hippocampus (215), may support the synapse (7). UCP2 may function to control reactive oxygen species (ROS) production during increased energy consumption. There is a critical need to identify genetic and imaging tracers to predict synaptic dysfunction before the appearance of substantial clinical symptoms of AD to initiate effective synaptic-preserving therapy. Pharmaceutical and neutraceutical interventions could then be tested on a shorter time scale than the 6–12 mo now required for clinical trials in AD.

METABOLIC DECLINE: EVIDENCE FROM IMAGING STUDIES

Advancing age is associated with decreased metabolism in frontal areas, temporal and parietal cortices, as measured by high-resolution positron emission tomography (PET) with $[^{18}F]$fluorodeoxyglucose imaging (151). Impairment of brain energy metabolism can lead to neuronal damage or facilitate the deleterious effects of some neurotoxic agents such as glutamate (226). On the other hand, reduced brain glucose metabolism, increased oxygen consumption, and reduced phosphocreatine levels in patients with AD have also been observed (205). Some of the most direct evidence of brain metabolism abnormalities associated with AD comes from in vivo PET. Autopsy-confirmed cases suggest that the PET diagnosis of AD is as good as the 90% specificity of clinical diagnosis. Consistent reports of reduced cerebral metabolism have been shown to occur in temporoparietal cortices of AD patients (259). Direct evidence for reductions in energy metabolism in the AD brain has been obtained from measurements of regional blood flow by imaging studies (218) and subsequently from determinations of $[^{18}F]$fluorodeoxyglucose incorporation or $^{15}$O$_2$ extraction by PET scanning (92, 15, 214). There are prominent and consistent decreases of the metabolic rate for glucose in the parietal and temporal cortices. These reductions appear early in AD, possibly even preceding the onset of clinical symptoms in some patients (132, 240), and increase in magnitude as the disease becomes more severe (242). Similar PET abnormalities have been observed in elderly people carrying the allele €4 for apolipoprotein E with no signs of cognitive impairment, known as a risk factor in late-onset AD (213). Although significant changes in energy metabolism are seen in AD, a major mechanistic issue remains regarding whether vascular aging or pathology causes the deficit or whether the reductions in metabolic rates measured in vivo arise from decreased neuronal function following neuronal loss or synaptic degeneration.

PYRIDINE NUCLEOTIDES AS PRIMARY ENERGY SUBSTRATES

The maintenance of cellular energy reserves is vital for cellular survival. The coenzyme NAD$^+$, a parent compound to NADH, NADP, and NADPH, is involved in many metabolic processes as an essential cofactor for enzyme-catalyzed oxidations (16), as the major donor of electrons for mitochondrial electron transport to power oxidative phosphorylation (9), and as an important contributor to ATP production. Because of this vital role, most cellular NAD$^+$ is located within the mitochondrial matrix, NAD$^+$ and NADP are key molecules involved in signal transduction, transcription, DNA repair, glutathione metabolism, and the NADPH-dependent thioredoxin system, which are important for the maintenance of the cellular antioxidant system and detoxification reactions (277).

NAD$^+$ is required for Sir2 deacetylase activity (103), a NAD-dependent histone deacetylase (9). Its activation is central to promoting increased life span in yeast and Caenorhabditis elegans (128). In humans and rodents, seven molecules that share the Sir2-conserved domain [sirtuin (SIRT) 1 to 7] have been identified (40). SIRT1 is located in the nucleus and is involved in chromatin remodeling and the regulation of transcription factors such as p53 (154), whereas other SIRT proteins are located within the cytoplasm and mitochondria (196, 194). Both Sirt1 and the Sir2 family of protein deacetylases and poly(ADP-ribos e) polymerase (PARP) are involved in major NAD-dependent nuclear enzymatic activities. Increased nuclear NAD$^+$ biosynthesis and consequent activation of the protein deacetylase SIRT1 have been found to protect against axonal degeneration (9). A decrease in NAD$^+$ levels could stimulate neurodegeneration.

NAD$^+$ is closely tied to cellular metabolism and genomic DNA repair (142). PARP (EC 2.4.2.30), a nuclear enzyme that is activated by double- or single-stranded breaks in DNA (229) and thought to assist in DNA repair by the ADP ribosylation of histones and other nuclear proteins (263), is another major consumer of the NAD$^+$ pool. PARP-1 activity is detected in both neuronal and nonneuronal cells in the central nervous system (CNS), and excessive PARP-1 activity is known to be detrimental to tissue because of the cellular energy loss (107). PARP and poly(ADP-ribos e) can be detected in the frontal and temporal cortex of AD patients, suggesting significant consumption of NAD$^+$ stores (153). Both NAD$^+$ and NADP are
substances for PARP (179). Depletion of intracellular NAD\(^+\) by PARP has been observed in conditions associated with excess free radical burden and DNA damage (229). The activated enzyme catalyzes the transfer of ADP-ribose units from NAD\(^+\) to a protein acceptor to produce ADP-ribose polymers. As a result, cellular NAD\(^+\) concentrations rapidly decline, thus posing a heavy demand on cellular ATP stores for resynthesis of NAD\(^+\) (206). When cells cannot overcome this energy crisis, cell death occurs via necrotic-type pathways (106). Other intracellular events that affect NAD\(^+\) levels or NAD\(^+\)/NAD\(^-\)-to-NADH ratios, such as energy transfer through respiration, may also affect physiological and pathological processes in the nervous system through SIRT1-dependent pathways (146). It is possible that alteration of NAD\(^+\) levels by manipulation of the NAD\(^+\) biosynthetic pathway, Sir2 protein activity, or other downstream effectors will provide new therapeutic opportunities for the treatment of diseases involving axonopathy and neurodegeneration (9).

CONSEQUENCES OF NAD DECLINE: ENERGY CRISIS

Cellular NAD\(^+\) holds a key position in the control of fundamental cell processes (16). Both acute and chronic neurodegenerative diseases have been linked to the loss of NAD\(^+\) stores. Mitochondrial NAD\(^+\) can be rapidly depleted under the influence of ROS and Ca\(^{2+}\) through the permeability transition pore (PTP) opening (77), a Ca\(^{2+}\)-dependent, high-conductance channel located in the inner mitochondrial membrane (19). PTP opening might be important for mitochondrial ionic homeostasis and intracellular signaling. Release of mitochondrial NAD\(^+\) is rapidly followed by its disappearance due to the abundance of NAD\(^+\)-consuming enzymes outside the mitochondrial matrix. A major metabolic pathway for the released NAD\(^+\) is hydrolysis into ADP-ribose and nicotinamide catalyzed by glycohydrolases (26, 277). Because glycohydrolases can also catalyze cADPribose could trigger the release of Ca\(^{2+}\) from the sarcoplasmic reticulum and initiate an amplification cycle that induces the permeability transition in the vast majority of mitochondria (79). However, the precise mechanisms of pore opening are not understood nor is the relationship to aging and AD.

The oxidation of NADH by complex I of the ETC is coupled to the phosphorylation of ADP by ATP synthase. The relationship between the rate of ATP production and the ratio of reduced NADH to oxidized NAD\(^+\), known as the redox ratio, has been used to measure metabolic states in single cells by fluorescence techniques (80). In neurons cultured from 24-month-old rats, we found an age-related reduction in NAD(P)H at rest that suggests an imbalance in production and demand (Fig. 2). Glutamate treatment that stimulates an influx of Ca\(^{2+}\) caused a robust decline in NAD(P)H in old neurons compared with that shown in middle-age and embryonic neurons, as complex I tries to keep up with the need for electrons to power the proton gradient and ATP production (Parihar and Brewer, unpublished observations). These findings could explain the vulnerability of old neurons to metabolic stressors (29). Considering the number of enzymes and transcription factors sensitive to the redox potential (232) or oxidized with age (42), the observed decreases in NAD(P)H, glutathione, and NAD(P)H redox ratio may be of pathophysiological relevance for aging and neurodegenerative diseases.

CHANGES IN EXPRESSION OF MITOCHONDRIAL GENES

The relationship between defects in the mitochondrial ETC and AD pathogenesis is still unknown. There are estimates of 1,500 human genes encoding mitochondrial proteins, of which only 13 are within the mtDNA (50, 144). The control of mitochondrial gene activity has a great impact on the susceptibility to various diseases (185). One of the mitochondrial abnormalities described is the change in the expression of mitochondrial and nuclear genes encoding polypeptide subunits of the cytochrome c-oxidase (complex IV) and NADH dehydrogenase (complex I) enzyme complexes of ETC (72, 159). To understand their role in AD, mRNA expression of mitochondrial-encoded genes in complexes I and IV and mRNA expression of nuclear-encoded mitochondrial genes in complexes IV and V have been investigated (1, 63, 237). Both complex I subunit 5 and cytochrome oxidase (COX) mRNAs, as well as mRNA for nuclear COX and ATPase genes, are reduced in the temporal cortex of AD patients (62, 159). As shown by quantitative RT-PCR analysis, a downregulation of mitochondrial genes in complex I and increased expression of genes in complexes III and IV of ETC in both early and definite AD brain specimens were detected (159). In addition, a trend for impaired NADH dehydrogenase (part of complex I) activity in homogenates of plaques has been found (173). A comparative analysis of mitochondrial genes in mice showed a differential expression occurring at different ages, upregulated in middle age and dramatically declined in old age (160). A decrease in gene expression of the ETC complex subunits in affected brain regions may reflect the impairment of oxidative metabolism affecting ATP and ROS production or may simply represent a physiological response to reduced neuronal activity (23). These causal relationships are yet to be resolved. In individual neurons from early AD, the neurons that had ROS
damage to nucleic acid showed correlated mitochondrial abnormalities (117). These features could increase neuronal susceptibility to cell death in AD. Whether oxidative damage to mitochondria leads to a decreased function or whether a decreased efficiency of the ETC results in excessive electron release and ROS formation is not clear. At some point, a circular futile cycle may accelerate beyond control.

**mtDNA MUTATIONS AFFECTING ENERGY TRANSFER**

Normal aging is accompanied by the accumulation of multiple point mutations in the control region for replication of mtDNA in fibroblasts (181). Patients with AD and elderly control subjects have a higher mtDNA somatic mutation rate in mitochondrial-encoded polypeptide genes than younger subjects (145). The aggregate levels of G:C to T:A and T:A to G:C transversions and of all point mutations increase with age in the frontal cortex (236). Mutations in mtDNA cause CNS abnormalities and mitochondrial myopathy. A potential mechanism that may contribute to the induction of these mutations is oxidative damage to mtDNA (236). Mutations in mtDNA, which may be maternally inherited or acquired with aging, can cause a variety of progressive or chronic degenerative diseases (265). Such mutations may either affect mtDNA-encoded subunits of respiratory complexes or mitochondrial tRNAs and rRNAs, thereby compromising the overall rate of mitochondrial protein synthesis. If these mutations occur in synaptic mitochondria of patients with AD, they may compromise energy supply at nerve terminals, ultimately leading to a loss of synaptic function (212). Postmitotic tissues such as brain typically show increased levels of mutant mitochondria due to the seeming inability of these tissues to select against cells containing mutant mtDNA genomes that occur in mitosis in other cells. It is generally recognized that mtDNA disorders present tissue-specific pathology even if a mtDNA mutation is present in all tissues. The situation of coexistence of both normal and mutated mtDNA in cells is termed heteroplasmy. One of the most important features shown by Wallace (265) in mitochondrial diseases is the existence of a threshold in the degree of a mitochondrial deficit for the expression of the disease, and these seem to be related to the balance between normal and mutant mtDNA and energy demands. As a result, it has been demonstrated that only 10% of wild-type DNA is enough to maintain a normal respiratory rate and also that 80–90% deleted mtDNA must be achieved before complex IV activity is compromised (65, 176). Furthermore, the finding by Coskun et al. (69) that 43% of AD brains have mutations in the mtDNA control region, compared with only 26% in controls, suggests that AD brain mitochondria may survive at just below threshold levels but may fail under stress to provide enough energy.

**DECLINES IN MITOCHONDRIAL ENZYMES**

Studies in autopsied AD brain have shown reduced activities of enzymes of energy metabolism such as hexokinase, which catalyzes the phosphorylation step by which glucose enters the pathway of glycolysis (143), and phosphofructokinase appears to be the major controlling step for glycolysis (24, 239). Further upstream, we (201) found deficits in upregulation of the neuronal glucose transporter Glut3 in response to energy demands in old rat neurons compared with results found in young rat neurons. Three mitochondrial components have been demonstrated to be deficient in AD (96): 1) the pyruvate dehydrogenase complex (PDHC), which catalyzes the entry of carbons derived from glucose into the TCA cycle; 2) the α-ketoglutarate dehydrogenase complex (KGDHC), which catalyzes a key step in the TCA cycle and is also an enzyme of glutamate metabolism; and 3) COX, the component of the ETC that uses molecular oxygen as one of its substrates.

Reductions in the key TCA cycle enzyme complexes, KGDHC (21) and PDHC (233, 247), are associated with chronic neurodegenerative disorders, including AD. The reduction in KGDHC activity may be responsible for the decreases in brain metabolism; its activity correlates better with the degree of cognitive impairment than does the amount of amyloid plaques or neurofibrillary tangles (97). Others agree that abnormally low levels of PDHC deficiency occur not only in regions of brain that are neuropathologically damaged in AD but also in regions that are histopathologically normal (45). COX is kinetically abnormal, and its activity is decreased in brain and peripheral tissue in late-onset AD (73). COX deficiency is found in the regions of brain that show histopathological damage in AD (225, 238). Mitochondria isolated from rat brain and other tissues showed a direct effect of micromolar Aβ on mitochondrial respiration (51). ATP synthesis (186), and activities of various enzymes involved in energy transfer (55, 113, 235). A direct interaction of Aβ with mitochondrial membranes could be the cause of perturbation of mitochondrial properties (187) and inhibition of COX activity in AD (51). These findings were strengthened by studies of Aleardi et al. (2), who observed a concentration-dependent decrease in ATP/O (number of moles of ATP produced per g-atom of oxygen consumed by the respiratory chain), respiratory chain complex inhibition, a potentiation of ROS production, and cytochrome c release. These abnormalities in brain mitochondrial metabolism precede the onset of neurological dysfunction as well as gross neuropathology of AD. As early events, they should be investigated as potential therapeutic targets.

Levels of mRNA for COX subunits I and III encoded by mtDNA that are sensitive to neuronal metabolic demands are decreased in AD brain regions that demonstrate neuropathology, such as midtemporal association neocortex, but are not changed in the relatively unaffected primary sensory and motor cortices (61, 189). The distribution of decreased COX activity and mRNA in the postmortem AD brain corresponds to pre-mortem-decreased glucose metabolism (211). Therefore, COX III mRNA levels and COX activity appear to be useful as indicators of impaired neuronal function in AD. Because the COX activity reduction under these conditions has been linked to a decrease in neuronal firing, a decrease in Na+/K+-ATPase activity, and presumably a decrease in utilization and demand for ATP (269), reductions in COX activity and mitochondrial mRNA transcripts in AD reflect a primary defect in mitochondrial energy metabolism (12). The question of whether down-regulation of various respiratory chain complexes with age and also in AD has a genetic or epigenetic basis for the shutting down of ATP production in glycolysis, the TCA cycle, and the ETC and/or consequence of an increased oxidant activity remains open for further investigation.
PROTON LEAKS AND UNCOUPLING

Mitochondrial H⁺ leak is defined as a lower than predicted H⁺/e⁻ stoichiometry. That is, for a given number of electrons flowing down the ETC to molecular oxygen, a lower number of protons are pumped than expected, and the membrane potential is less polarized (34). In isolated hepatocytes from 30-mo-old mice, aging increased the proton leak rate and decreased ATP turnover reactions compared with that shown in 3-mo-old mice (112). The age-related decrease in mitochondrial proton motive force in intact hepatocytes is coincident with increased production of hydrogen peroxide (108, 224). Under baseline physiological conditions, H⁺ leak is beneficial because it decreases ROS generation. Such a role for H⁺ leak is supported by data showing that the uncoupler carbonyl cyanide m-chlorophenylhydrazone protects from enhanced oxidative stress (152). A cytoprotective strategy of mitochondrial uncoupling has been identified in aging (248). Expression of UCP1 and UCP2 has been shown to protect in brain models of ischemia-reperfusion (stroke) (170). Thus uncoupling of mitochondria has been shown to decrease ROS production; however, uncoupling of inhibited mitochondria enhances ROS (34). ROS itself can increase H⁺ leak in isolated brain mitochondria (35, 87), associated with phospholipid oxidation (124). Thus a feedback loop has been proposed to exist between ROS and H⁺ leak (34), but the role of UCPs in this phenomenon remains to be demonstrated. Efforts to determine the molecular mechanism of the basal H⁺ leak have been largely unsuccessful. There are currently four acknowledged pathways of H⁺ leak: 1) the basal leak via UCPs (249), 2) allosteric activation of the adenine nucleotide translocase by AMP (47), 3) the PTP (70), and 4) transmembrane cycling of protonated/unprotonated nonesterified fatty acids (94).

REGULATION AND DYSREGULATION OF CALCIUM DYNAMICS

Calcium has a profound impact on mitochondrial energy function by 1) activation of mitochondrial matrix dehydrogenases (for review, see Ref. 178) to produce more NADH, which 2) donates more electrons to the ETC through complex I to 3) drive the synthesis of more ATP. At the same time, large amounts of calcium influx depolarize the mitochondrial membrane potential (ΔΨm) in general (68) and the plasma membrane potential in particular. Ca²⁺ activates neurotransmitter vesicle release after action potential through voltage-gated calcium channels (133). Elevated intracellular calcium affects enzymes involved in normal cellular and physiological processes, including several protein kinases and phospholipases (257), cytoskeletal integrity (227), and synaptic transmission between neurons (36, 220, 221). The major fast metabolic effect of elevated mitochondrial calcium is activation of dehydrogenases of the TCA cycle (177, 219). However, increases in calcium also play a critical role in pathophysiological events, such as excitotoxicity in several neurodegenerative diseases. The mechanism of events occurring downstream of neuronal calcium overloading and neurodegeneration is being actively investigated.

Dysregulation of intracellular calcium signaling has been implicated in the pathophysiology of AD (175), which occurs during the initial phase of the disease, before the development of symptoms (254). Abnormal calcium regulation and an increased production of neurotoxic Aβ and both APP and presenilin-1 (PS1) mutations in AD patients support a role for perturbed calcium homeostasis in AD. Increased accumulation of intracellular calcium elicits the accumulation of Aβ and the hyperphosphorylation of tau and neuronal death (137). Because disruption of calcium homeostasis is an important mechanism underlying such loss of neurons, this function of PS1 may lead to apoptotic neuronal cell death in AD (59). PS1 is intimately linked to cellular calcium homeostasis, and AD-related mutations of PS1 can alter inositol trisphosphate-coupled intracellular calcium stores and calcium influx pathways (273). Dysregulation of calcium homeostasis has also been demonstrated in fibroblasts from patients with AD and in fibroblasts and neurons from transgenic mice bearing a PS1 mutation (122). The deregulation of calcium homeostasis in aging is evident from our report of an age-related increase in resting intracellular calcium in hippocampal neurons that becomes profoundly dysregulated after glutamate exposure (33). Interestingly, we found that the dysregulation, glutamate toxicity, and Aβ toxicity could all be reversed by pretreatment with estrogen. Therefore, rapid inactivation of calcium currents is the critical step terminating calcium influx and preventing calcium overloading of the cell (234).

ROS PRODUCTION AND OXIDATIVE STRESS

Mitochondria are the major endogenous source of ROS as a natural cost of a 2% inefficiency of uncontrolled electrons that leak and encounter O₂ to form O₂⁻ (60). Low levels of ROS are important for many life-sustaining processes of cells and tissues (95, 261), but they induce cell damage and death at higher levels. In normal aging, ROS production increases, whereas the antioxidant capacity fails to increase sufficiently (163, 208). Observations that support the concept of oxidative stress in aging have been well supported by evidence of oxidative damage in isolated mitochondria (246). A number of related questions concern the nature of the ROS present in mitochondria and what are the steady-state concentrations of the O₂⁻ vs. H₂O₂ in AD patients (91). The primary ROS generated in the organelle is O₂•⁻, which is rapidly converted to H₂O₂ by mitochondrial manganese superoxide dismutase or cytosolic copper/zinc superoxide dismutase enzymes. The resulting H₂O₂ is reduced to water by glutathione peroxidase or catalase. Ubisemiquinone, localized at the CoQ binding sites of complexes I, II, and III, appears to be the primary electron donor (136, 256). However, H₂O₂, in the presence of reduced transition metals, is converted to the highly reactive hydroxyl radical (·OH). The redox state of the mitochondrial respiratory chain is the primary factor governing mitochondrial ROS generation, and this property is inherently governed by the trans-membrane proton gradient (ΔpH) and the ΔΨm. Inhibitors such as rotenone (complex I) or antimycin (complex III) increase mitochondrial ROS production by raising the concentration of electron donors. The pathophysiological inhibition of any of these sites results in an upregulation of ROS formation (34). The Fe-S centers of these complexes are major targets for acute ROS toxicity. Hence, mitochondria are particularly sensitive to oxidative stress (11, 99).

Because age is the major risk factor for AD, it is reasonable that the aging process itself plays an important role in promoting oxidative stress in the aging brain. The major question in
these studies is whether oxidative damage increases in mitochondria with age, which terminal-oxidizing species are most likely to cause it, and which targets (proteins, lipids, DNA) are most susceptible to damage. The brain is particularly susceptible to the damaging effects of ROS because of its high metabolic rate, its high unsaturated lipid-to-volume ratio, and its reduced capacity for cellular regeneration compared with other organs (6). Thus neurons are particularly susceptible to life-long accumulation of oxidative damage. Oxidative stress and free radical damage have been consistently associated with AD pathogenesis (44, 164, 198, 203). Proteins that are involved in proteolysis might themselves produce oxidative stress. This assumption is based on the findings of the occurrence of Aβ within brain mitochondria of human AD patients (71, 155) and transgenic mice (5). The accumulation of Aβ in mitochondrial membranes inhibits enzymatic activity of respiratory chain complexes (III and IV) (56, 71, 161) in the presence of Cu²⁺ in a dose-dependent manner (71), causes reduction in the rate of oxygen consumption (56), and enhances ROS production (161). An increase in Aβ deposition resulted in the induction of oxidative stress in transgenic mice overexpressing mutant APP and PS1 (168) and has been demonstrated to produce H₂O₂ in cultured cells (14).

Age-related alterations in proteolytic processing of APP have been suggested to play a major role in the increased levels of oxidative stress in neurons in AD (171). This abnormality leads to neuronal death, which manifests as cognitive impairment and the development of brain pathology, as in AD. Thus both protein aggregation and oxidative stress clearly have an important impact on one another; however, it is difficult to determine whether oxidative stress or protein aggregation is the initiating event in neurodegeneration. It is still unclear whether oxidative stress is the primary initiating event or is the result of a secondary response associated with neurodegeneration in AD. Results emerging from cell and animal model studies will continue to have an enormous impact on the development of future human clinical antioxidant trials for these disorders.

Much evidence for increased levels of cellular oxidative stress in vulnerable regions of AD comes from analyses of tissue homogenates from postmortem brain tissue, which were compared with the same brain regions from age-matched controls and to less vulnerable brain regions from the same patients (241). For example, markers for lipid peroxidation, including 4-OH-nonenal and malondialdehyde, and protein nitration as a marker of protein oxidation have been identified in the cortex and hippocampus of patients with AD (8, 43, 243). Protein carbonyls in brain samples of AD patients are greater than those in age-matched controls and correlate well with tangles (244). Cellular injury during AD marked by the presence of 8-hydroxy-2'-deoxyguanosine, a marker of oxidative damage in intact DNA, was examined in the cerebrospinal fluid of AD patients, suggesting that these patients suffer from impaired DNA repair. mtDNA damage could also have greater implications than damage to nuclear DNA, since the whole mitochondrial genome codes for genes that are expressed, whereas nuclear DNA contains a large number of sequences that are not transcribed in the brain (258). The accumulation of free radicals and mtDNA mutations during the life span can lead to progressive mitochondrial dysfunction and decreased ATP levels, which in turn might influence the neuropathology of late-onset neurodegenerative diseases such as AD. The resultant oxidative damage to mitochondria may increase oxidized proteins with age (18, 25), and these may also cause mitochondrial decline in energy transfer perhaps due, in part, to altered conformation of critical enzymes (98). The significant loss of mitochondrial cardiolipin, coupled with oxidation of critical thiol groups in key proteins, may adversely affect cytochrome c-oxidase activity necessary for mitochondrial function (199). Levels of malondialdehyde in the neocortex of the brain of individuals with AD (197) and 4-OH-nonenal in the plasma of patients with AD (180) are elevated. Chronic oxidative stress can also reduce expression of metabolic enzymes as part of the stress response mediated by signaling pathways through redox-sensitive transcription factors, kinases, and phosphatases (214, 254). These changes could directly impact the ability of mitochondria to maintain their membrane potential and energy transfer.

In addition to ROS, reactive nitrogen species such as nitric oxide (NO) and peroxynitrite (ONOO⁻) are also synthesized in mitochondria. NO is synthesized from l-arginine by a constitutive isoform of nitric oxide synthase (NOS) called mitochondrial NOS (mtNOS) (251, 275). mtNOS has been identified as the neuronal NOs isoform (89), and it is likely coded by the same neuronal NOS gene (129). The level of basal NO appears to be a key factor in this regulatory process (74). The activation of NOS in mitochondria brings about a decrease in oxygen consumption and regulation of cellular energetic metabolism (193). In addition, NO causes reversible inhibition of COX activity (37), a regulation that can explain the existence of mtNOS (184). The mitochondrial respiratory chain is sensitive to both NO- and ONOO⁻-mediated damage (210). NO is suggested to play an important role in Aβ-induced mitochondrial dysfunction and cell death (131). However, the role of NO in regulation of mitochondrial functions, including energy homeostasis, has not been fully explored. The threshold level of O₂⁻*, which favors reaction with NO to generate ONOO⁻ and plays a significant role in mitochondrial decay, warrants study in greater depth.

DEPLETION OF ENERGY AND GLUTAMATE EXCITOTOXICITY

The brain contains high concentrations of glutamate as the principal excitatory neurotransmitter and as an important astrocytic metabolite interconverted to glutamine. Excitotoxicity, a term coined by Olney (195), occurs in part because of overactivation of NMDA receptors, excessive influx of calcium, and consequent production of damaging free radicals together with activation of proteolytic processes that contribute to neuronal injury and cell death (64, 147). Several lines of evidence link excitotoxicity with the pathogenesis of AD, including 1) oxidative stress and increased intracellular calcium generated in response to Aβ, 2) increases in NMDA receptor activation in response to Aβ, 3) downregulation of glutamate transporters in AD, 4) inhibition of glutamate reuptake by Aβ, 5) increased hyperphosphorylation of tau by NMDA receptor activation, 6) protection conferred by the NMDA receptor antagonist memantine from intrahippocampal injection of Aβ (148), and 7) clinical efficacy of memantine (148).

During acute and chronic neurodegenerative disorders, disruption of energy metabolism impairs the clearance of glut-
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Invited Review

WHAT FACTORS DETERMINE HOW A NEURON WILL DIE?

The intermembrane space contains a number of cell death-promoting factors, including cytochrome c; procaspases-2, -3, and -9; apoptosis initiating factor (AIF); and the caspase-activated DNase (158, 85, 251). On release, cytochrome c activates the cytosolic Apaf-1, which activates the procaspases to destroy the cytoplasm. AIF and caspase-activated DNase are transported to the nucleus where they degrade chromatin (265). Recent data showed that caspases-3 and -7 control the loss of ΔΨm and AIF release from mitochondria (139). Our study (32) of age-related elevation in caspase-3 in old neurons at rest supports a head start on the path to apoptosis.

The mitochondrial permeability transition can be stimulated to open by uptake of excessive calcium, increased oxidative stress, and decreased ΔΨm, ADP, and ATP (100). Thus inhibition of oxidative phosphorylation in disease states increases the susceptibility of cells to undergo apoptosis (38, 165). The energetic state of the cell determines whether a given stress can be tolerated or whether this leads to energetic failure based on certain thresholds (192). Dropping ATP levels too low results in necrotic cell death; however, without severe ATP depletion, apoptosis develops instead. If ATP is depleted during the progression of apoptosis, necrotic cell death will intervene to produce the secondary necrosis that is so often associated with apoptosis (41).
ANTIOXIDANT DEFENSES

The cell has several antioxidant defenses and repair mechanisms to deal with oxidative stress and associated oxidative damage; however, in many neurodegenerative disorders, the activities of various antioxidant defense molecules are not reduced (31) that would normally counteract the injurious effects of ROS. Affected brain regions in AD do not exhibit reduced activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase (273). However, concentrations of uric acid, a potential scavenger of ONOO\(^{-}\), and activity of the enzyme methionine sulfoxide reductase, which reverses oxidation at protein methionine residues, are decreased (93). Together, there is no support for drastic deficits of antioxidant defenses in AD. The best that can be said is that defenses are inadequate for the increased rates of ROS generation.

THERAPEUTIC TARGETS

Identification of novel therapeutic targets for the treatment of neuronal injury would be extremely beneficial to reduce or eliminate disability from CNS disorders. Significant insights concerning the cellular and molecular bases of AD have illuminated the potential causes and consequences of AD pathogenesis in the human brain. Assigning AD pathogenesis to a single cause may not be appropriate, as increasing lines of evidence indicate that multiple factors likely contribute to the clinical manifestation of AD. This suggests that one specific treatment may not be able to prevent or reverse AD. Because mitochondrial ROS is thought to be critical in the pathogenesis of AD, antioxidants that accumulate within mitochondria might be useful in controlling excessive generation of metabolic toxicants. By taking advantage of the large \( \Delta \Psi_m \) (−150 to −170 mV), drugs effective in controlling ROS production and detoxification can be delivered by covalent attachment to a lipophilic cation (188). Mitochondrial-targeted derivatives of vitamin E and coenzyme Q show efficacy in protecting mitochondria from oxidative damage (101). Heat shock proteins (70-kDa heat shock protein and 78-kDa glucose-regulated protein) that can be increased by dietary restriction are emerging in cortical, striatal, and hippocampal neurons in AD (209) and confer cytoprotection in neurodegenerative disease and aging (49). Recently, several in vivo (169) and in vitro (76) studies have suggested the neuroprotective potential of some energy substrates against neuronal damage induced by excitotoxicity, oxidative stress, and metabolic inhibition. Therapeutic strategies for reducing neurodegeneration could address restoration of \( \Delta \Psi_m \) and reduction of elevated A\( \beta \) secretion. Creatine is neuroprotective against A\( \beta \)-induced neurotoxicity in hippocampal cultured neurons (30) and shows promise for treatment of Huntington disease (114). Pyruvate is neuroprotective against glutamate-mediated neuronal damage (167). \( N \)-acetyl-L-carnitine and lipoic acid given to old rats improve memory loss and lower nucleic acid oxidation (4). In an APP + PS1 mouse model of AD, supplementation with blueberry extract prevented the behavioral decline in these animals (126). Does this represent the slowing of a mitochondrial function or the delaying of the pathological process of mitochondrial damage? Understanding the dynamic stages and subsequent loss of mitochondrial membrane permeability and the eventual induction of apoptotic and inflammatory activation may ultimately serve to elucidate therapeutic strategies linked to brain cellular metabolism.

CONCLUSION

The precise sequence of events in AD pathogenesis is uncertain. We know relatively little about the signals from either developmental cues or damage signals transduced to and integrated in the mitochondria during aging and neurodegenerative diseases. However, impaired intramitochondrial metabolism associated with respiratory chain dysfunction and the consequent oxidative stress are being considered as a possible pathogenic mechanism in a number of neurodegenerative disorders, including AD. Oxidative stress is intimately linked with an integrated series of cellular phenomena, which all seem to contribute to neuronal demise. It is less clear from such studies on mitochondrial function whether inefficiency in energy transfer produces more ROS that correlates with disease progression, as some mitochondria are destroyed and release apoptotic-inducing factors. We need to tilt the balance back toward mitochondrial turnover for healthier mitochondrial function so that energy homeostasis can be improved, rather than bioenergetic catastrophe, resulting in abortive apoptosis or necrosis. The creation of numerous cell and animal models has aided emulation of the protective effects of key enzymatic components that regulate oxidative stress in neurodegenerative diseases, with the aim of developing rational drugs or genetic therapy. It is clear that mitochondrial failure needs to be part of the treatment target mix.

GRANTS

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