MITOENERGETIC FAILURE IN ALZHEIMER DISEASE

Mordhwaj S Parihar¹ and Gregory J. Brewer¹CA, ²

From the ¹Department of Medical Microbiology, Immunology and Cell Biology,
²Department of Neurology, Southern Illinois University School of Medicine,
Springfield, IL 62794-9626 USA

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CAAddress correspondence to: Gregory J. Brewer, Tel. 217-545-5230; Fax, 217-545-3227; E-Mail: gbrewer@siumed.edu
ABSTRACT

Brain cells are highly energy-dependent for maintaining ion homeostasis during high metabolic activity. During active periods, full mitochondrial function is essential to generate ATP from electrons that originate with the oxidation of NADH. Decreasing brain metabolism is a significant cause of cognitive abnormalities of Alzheimer’s disease (AD), but it remains uncertain whether this is the cause of further pathology or whether synaptic loss results in a lower energy demand. Synapses are the first to show pathological symptoms in AD prior to the onset of clinical symptoms. As synaptic function has high energy demands, interruption in mitochondrial energy supply could be the major factor in synaptic failure in AD. A newly discovered age-related decline in neuronal NADH and redox ratio may jeopardize this function. Mitochondrial dehydrogenases and several mutations affecting energy transfer are frequently altered in aging and AD. Thus, with the accumulation of genetic defects in mitochondria at the level of energy transfer, the issue of neuronal susceptibility to damage as a function of age and age-related disease becomes important. In an aging rat neuron model, mitochondria are both chronically depolarized and produce more ROS with age. These concepts suggest that multiple treatment targets may be needed to reverse this multifactorial disease. This review summarizes new insights based on the interaction of mitoenergetic failure, glutamate excitotoxicity and amyloid toxicity in the exacerbation of AD.
Alzheimer's disease (AD) is a neurodegenerative disorder characterized primarily by a progressive deterioration of cognitive function with memory loss. Most vulnerable regions of brain in AD include the basal forebrain, amygdaloid body, hippocampus, entorhinal cortex, neocortex and certain brainstem nuclei (10, 231). Most cases are sporadic with no known genetic linkage, while about 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, 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. β-amyloid deposition in the plaques is composed of a 39–42 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 pathophysiological 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) that include calcium dysregulation and oxidative stress. Oxidative stress and therapeutic interventions have been extensively reviewed (121, 204). Given the known differences in energy metabolism between normal and AD brain (22, 207) we discuss the need for further investigation of the neural mechanisms through which brain cell functions influence 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 NAD$^+$ oxidizing power (83, 223). Facilitation of intracellular energetic communication requires contributions of cytoplasmic streaming, positioning of mitochondria and their movement in response to changes in energy utilization, along with formation of enzymatic complexes (118, 140). Mitochondria contribute ~90% of the required energy for cellular functions (264) by two linked metabolic processes: the citric acid cycle (anaerobic) and the electron transport chain (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 upon a rise in AMP/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 (265, 223, 82). 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 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 mitochondrial membrane, intramitochondrial ATP is actually released from mitochondria by interconversion to phosphocreatine (228).

The importance of phosphocreatine to the heart and protection from heart attacks (222), probably also applies 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 glia 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 electron transport chain (ETC) coupled to oxidative phosphorylation (OXPHOS) from the oxidation of NADH, a substrate for complex I and FADH₂, 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 the Kreb's 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 leads to assessment of the mitochondrial ATP/O$_2$ or P/O ratio (162). P/O 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). While glucose is the primary energy substrate, brain cells metabolize ketones during fasting reductions of blood glucose (48, 102). Ketone bodies, consisting of acetoacetate, and β-hydroxybutyrate, derived from fat metabolism in the liver and transported into the brain through the blood-brain barrier, monocarboxylic
transporters (202, 134). The basal rate of glucose utilization in astrocytes is higher than in neurons (156). A metabolic compartmentation exist whereby glucose is 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 characteristically 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 electron transport chain and interact with oxygen to produce superoxide anion and other reactive oxygen nitrogen species (RONS). Leakage of electrons can increase significantly with mitochondrial dysfunction with age. The close proximity of mtDNA to the flux of RONS and the relative lack of mtDNA protection and repair mechanisms, leads 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 that 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 is the case largely because glutamate receptors and calcium channels are concentrated in synaptic compartments, and membrane depolarization and calcium influx resulting from activation of these ion channels results 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 following 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 APP and/or PS1 mutations have provided considerable support to 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 non-competitive NMDA receptor antagonist could support either of these possibilities. Our studies of aging rat neurons support the increased susceptibility hypothesis as an age-related contributor to the problem (29). The basic question is where does synaptic pathology start? Distal dendrites are first to be affected prior to the appearance of neurofibrillary tangles (NFTs) (28). Synaptophysin as a presynaptic marker is lost early in frontal cortex in AD (166).

Synaptic plasticity is likely dependent on the capacity of neurons to meet energy demands to maintain ionic 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 cyclic AMP-response element-binding protein (CREB), 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 uncoupling proteins located in the mitochondrial
inner membrane and abundantly expressed in the hippocampus (215), may support the synapse (7). UCP2 may function to control ROS production during increased energy consumption. There is a critical need to identify genetic and imaging tracers to predict synaptic dysfunction prior to the appearance of substantial clinical symptoms of AD in order to initiate effective synaptic preserving therapy. Pharmaceutical and neutraceutical interventions could then be tested on a shorter time scale than the 6-12 months 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 with $^{18}$F-fluorodeoxyglucose (FDG PET) 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 Alzheimer’s patients have also been observed (205). Some of the most direct evidence of brain metabolism abnormalities associated with AD comes from in vivo positron emission tomography (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 brain in Alzheimer’s disease has been obtained from measurements of regional blood flow by imaging studies (218) and subsequently from determinations of \[^{18}\text{F}\]fluorodeoxyglucose incorporation or \[^{15}\text{O}\] extraction using PET scanning (92, 15, 214). There are prominent and consistent decreases of the metabolic rate for glucose in the parietal and temporal association cortices. These reductions appear early in the disease, 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 e4 for apolipoprotein E with no signs of cognitive impairment, known as a risk factor in late-onset AD (213). While significant changes in energy metabolism are seen in AD, a major mechanistic issue remains 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 nicotinamide adenine dinucleotide (NAD\(^+\)), a parent compound to NADH, NADP, and NADPH, is involved in many metabolic processes as both 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. Due to this vital role, most
of 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, both of 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 Sir2 family of protein deacetylases and poly(ADP-ribose) polymerase (PARP) are involved in major NAD-dependent nuclear enzymatic activities. Increased nuclear NAD$^+$ biosynthesis and consequent activation of the protein deacetylase SIRT1 has 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). Poly(ADP-Ribose) polymerase (PARP; EC 2.4.2.30), a nuclear enzyme which 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 non neuronal cells in the CNS, and excessive PARP-1 activity is known to be detrimental to tissue because of the cellular energy loss (107). PARP and poly(ADP-ribose) can be detected in the frontal and temporal cortex of AD patients, suggesting significant consumption of NAD$^+$ stores (153). Both NAD$^+$ and NADP are substrates 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$^+$/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\textsuperscript{2+} through the permeability transition pore (PTP) opening (77), a Ca\textsuperscript{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\textsuperscript{+} is rapidly followed by its disappearance due to the abundance of NAD\textsuperscript{+} consuming enzymes outside the mitochondrial matrix. A major metabolic pathway for the released NAD\textsuperscript{+} is hydrolysis into ADP-ribose and nicotinamide catalyzed by glycohydrolases (26, 277). Since glycohydrolases can also catalyze cyclization reactions, formation of cyclic ADP-ribose could trigger the release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum, and initiate an amplification cycle inducing 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 electron transport chain 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\textsuperscript{+}, known as the redox ratio has been used to measure metabolic states in single cells using 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\textsuperscript{2+} caused a robust decline in NAD(P)H in old neurons compared to middle-age and embryonic, as complex I tries to keep up with the need for electrons, to power the proton gradient and ATP production.
(Parihar and Brewer, submitted). 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, GSH, 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 of nuclear-encoded mitochondrial genes in complexes IV and V have been investigated (1, 63, 237). Both complex I subunit 5 and COX mRNAs, as well as mRNA for nuclear COX and ATPase genes, are reduced in the temporal cortex of AD patients (62, 159). Using quantitative RT-PCR analysis, a down regulation 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 a dramatic decline in their expression 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 showing 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.

MITOCHONDRIAL DNA MUTATIONS AFFECTING ENERGY TRANSFER

Normal aging is accompanied by the accumulation of multiple point mutations in the control region for replication of mitochondrial DNA (mtDNA) in fibroblasts (181). AD patients and elderly control subjects have higher mtDNA somatic mutations in mitochondrial-encoded polypeptide genes compared to the mutation rate of 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
Mutations in mtDNA cause central nervous system abnormalities as well as 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 AD patients, they may compromise energy supply at nerve terminals which ultimately leads 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 occurs in mitosis in other cells. It is generally recognized that mitochondrial DNA disorders present tissue specific pathology even if a mitochondrial DNA 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 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 to 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 found deficits in upregulation of the neuronal glucose transporter Glut3 in response to energy demands in old rat neurons compared to young rat neurons (201). 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 (KGDH), which catalyzes a key step in the TCA cycle and is also an enzyme of glutamate metabolism; and

3) cytochrome oxidase (COX), the component of the electron transport chain which uses molecular oxygen as one of its substrates.
Reductions in the key TCA cycle enzyme complexes, the 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 which 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 Alerdi et al. (2) where 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 has been observed. 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 mitochondrial DNA 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 post mortem AD brain corresponds to premortem decreased glucose metabolism (211). Therefore, COX III mRNA levels and COX activity appear to be useful as indicators of impaired neuronal function in AD. As 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), therefore, the reduction in COX activity and mitochondrial mRNA transcripts in AD reflect a primary defect in mitochondrial energy metabolism (12). The question 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 electron transport chain 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 electron transport chain to molecular oxygen (O₂), a lower number of protons are pumped than expected, and the membrane potential is less polarized (34). Aging increases the proton leak rate and decreases ATP turnover reactions in isolated
hepatocytes from 30-month old mice compared with 3-month old mice (112). The age-related decrease in mitochondrial proton motive force in intact hepatocytes is coincident with increased production of hydrogen peroxide (224, 108). Under baseline physiological conditions, $H^+$ leak is beneficial by decreasing ROS generation. Such a role for $H^+$ leak is supported by data showing that the uncoupler carbonyl cyanide $m$-chlorophenylhydrazone (CCCP) protects from enhanced oxidative stress (152). A cytoprotective strategy of mitochondrial uncoupling has been have 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, but, 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 (uncoupling proteins) (249); 2) allosteric activation of the adenine nucleotide translocase (ANT) by AMP (47); 3) the PT (permeability transition) pore (70) and 4) transmembrane cycling of protonated/unprotonated non-esterified fatty acids (94).

REGULATION AND DYSREGULATION OF CALCIUM DYNAMICS
Calcium (Ca^{2+}) has a profound impact on mitochondrial energy function by 1) activation of mitochondrial matrix dehydrogenases (for review, see 178) to produce more NADH, that 2) donates more electrons to the electron transport chain through complex I to 3) drive the synthesis of more ATP. At the same time, large amounts of Ca^{2+} influx depolarizes the ΔΨ_m in general (68) and on membrane potential in particular. Ca^{2+} activates neurotransmitter vesicle release after action potential through voltage-gated Ca^{2+} channels (133). Elevated intracellular Ca^{2+} affects enzymes involved in normal cellular and physiological processes include 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 Ca^{2+} is activation of dehydrogenases of the TCA cycle (177, 219). However, increase in Ca^{2+} also play a critical role in pathophysiological events such as excitotoxicity in several neurodegenerative diseases. The mechanism of events occurring downstream of neuronal Ca^{2+} 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, prior to the development of symptoms (254). Abnormal Ca^{2+} regulation and an increased production of neurotoxic Aβ and both APP and presenilin-1 mutations in AD patients, support a role for perturbed Ca^{2+} homeostasis in AD. Increased accumulation of intracellular calcium elicits the accumulation of Aβ and the hyperphosphorylation of tau and neuronal death (137). Since disruption of Ca^{2+} 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 Ca\(^{2+}\) homeostasis, and that AD-related mutations of PS1 can alter inositol trisphosphate (IP\(_3\))-coupled intracellular Ca\(^{2+}\) stores as well as Ca\(^{2+}\) 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 PS-1 mutation (122). The deregulation of Ca\(^{2+}\) homeostasis in aging is evident from our report of an age-related increase in resting intracellular Ca\(^{2+}\) 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 Ca\(^{2+}\) currents is the critical step terminating Ca\(^{2+}\) influx and preventing Ca\(^{2+}\) 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 O\(_2\) to form O\(_2^-\) (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 while the antioxidant capacity fails to increase sufficiently (163, 208). Observation that supports the concept of oxidative stress in aging has 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 $\text{O}_2^{\cdot-}$ versus $\text{H}_2\text{O}_2$ in AD patients (91). The primary ROS generated in the organelle is $\text{O}_2^{\cdot-}$, which is rapidly converted to $\text{H}_2\text{O}_2$ by mitochondrial manganese superoxide dismutase (MnSOD) or cytosolic copper/zinc superoxide dismutase (Cu/ZnSOD) enzymes. The resulting $\text{H}_2\text{O}_2$ is reduced to water by glutathione peroxidase (GPx) 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, $\text{H}_2\text{O}_2$, in the presence of reduced transition metals, is converted to the highly reactive hydroxyl radical ($^\cdot\text{OH}$). 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 ($\Delta\text{pH}$) and the $\Delta\psi_m$. 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 up-regulation 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).

Since 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 if 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 affects 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 protofibril formation 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 Cu^{2+} 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 secondary response that is associated with neurodegeneration in AD. Results emerging from current 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 compared to 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-HNE and malondialdehyde (MDA), 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 in age-matched controls and correlate well with tangles (244). Cellular injury during AD marked by the presence of 8-hydroxy-2'-deoxyguanosine (8-OHdG), 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. Mitochondrial DNA damage could also have greater implications than damage to nuclear DNA since the whole mitochondrial genome codes for genes that are expressed while 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 MDA in the neocortex of the brain of individuals with AD (197) and 4-HNE 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 nNOSα isoform (89) and it is likely coded by the same nNOS 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 cause reversible inhibition of COX activity (37), a regulation that can explains 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 O$_2^-$ 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 N-methyl-D-aspartate (NMDA) receptors, excessive influx of Ca$^{2+}$ and consequent production of damaging free radicals together with activation of proteolytic processes that contribute to neuronal injury and cell death (147, 64). Several lines of evidence link excitotoxicity with the pathogenesis of AD including:

1) oxidative stress and increased intracellular Ca$^{2+}$ generated in response to Aβ;

2) increases in NMDA receptor activation in response to Aβ;

3) down regulation of glutamate transporters in AD

4) inhibition of glutamate reuptake by Aβ;

5) increased hyperphosphorylation of tau by NMDA receptor activation and

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 glutamate (78, 58, 157). Excitotoxicity propagates in a chain like manner as dying neurons release more glutamate to neighboring neuronal cells particularly during ischemia and stroke. Does improving energy metabolism also prevent glutamate toxicity in neurodegenerative disorders? One of our studies showed protection from glutamate and Aβ toxicity by preloading neurons with creatine (30). We hypothesize that long term energy deficiencies lead to failure to reduce intracellular Ca²⁺ and over activation of NMDA receptors to cause excitotoxicity since short term energy failure is not toxic to neurons with healthy energetics (148).

Electron paramagnetic resonance spectroscopy provided direct evidence that NMDA receptor activation leads to the generation of superoxide radicals (138). Schulz et al. (230) provided the first in vivo evidence that excitotoxic neuronal injury is linked to free radical generation and showed the depletion of antioxidant glutathione in neurodegeneration. Glutamate toxicity may involve defects in electron transport complexes or other perturbations of mitochondria which can be responsible for excess production of ROS (109, 81. Expression of AD-linked PS-1 mutations in cultured neural cells and transgenic mice results in aberrant processing of APP (111) and increased vulnerability of neurons to apoptosis and excitotoxicity (13). Glutamate and Aβ insults cause impairment of calcium homeostasis in neurons expressing mutant PS-1 (104). This perturbed calcium homeostasis appears to contribute to increased levels of cellular
oxidative stress and mitochondrial dysfunction in neurons subjected to apoptotic insults. A strong indication of the mitochondrial ROS generation in glutamate neurotoxicity derives from the evidence that the enhanced cellular $O_2^-$ production and cytotoxicity are abolished in the presence of rotenone/oligomycin, inhibitors of mitochondrial complexes I and IV (57). In addition, nitric oxide synthase, which is induced by activation of the NMDA receptor, generates nitric oxide as well as $O_2^-$ and $H_2O_2$. The immediate consequence of glutamate excitotoxicity and reduced respiration may be the depolarization of mitochondrial membrane potential ($\Delta \Psi_m$).

Several studies have identified depolarized $\Delta \Psi_m$ in brain mitochondria after $N$–methyl-D-aspartate (NMDA) receptor activation and elevated intracellular Ca$^{2+}$ (226). The relationship of ROS production and membrane depolarization could be clarified from the time course of the ROS production in relation to $\Delta \Psi_m$ in response to glutamate exposure. We used laser scanning confocal microscopy to simultaneously observe both ROS production and changes in $\Delta \Psi_m$ in individual mitochondria as a function of age of the rat neurons (Parihar and Brewer, in preparation). Fluorescent labeling with tetramethylrhodamine ethyl ester (TMRE) monitors $\Delta \Psi_m$ while simultaneously monitoring ROS with 2', 7' – dichlorofluorescin diacetate (H$_2$DCFDA). In response to 5, 10 and 15 min exposure to glutamate, individual mitochondria both increased their ROS levels simultaneous with depolarization of $\Delta \Psi_m$ for all ages (Fig. 3). However, age-related deficits were observed before glutamate (0 time) that give old neurons a head start in $\Delta \Psi_m$ depolarization and ROS production. This mitochondrial head
start may prove to be the catastrophic age-related deficit that makes old neurons more susceptible to glutamate and Aβ (29). Comparing p53 overexpressing senescent cells with pre-apoptotic PC12 cells, Sugrue et al. (249) also found decreased $\Delta \psi_m$ in aged cells. Fluctuations in $\Delta \psi_m$ reflect an intermediate, unstable state of mitochondria that may lead to or reflect mitochondrial permeability transition (MPT) or calcium cycling (216). In addition to causing mitochondrial membrane depolarization, increased mitochondrial oxidative stress and dysregulation in calcium homeostasis, Aβ can cause damage to mitochondrial DNA (27), although it is not known if DNA damage precedes or follows mitochondrial dysfunction. Overall, our results showed a high rate of ROS production and depolarization of membrane potential in old neurons which may be one of the most relevant causes of cell impairment during excitotoxicity. In particular, these events could impair mitochondrial energy transfer, enhancing depletion of cellular energy stores, and leading to metabolic catastrophe.

**WHAT FACTORS DETERMINE HOW A NEURON WILL DIE?**

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

The MPT can be stimulated to open by uptake of excessive Ca$^{2+}$, increased oxidative stress, decreased $\Delta \Psi_m$, ADP, and ATP (100). Thus inhibition of OXPHOS 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 lead to energetic failure based on certain thresholds (192). Dropping ATP levels too low results in necrotic cell death, but 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, but 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 SOD, glutathione peroxidase (GSH-Px), glutathione reductase (GSHRd) 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 basis 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. Taking advantage of its 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). A mitochondrial targeted derivative of vitamin E (MitoVit E) and Coenzyme Q (MitoQ) show efficacy in protecting mitochondria from oxidative damage (101). Heat shock proteins (HSP-70 and GRP-78) that can be increased by dietary restriction are expressed 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
potentiality 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 Aβ secretion. Creatine is neuroprotective against Aβ-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 improves memory loss and lowers 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 delaying the pathological process of mitochondrial damage? Understanding the dynamic stages and subsequent loss of mitochondrial membrane permeability, 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 is 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 that 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 as to improve energy homeostasis instead of 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 mitoenergetic failure needs to be part of the treatment target mix.

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FIGURE LEGENDS

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 Ca$^{2+}$ and Na$^+$. The two main types of glutamate receptors, ionotrophic (further classified as NMDA, AMPA and kainite) and metabotropic (MTG-R) in the post synaptic neuron bind glutamate in the synapse. The ion flux activates the Na$^+$/K$^+$-ATPase and the Ca$^{2+}$-ATPase, leading to increased energy (ATP) demand. This increased energy demand together with the Ca$^{2+}$ influx stimulates oxidative phosphorylation which forms ATP by consumption of NADH and also produces toxic reactive oxygen species such as superoxide anion (O$_2^{•-}$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (’OH), nitric oxide (NO) and peroxynitrite (ONOO$^-$). NADH is produced by glycolysis and the mitochondrial TCA cycle dehydrogenases. Activation of biochemical cascades during amyloid beta (Aβ) and glutamate excitotoxicity result 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, reducing their density at the synapse (245). This will impair glutamatergic transmission.

Fig. 2- Resting NAD(P)H autofluorescence in hippocampal neurons declines with age and is reduced further by the treatment with glutamate. Intrinsic
fluorescence of NAD(P)H was collected from individual embryonic (E18, open squares), middle-age (open circles) and old-age (closed circles) neurons in culture. After collecting resting fluorescence, glutamate (200µM) was applied to the cell causing an immediate stimulation of NAD(P)H, but NAD(P)H declines after 33 min. of exposure to glutamate. Note that old neurons start with the lowest levels of NAD(P)H (from Parihar and Brewer, submitted).

**Fig. 3-** Simultaneous decline in mitochondrial membrane potential ($\Delta \Psi_m$) with an increase in reactive oxygen species (ROS) occurs at the same rates for E18 (open squares), middle-age (open circles) and old neurons (closed circles), but starts out depolarized and at the highest rate of ROS production for old neurons. The points represent 0, 5, 10 and 15 min of glutamate treatment (from Parihar and Brewer, submitted).
Fig. 1 Brewer

Presynaptic neuron

mitochondrion

O₂ → NADH → TCA

ROS (NO, O₂⁻, H₂O₂, ·OH)

Ca²⁺

Oxidative stress

Synaptic vesicle

Metabotropic glutamate receptor

NMDA-R

Glutamate transporter

Ca²⁺-ATPase

APP

α7-nicotinic receptor

Endocytosis

Ca²⁺

ADP

IP₃

Ca²⁺

ATP cycle

NADH

NAD⁺

Ca²⁺

Outer membrane

mTCK

Adenine nucleotide transporter

Inner membrane

mitochondria