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Review

Mechanisms of mitochondrial dysfunction and energy deficiency in Alzheimer's disease

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Abstract

Several studies have demonstrated aberrations in the Electron Transport Complexes (ETC) and Krebs (TCA) cycle in Alzheimer's disease (AD) brain. Optimal activity of these key metabolic pathways depends on several redox active centers and metabolites including heme, coenzyme Q, iron-sulfur, vitamins, minerals, and micronutrients. Disturbed heme metabolism leads to increased aberrations in the ETC (loss of complex IV), dimerization of APP, free radical production, markers of oxidative damage, and ultimately cell death all of which represent key cytopathologies in AD. The mechanism of mitochondrial dysfunction in AD is controversial. The observations that Aβ is found both in the cells and in the mitochondria and that Aβ binds with heme may provide clues to this mechanism. Mitochondrial Aβ may interfere with key metabolites or metabolic pathways in a manner that overwhelms the mitochondrial mechanisms of repair. Identifying the molecular mechanism for how Aβ interferes with mitochondria and that explains the established key cytopathologies in AD may also suggest molecular targets for therapeutic interventions. Below we review recent studies describing the possible role of Aβ in altered energy production through heme metabolism. We further discuss how protecting mitochondria could confer resistance to oxidative and environmental insults. Therapies targeted at protecting mitochondria may improve the clinical outcome of AD patients.

Keywords: Heme; Energy; TCA cycle; Mitochondria; Amyloid-β; Nutriceutical

1. Introduction

Sporadic Alzheimer's disease (AD) now has a prevalence approaching 40% among people 80 years of age or older. At present, 4 million Americans are affected with AD, and the estimated annual health care cost is almost 100 billion dollars. Due to the expected increase in the number of individuals 65 years or older, it is estimated that the total incidence of AD will quadruple by the year 2050 (Brookmeyer et al., 1998). Thus, there is an urgent need to find a means of preventing, delaying the onset, or reversing the course of AD. Compared to other disorders, the development of drugs to treat age-related neurodegenerative diseases is relatively slow. One contributing reason is the difficulty of developing safe treatments that can cross the blood brain barrier. Additionally, and probably most significantly, for most age-related disorders, the mechanism of action that results in neurodegeneration and specific metabolic pathways involved have not been elucidated. These are major obstacles that must be overcome in order to find suitable therapeutic targets for the design of appropriate drugs or the development of preventative therapies.

Amyloid-β (Aβ) peptides (Aβ40 and Aβ42) are viewed by the majority in the field as the culprit peptides

Abbreviations: BACE1, β-site amyloid precursor protein-cleaving enzyme1; APP, amyloid-β precursor protein; BBB, blood brain barrier; AD, Alzheimer disease; NFT, neurofibrillary tangles; SP, senile plaques; ETC, electron transport complexes; HO, heme oxygenase; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; αKGDH, α-ketoglutarate dehydrogenase.

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implicated in AD (Selkoe, 2000; Smith et al., 2000). There has been progress in defining some of the key biochemical pathways that appear affected by excessive Aβ peptides in the brain. The neurotoxicity of Aβ has been recently linked to multimeric forms that are also intraneuronal (Walsh et al., 2002; Walsh et al., 2000). Aβ is also found in the mitochondria (Crouch et al., 2005; Lustbader et al., 2004; Yamaguchi et al., 1992). These findings may provide a direct link between Aβ and key cytopathologies of AD such as mitochondrial dysfunction (e.g., low complex IV or TCA cycle). Aβ also binds with heme, which is synthesized in the mitochondria, to form the complex Aβ-heme, which exhibits peroxidase activity (Atamna, 2006; Atamna and Boyle, 2006; Atamna and Frey, 2004). The presence of intracellular and mitochondrial Aβ is also consistent with the view that heme metabolism is abnormal in AD brain (Atamna, 2006).

Because intracellular Aβ and specific cell pathologies associated with AD (e.g., energy hypometabolism) appear before the presence of senile plaques or neurofibrillary tangles (NFT), therapeutic interventions to protect the mitochondria in AD may help prevent neuropathology and clinical decline in patients with AD. Here we review key aspects of mitochondrial dysfunction in AD and propose a molecular link between these aspects, heme metabolism, and the accumulation of Aβ peptides.

2. Energy metabolism and Alzheimer's disease

Mitochondria are the most complex and metabolically active organelles in the cell. They respond to physiological and environmental cues in order to meet cellular energy and metabolic demands (McBride et al., 2006; Wallace, 2005; Wallace et al., 2003). There are three key consequences of mitochondrial dysfunction germane to AD: compromised energy metabolism (e.g., low ATP); oxidative stress and oxidative damage (e.g., DNA oxidation); and metabolic consequences (e.g., low regulatory heme). If the mitochondrial dysfunction is severe, cell death may occur by apoptosis or necrosis (Armstrong, 2006; Naoi et al., 2005; Schapira, 2006). Inheritable mitochondrial DNA (mtDNA) deletions that cause diseases with severe clinical presentations usually appear at younger ages (Wallace, 1999). Mitochondrial dysfunction also occurs during aging in individuals with a history of a normal and healthy life (Barja, 2002; Cortopassi and Wong, 1999; Lee et al., 1997; Mecocci et al., 1993). Mitochondrial dysfunction in healthy individuals during aging is usually latent until a threshold of aberration is reached, which eventually results in the disruption of cellular function. Clinical presentations of the age-related aberrations to the mitochondria are not the same as the inheritable disorders. The primary cause for mitochondrial dysfunction with age is still controversial.

Mitochondrial dysfunction with age may be accelerated in AD patients or the threshold of cell impairment in response to abnormal mitochondria may be lower in AD patients. Mitochondrial dysfunction in AD may be influenced by environmental, metabolic, dietary, and/or genetic factors.

2.1. Energy hypometabolism in Alzheimer's disease brain

Energy hypometabolism is one of the most consistent and earliest abnormalities seen in AD and in mild cognitive impairment. Positron emission tomography imaging combined with 18F-2-deoxy-2-fluoro-D-glucose have shown a decline of 21–28% in regional glucose uptake and utilization in AD brain relative to elderly normal controls (de Leon et al., 1983a, 1983b), indicating impaired energy metabolism in the brain of AD patients. More, recent studies provided additional evidence of reduced brain glucose metabolism in patients with mild cognitive impairment and AD (Faulstich, 1991; Jagust, 2004; Johnson et al., 2005; Small et al., 2006). The decline in glucose metabolism occurs in the areas that are known to be affected early in AD (e.g., posterior cingulate, parietal, and temporal cortices (Reiman et al., 1996; Small et al., 2000). These decrements in glucose metabolism appear before the onset of memory deficits (Hsia et al., 1999; Mucke et al., 2000), and seem to sensitize the neurons to energy deficiency (Arias et al., 2002).

2.2. The effect of energy metabolism on amyoild-β

A possible link between abnormal energy metabolism and Aβ, the culprit peptide in AD pathology, has been demonstrated by several studies. Energy deficiency causes an increase in the levels of intracellular amyloidogenic APP fragments *in vitro* (Gabuzda et al., 1994; Gasparini et al., 2001). Energy metabolism inhibition also leads to an increase in the amyloidogenic APP processing *in vivo* (Velliquette et al., 2005). The link between energy deficiency and Aβ production seems to be mediated by BACE1. Energy deficiency, induced by specific inhibitors, increases the level and activity of BACE1 (Velliquette et al., 2005). This effect of energy deficiency lasted for 7 days. Reactive oxygen species were not measured in this study, though they may contribute to the long lasting effect of energy deficiency on induction of BACE1. Indeed, oxidative stress elevates BACE1 levels in cultured neurons (Tamagno et al., 2002; Tamagno et al., 2005), which supports a potential role for ROS in the BACE1 increase in AD and in energy deficiency. Importantly, BACE1 levels were also elevated after traumatic brain injury (Blasko et al., 2004), ischemia (Lee et al., 2006b; Wen et al., 2004), and oxidative stress (Tamagno et al., 2005; Tong et al., 2005), all of which can cause an energy deficiency. These studies indicate the possible influence of the energy charge and oxidative stress on APP processing. However, recent work showed that the cleavage of APP by γ-secretase to form Aβ appear to require ATP(Netzer et al., 2003). This dependency does not support the view that energy deficiency is a trigger for increased amyloidogenic Aβ production because low ATP levels due to an energy

deficiency would decrease γ-secretase cleavage and Aβ formation. Furthermore, it has been demonstrated that energy deficiency caused a decrease in the level of APP and its processing products of Aβ and sAPP *in vitro* (Hoyer et al., 2005). These studies indicate that the effect of energy metabolism on APP is complex. Future research should provide answers to questions regarding the differential effects of energy on Aβ production by BACE and γ-secretase, and may contribute to our understanding of the biological functions of APP and Aβ.

2.3. Factors that may cause energy hypometabolism in Alzheimer's disease brain

The molecular events that drive the energy hypometabolism and typical cellular pathologies seen in AD are not clear. Linking the molecular mechanism for the energy hypometabolism in AD to the excessive levels of Aβ would help provide an explanation for the typical cytopathologies seen in AD, and should offer new therapeutic strategies for AD. Several mechanisms can cause energy deficiency in AD.

Energy metabolism is the most important function of mitochondria in all types of cells (Tyler, 1992). The brain, especially neurons, have a high demand for energy (Raichle, 2006). Thus, impaired mitochondrial function is one possible mechanism responsible for the energy deficiency in AD (Blass et al., 2000; Mosconi et al., 2005; Parihar and Brewer, 2007; Valla et al., 2001). A variety of biochemical aberrations can lead to the abnormal energy metabolism by the mitochondria. The electron transport complexes (I, II, III, and IV) and the Krebs cycle (TCA) cycle are the mitochondrial metabolic pathways that are essential for generating the proton gradient across the inner membrane of the mitochondria that is used to produce ATP. Cytochrome *c* oxidase (complex IV) is an intracellular measure of oxidative energy metabolic capacity and respiration (Villani and Attardi, 2000). Complex IV, which is decreased in the mitochondria from AD patients (Parker et al., 1990, 1994b), is a strong molecular candidate as a contributor to the energy hypometabolism. One possible mechanism for the decrease in complex IV and the resulting mitochondrial dysfunction is a deficiency of heme ((Atamna, 2004, 2006), see Section 5).

The activity of the electron transport complexes (ETC) depends on cardiolipin, coenzyme Q, copper, heme, and iron-sulfur clusters for adequate functioning. The TCA cycle extracts the electrons form the intermediate metabolites to reduce NAD and FAD. The reduced NAD and FAD are the major source of electrons for the ETC and pass the electrons to the complexes I and II, respectively. Thus, malfunctioning of any of the energy extracting mechanisms of the mitochondria may contribute to the energy hypometabolism in AD (see Section 3.1 and 5.2).

A different mechanism for energy hypometabolism in AD may be the reduced cerebral blood flow due to abnormal microvasculature in specific areas in the brain (reviewed in Chaney et al., 2003). Hypoperfusion of the posterior cingulate cortex, which is a brain region that shows impairment in early stages of AD, occurs in subjects with mild cognitive decline who later develop AD (Kogure et al., 2000). Additionally, treatment that increase the cerebral blood flow have beneficial effects on AD patients probably due to increased neuronal energy production, which leads to cognitive improvement (Goldsmith, 2001). The breakdown of microvasculature, which leads to internal hemorrhages in certain areas in the brain, could reduce energy metabolism. Vascular dementia is suspected to be one primary cause of AD (Shi et al., 2000). The "silent" brain infarcts in healthy elderly people, which reduce blood supply to the brain, might be associated with dementia (Vermeer et al., 2003a, 2003b). Mitochondrial dysfunction in brain endothelial cells may also contribute to the microvasculature aberration and increase their breakdown (see section below, Aliev et al., 2003).

Several lines of evidence indicate altered insulin metabolism and decreased transport of insulin to the brain may contribute to impaired brain glucose uptake and metabolism in AD patients. Reported decreases in the levels of both insulin and IGF-I in the brains of AD patients (Rivera et al., 2005) likely contribute to the energy hypometabolism and cognitive impairment found in AD. The role of insulin in maintaining memory function has been recently demonstrated by intranasal administration of insulin, which bypasses the blood–brain barrier to selectively increase insulin in the CNS, without altering insulin or glucose levels in the blood. Intranasal insulin significantly improved verbal memory in patients in the early stages of AD or with amnestic mild cognitive impairment compared to placebo (saline-treated controls) (Reger et al., 2006). Intranasal insulin has also been shown to improve memory in normal human adults (Benedict et al., 2007).

Insulin resistance (a metabolic condition of abnormal glucose metabolism) is associated with an increased relative risk for AD. Amyloidogenic processing of APP to form Aβ in the Tg2576 mouse model for AD was enhanced by insulin resistance (Ho et al., 2004). Furthermore, *in vitro* studies showed that insulin was needed to prevent the accumulation of intracellular Aβ (Gasparini et al., 2001), indicating the importance of brain insulin for maintaining healthy neurons in the brain. The degeneration of specific neurons in AD brain may contribute to the decline in insulin in the brain. Mitochondrial dysfunction and oxidative stress may also contribute to the disturbed insulin metabolism in AD brain. Enhanced memory in AD patients and normal adults with intranasal insulin may be a direct effect of enhancing glucose uptake and metabolism in specific neurons, insulin's influence on acetylcholine, or NMDA receptor activation.

3. Mitochondrial dysfunction in Alzheimer's disease

AD is characterized by abnormal metabolism of APP and excessive levels of Aβ peptides, key players in AD

neuropathology. Aging is the major risk factor for AD. Of particular significance is that Aβ can be found in the cells and the mitochondria. These findings together with the pathological role of soluble multimeric complexes of Aβ in the early onset of AD may be key clues to the molecular mechanism of Aβ's neurotoxicity. The described specific mitochondrial dysfunctions are probably consistent with the key role in energy hypometabolism and increase in the production of free radicals observed in the brain in the early stages of AD. However, no unifying mechanism has been proposed to link Aβ to specific key cytopathologies (e.g., loss of iron homeostasis), mitochondrial dysfunction (e.g., decrease in complex IV), and the neurodegeneration seen in AD.

Mitochondria benefit their host cells by producing energy efficiently, detoxifying oxygen, maintaining the cellular redox potential, controlling calcium homeostasis, synthesizing heme and iron-sulfur clusters, and other key metabolites. Mitochondria are also implicated in the production of free radicals, particularly, under pathological conditions where an imbalance in the energy extracting mechanism (i.e. ETC) occurs due to a dysfunction of a specific component (e.g., selective decrease in complex IV). The biochemistry and physiology of the mitochondria appears abnormal in the AD brain. Dysfunctional mitochondria are presumed to compromise neuronal plasticity and neuronal response to metabolic challenges, physiologic and environmental cues, and the encoding of new memories by lowering the energy charge in neurons (Li et al., 2004).

3.1. Decline in key macromolecules of mitochondria in Alzheimer's disease

Several studies have demonstrated changes to the ETC and TCA cycle, which are the two most important metabolic pathways in the mitochondria. Researchers have demonstated a 30–40% decrease in complex IV activity (Cottrell et al., 2001; Maurer et al., 2000; Mutisya et al., 1994; Parker et al., 1994b) and in α-ketoglutarate dehydrogenase (αKGDH) (Gibson et al., 2000b; Ko et al., 2001; Mastrogiacoma et al., 1993, 1996). A more comprehensive screening of the activities of the TCA cycle enzymes in AD (Bubber et al., 2005) found that some decreased (e.g., PDH, αKGDH, ICDH), others increased (e.g., SDH and MDH), while the other four enzymes did not change (e.g., aconitase). These changes presumably would result in a decline in succinyl-CoA, the intermediate of the TCA cycle that is produced by αKGDH and consumed by the subsequent reactions of SDH and MDH. Succinyl-CoA is the precursor for heme (Furuyama and Sassa, 2000; Schulman and Richert, 1957), thus, a decline in succinyl-CoA would be expected to lead to a decline in heme (Atamna et al., 2007). In addition to the biochemical changes, the mitochondria from AD brain patients exhibits a substantial structural changes that included the cristae, accumulation of osmiophilic material, and smaller size compared to normal controls (Baloyannis, 2006).

3.2. Direct interaction between Aβ, APP, APOE, and their fragments with mitochondria

A direct link between Aβ and mitochondria was demonstrated as early as 1992. The first localization of APP to the mitochondria was seen in the outer membrane of mitochondria from the brain of AD patients using immunohistochemistry (Yamaguchi et al., 1992). This finding was followed by more recent observations of the interaction of APP and fragments of APP with the mitochondrion. APP or its fragments were shown in mitochondria (Crouch et al., 2005) to bind to the mitochondrial protein import machinery (Anandatheerthavarada et al., 2003; Devi et al., 2006), HtrA 2 serine protease in the intermembrane space (Park et al., 2006), or ABAD in the mitochondrial matrix (Lustbader et al., 2004). Furthermore, Aβ monomers and multimeric forms can be isolated with mitochondrial preparations from Tg2576 mice overexpressing human APP (Manczak et al., 2006). The functional implications of the above indicate binding of Aβ to the mitochondria, but the direct relevance of this to mitochondrial dysfunction in AD still needs to be demonstrated in an experimental setting that faithfully represents AD's pathology.

Interestingly, a decline in complex IV, a decline in energy charge, and an increase in free radical production were observed in several studies by treating cells with Aβ or by overexpressing APP (Bennett et al., 1992; Keil et al., 2004). A direct interaction of Aβ with several mitochondrial components has been shown, however no unifying mechanism has been proposed to link the binding of Aβ and mitochondria to the key cytopathologies specific for AD (see Section 5).

ApoE 4 is a major risk factor for late onset AD (Reiman et al., 1996). ApoE is produced in neurons in response to stress (oxidative stress), excessive Aβ, or head trauma (Mahley et al., 2006). Interestingly, mitochondrial dysfunction in AD appears to vary with the genotype of apoE (Gibson et al., 2000a). Several biochemical observations show the binding of specific apoE4 fragments with mitochondria (Chang et al., 2005). These interactions cause mitochondrial dysfunction as demonstrated by low ATP and increased free radical production. Mitochondrial dysfunction in AD brain that occurs as a result of mechanisms not related to Aβ may be exaggerated in the presence of Aβ.

3.3. Mitochondrial dysfunction in non-neuronal cells

Neuronal tissue is not the only tissue to exhibit mitochondrial abnormalities in AD patients. Platelets and fibroblasts (Mancuso et al., 2003; Naderi et al., 2006; Parker et al., 1994a; Sheu et al., 1994) from AD patients also exhibit mitochondrial aberrations. These findings suggest there

are possibly systemic effects of the disease on mitochondria in AD patients, and support the view that brain endothelial and smooth muscle cells also exhibit mitochondrial aberration (Aliev et al., 2003; Aliyev et al., 2005). Mitochondrial dysfunction leads to cellular senescence (Atamna et al., 2000, 2001b; Kalaria, 1996; Liu et al., 2002a), increasing ROS production, lowering energy charge, and decreasing metabolic efficiency. Although, damage to neuronal cells are the proximal cause of degeneration in AD, mitochondrial dysfunction in brain microvasculature and BBB seen with age may contribute to the brain pathology in AD (Mooradian, 1988; Zlokovic, 2005). The accumulation of Aβ in the CNS and its effect on mitochondria may exacerbate mitochondrial dysfunction in non-neuronal cells in the brain.

4. Increased production of free radicals in AD brain

An additional problem in AD brain that has been attributed to mitochondria is increased oxidative stress (Markesbery, 1997; Nunomura et al., 2001; Ohta and Ohsawa, 2006) due to abnormal production of reactive oxygen species (ROS) (Cadenas and Davies, 2000). Production of ROS by the mitochondria is viewed as a key outcome of mitochondrial decline with age (Cadenas and Davies, 2000). Increase in ROS production by the mitochondria may cause oxidative damage to mitochondrial proteins, lipids, and DNA, thereby further disrupting mitochondrial function and energy production if the mitochondrial mechanisms of repair (protein turn-over, DNA repair) and defense (antioxidants) fail.

Two different views on the interplay between the accumulation of Aβ and oxidative stress in AD have been proposed. Although, increased levels of Aβ42 is viewed by the majority as neurotoxic, the Aβ40 form, which is more abundant in the brain has been argued to be either neuroprotective (Lee et al., 2006a; Teng and Tang, 2005) or neurotoxic. Neuroprotective Aβ is viewed as one additional defense mechanism that neurons use against oxidative stress. The role of neurotoxic Aβ in producing oxidative stress could be mediated through the induction of mitochondrial dysfunction. Complexation of extracellular Aβ with copper and iron (Bush, 2003) is also proposed to contribute to the increase in oxidative damage. Loss of iron homeostasis is one of the key cytopatholgies in AD brain (Connor et al., 1992; Smith et al., 1998). Abnormal heme metabolism is proposed as the proximal cause for disturbed iron homeostasis in AD brain (Atamna, 2006).

Several lines of evidence indicate that electron transport complexes I and III are major sources for production of ROS by mitochondria that increase with age (Cadenas and Davies, 2000; Liu et al., 2002b). A selective decrease in complex IV (as in AD), while complexes I and III remain intact, increases the production of ROS (Atamna et al., 2001a; Sohal, 1993). Sodium azide, which inhibits mitochondrial complex IV, increases the production of ROS from mitochondrial ETC and impairs learning and memory when administered to rats (Callaway et al., 2002; Cassarino and Bennett, 1999). A decline in complex IV is not the sole cause of AD. A combined decrease in complex IV and αKGDH, in conjunction with Aβ accumulation and certain effects of aging, in addition to many other genetic, environmental, and dietary factors lead to AD (Hoyer, 2004). The role of accumulation of Aβ and heme deficiency in mitochondrial dysfunction will be presented in Section 5.

4.1. Oxidative damage to mtDNA from AD patients

Additional direct changes to mitochondria were demonstrated by an increase in the incidence of mtDNA mutations in AD brain that exceeds the level expected due to normal aging (Coskun et al., 2004; Hamblet et al., 2006; Wallace et al., 1995). The functional consequence of these biochemical changes can be impaired energy production (Porteous et al., 1998). Because each mitochondrion can have between 2 and 10 copies of mtDNA and each cell contains several hundreds of mitochondria, mtDNA damage must exceed a certain threshold to have functional consequences for AD brain pathology (Gellerich et al., 2002). The threshold level of mtDNA mutation needed to exhibit a biological effect remains the subject of debate. Interestingly, only a few studies show no difference in the occurrence of mtDNA mutations between AD and age-matched non-demented controls (Coon et al., 2006). Deletions of mtDNA have severe consequences on cellular energy charge, thus they exist in heterplasmic form (where both normal and mutated mtDNA exist in the same cell) (Alemi et al., 2007; Gellerich et al., 2002; Porteous et al., 1998). What constitutes the threshold number of age-associated mutations to mtDNA that is required before there are functional consequences is less clear.

Although mitochondria can only repair oxidized nucleotide bases in the mtDNA (Stuart et al., 2005), activity of mitochondrial DNA base excision repair increases with aging (Dianov et al., 2001); this may represent an adaptive response to counter oxidative damage to mtDNA. Relative to nuclear DNA, mtDNA is more susceptible to endogenous and exogenous oxidative damage, which is probably why there is increased mutation in mtDNA relative to nuclear DNA in AD patients.

5. Proposed unifying mechanism to explain Aβ neurotoxicity

The binding of Aβ (Aβ40 and Aβ42) with heme, a key metabolite, forming Aβ-heme complex has recently been demonstrated (Atamna and Boyle, 2006; Atamna and Frey, 2004). Heme (ferroprotoporphyrin IX), the major functional form of iron, is synthesized in all nucleated cells including brain cells by ferrochelatase, which is located in the inner membrane of mitochondria (Burden et al., 1999). Each tissue synthesizes its own heme; heme is not

interchanged between tissues or cells. Heme, although synthesized in the mitochondrial inner membrane, can reach all the compartments of the cell, probably carried by specific proteins (Hamza, 2006). Amino acids, peptides, and proteins are also believed to transiently bind the newly synthesized heme (Dias et al., 2006; Latunde-Dada et al., 2006). The pool of this transiently bound heme will be referred to in the remaining sections as "regulatory heme". We propose that Aβ may primarily complex with regulatory heme and reduce its availability. Aβ may also form a complex with heme in the mitochondria in addition to other cellular compartments.

The concentration of regulatory heme is inferred to be less than 30 nM (Sassa, 2004), however, it has not been directly measured. The function of regulatory heme in brain cells (and in other cells in general[1]) is to deliver heme to various metabolic destinations: Complex IV (after conversion to heme-*a*), *b*-cytochromes, *c*-cytochromes, catalases, and heme oxygenases (for degradation). In addition, regulatory heme delivers heme to Heme Regulatory Motifs (HRM) in various regulatory proteins. Heme bound to HRM appears interchangeable with that present in regulatory heme, but not with heme bound to cytochromes, which is essentially irreversible (Zhang and Guarente, 1995). Examples of proteins regulated by heme are: RNA-binding protein DiGeorge critical region-8 (DGCR8) (Faller et al., 2007), BTB and CNC homology 1 (Bach1) (Dhakshinamoorthy et al., 2005; Suzuki et al., 2004), neural PAS domain protein 2 (NPAS2), E75 (Reinking et al., 2005), IRP2 (Goessling et al., 1998; Ishikawa et al., 2005), δ-aminolevulinat synthase (Goodfellow et al., 2001; Lathrop and Timko, 1993; Munakata et al., 2004), indoleamine (tryptophan) dioxygenase (Badawy, 1978; Ren and Correia, 2000), and cystathionine β-synthase (Meier et al., 2001). These diverse and important roles of regulatory heme in cellular metabolism may account for some of the metabolic consequences of heme deficiency. Heme deficiency occurs in senescent neurons (Chernova et al., 2006) and contributes to mitochondrial dysfunction and oxidative stress in Friedreich's ataxia (Napoli et al., 2006; Schoenfeld et al., 2005). Heme-deficient human neuroblastomas or astrocytoma die after stimulation with fresh serum (Atamna and Boyle, 2006; Atamna et al., 2002). In addition, a heme deficiency also halts the NGF-dependent signaling pathway and causes neuronal death (Zhu et al., 2002). Thus, it is likely that a reduced availability of regulatory heme by sequestration in excess Aβ disturbs specific signaling pathways and expression of various genes, which might have additional implications for AD. The most pronounced effect is loss of mitochondrial complex IV through the shortage of heme-*a*.

[1] Heme that is synthesized in bone marrow is mainly used for production of hemoglobin and is distinct from the heme that is synthesized in brain cells.

5.1. Heme oxygenase is induced in AD and may contribute to heme deficiency through Aβ-heme

Induction of heme oxygenase in liver has been shown to create a heme deficiency by accelerating heme degradation (Converso et al., 2006). Induction of heme oxygenase in human brain cells in tissue culture also causes heme deficiency (Atamna et al. in preperation). Changes in heme oxygenase (HO) is one of the key biochemical changes observed very early in the research on AD (Schipper et al., 1995; Smith et al., 1994). It is known that HO activity requires the heme to be bound to a protein carrier/s, the identity of which has not been discovered (Schacter et al., 1972; Vreman and Stevenson, 1988). We propose that induction of HO might be a response to increase Aβ-heme, which in turn increases the degradation of heme by HO. Since HO is located mainly in the ER, Aβ-heme may enhance the shuttle of heme from the various compartments to HO. Thus, an increase in heme oxygenase in AD brain may exacerbate the heme deficiency proposed in AD brain.

5.2. Reduced metabolic sufficiency of TCA cycle contributes to heme deficiency

The metabolic activity of the TCA cycle decreases in AD mitochondria due to a decline in several of the enzymes of the cycle (Bubber et al., 2005). We think this decline in enzymes, in turn, depletes the TCA intermediate succinyl-CoA. Succinyl-CoA and Glycine are the precursors for heme synthesis (Ponka, 1999). Such a depletion in TCA cycle intermediates lowers the capacity of the cell to synthesize heme, which is essential, among other things, for the integrity of the ETC especially that of complex IV. We have recently demonstrated that inhibition of the anaplerotic reactions in the mitochondria (which replenish the TCA intermediates) selectively inhibits heme production (Atamna et al., 2007). In these experiments, decreasing the catabolism of branched chain amino acids, which can be achieved by biotin deficiency, inhibited the anaplerotic reactions.

5.3. Aβ-heme participates in reduction–oxidation reactions

A further important discovery that connects Aβ to the oxidative stress seen in AD (Markesbery, 1997; Nunomura et al., 2001) is that Aβ-heme has a peroxidase activity which oxidize several brain metabolites and neurotransmitters, such as serotonin and DOPA (Atamna and Boyle, 2006) (and CoQ, unpublished observations). Aβ-heme may also be found in the mitochondria as both Aβ and heme co-localize to this organelle. The ability of Aβ-heme to catalyze the electron transfer form H_2O_2 to an organic substrates, suggests it may also participate or interfere with the electron transfer at the level of ETC or by oxidation of specific mitochondrial redox-dependent enzymes such as αKGDH, which is prone to oxidative damage (Gibson

et al., 2000b). Both Aβ40 and Aβ42 can form a complex with heme. Future research should address whether the forms of Aβ-heme have differential metabolic consequences.

5.4. Heme colocalization to the senile plaques and the increase in heme in AD autopsies

Consistent with our hypothesis that Aβ-heme is present in AD brain, heme has been recently demonstrated (using histochemical methods) to colocalize to the senile plaques (SP) (Cullen et al., 2006). The source of heme in SP is presumed to originate from hemoglobin that leaks to the brain from vascular breaches, while Aβ may originate from platelets. Although feasible, we argue that the heme in the senile plaques is complexed to form Aβ-heme. If this is the case, then Aβ-heme may also be formed outside the cells (i.e. neurons) in addition to Aβ-heme proposed to be formed inside the cells (and mitochondria). Thus, there may be two sources of the same toxic agent, Aβ-heme, that enhance each other.

One might ask why was not the heme in senile plaques detected before? The answer might be that the chemicals that are routinely used for the isolation of Aβ and senile plaques are SDS, β-mercaptoethanol, or formic acid (Selkoe et al., 1986). These reagents release heme from heme proteins and cause heme degradation and iron release (Atamna and Ginsburg, 1995). This may explain why heme has not been implicated before in AD pathology.

An additional implication of heme colocalizing to SP (Cullen et al., 2006) is that excess heme, which we found previously in AD brain (Atamna and Frey, 2004; Venters et al., 1997) may partially exist at the extra cellular milieu and originate from the heme trapped in the senile plaques. If this is the case, then one may assume that the intracellular heme may be decreased. Indeed, we found that heme-*a*, which mainly is inside the cells exhibited a trend of about 25% decline, which was consistent with the previous findings by Parker who measured cytochromes *a* and a_3, which are the protein forms of heme-*a* (Parker et al., 1994b). More research, especially research focused on regulatory heme, is needed regarding the compartmentalization of heme in the brain.

5.5. Possible relevance of Aβ-heme to neurovascular dysfunction in AD

Aβ-heme could be also relevant to the neurovascular dysfunction in AD. The senile plaques, which seem to colocalize with heme, might be an extracellular source of Aβ-heme. We know that heme can dismantle senile plaques to smaller aggregates, which themselves might be soluble forms of monmers and multimeric forms of Aβ-heme (unpublished observation). Thus, Aβ-heme, if formed in the senile plaques, may diffuse and reach membranes in the neuronal or brain endothelial cells. Aβ-heme having peroxidase activity, may also contribute to the damage and dysfunction of brain endothelial cells and BBB. Aβ itself is amphebatic peptide that interact with lipid bilayers (Kagan et al., 2004). Aβ-heme complex retains some of the hydrophobic features of Aβ (especially Aβ42-heme) that allows its association with the membrane either by insertion in the bilayer or by association with the membrane surfaces (Atamna et al in preparation).

6. Heme deficiency is a phenocopy of AD

A unique feature of the proposed mechanism is that it produces a phenocopy of AD key cytopathologies when heme deficiency is applied to cells *in vitro*. We created a heme deficiency in culture cells to determine if the consequences matched the key cytopathologies of AD (Atamna et al., 2002, 2001a). We found that the consequences include a decline in the level of Complex IV, induction of heme synthesis, iron accumulation, mitochondrial dysfunction, increased production of oxidants (e.g. H_2O_2), dimerization of APP (suggesting abnormal processing, Scheuermann et al., 2001); and neuronal cell death after induction of division or differentiation (some of these are described in Fig. 1) (Atamna et al., 2001a, 2002).

Because this paper is concentrating on the mitochondrial dysfunction in AD, we will focus on the decline in complex IV. The decline in complex IV might be explained by a decrease in the levels of heme-*a* that results from decrease in regulatory heme. Heme-*a* is essential for the assembly and activity of Complex IV, which is the only enzyme that contains heme-*a*. Regulatory heme can be directly used to form heme-*b* or heme-*c* when tightly associated with cytochromes *b* and *c*, respectively (in the case of cytochrome *c*, heme is covalently bound to the protein). However, regulatory heme needs biochemical modifications to form heme-*a* through two reactions: heme-O synthase (cox10p) (Barros et al., 2002; Saiki et al., 1992; Tzagoloff et al., 1993)), which adds a farnesyl group to give heme-O (Mogi et al., 1994; Weinstein et al., 1986), and heme-*a* synthase (cox15p), which oxidizes the methyl group

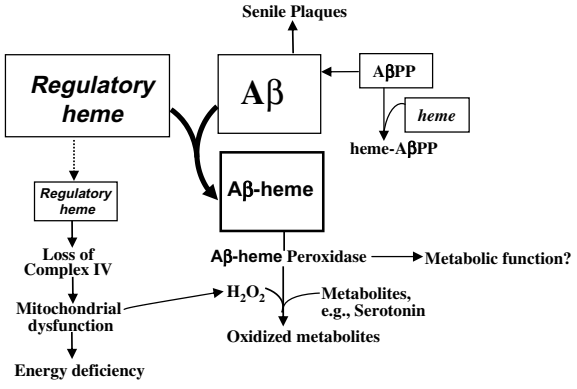

Fig. 1. Proposed sequestration of regulatory heme by excessive Amyloid-β (Aβ) and the formation of Aβ-heme.

to produce heme-*a* (Barros et al., 2002; Barros and Tzagoloff, 2002; Brown et al., 2002).

6.1. Heme may be important for the processing of APP

The unexpected dimerzation of APP in response to heme deficiency that was SDS resistant is intriguing. Dimers of APP and Aβ were found in membranes *in situ* (Scheuermann et al., 2001) and Aβ dimers in AD brain patients (Enya et al., 1999). Additionally, APP and Aβ dimers were also found in mice transgenic for AD (Kawarabayashi et al., 2004). The production of Aβ has been suggested to be positively regulated by dimerization of APP *in vivo* (Scheuermann et al., 2001). The implication of APP dimerization on its processing and AD pathology has been recently discussed (Marchesi, 2005). Although, the binding of heme to APP *in vivo* has not yet been demonstrated, we view the effect of heme deficiency on APP as an additional support for the proposed role of heme metabolism in AD.

6.2. Heme deficiency and the other proposed mechanisms for AD

Our proposed model of heme binding with Aβ may work in conjunction with other mechanisms that were previously proposed to explain Aβ toxicity: (1) the interference of Aβ with branched-chain fatty acid metabolism and thus the TCA cycle, through complexing with ABAD (Yan and Stern, 2005); (2) the proposed direct damaging effect that the proteolytic fragments of APP and ApoE4 have on the mitochondria (Anandatheerthavarada et al., 2003; Chang et al., 2005); and (3) the role of oxidative stress in AD pathology through the peroxidative activity of Aβ-heme (Atamna, 2006). All these proposed mechanisms may work in conjunction to account for mitochondrial dysfunction that may trigger the early events that lead to AD. The interaction of ABAD may exacerbate the metabolic insufficiency of the TCA cycle leading to decrease in succinyl-CoA, the precursor for heme (Atamna et al., 2007). Similarly, the binding of APP fragments to protein import machinery is presumed to compromise the mitochondrial function, if the dysfunctional protein import complexes or damaged mitochondria were not efficiently removed through turnover or autophagy, respectively (Alemi et al., 2007; Lee and Wei, 1997; Lemasters, 2005). Smith et al. has found fragments of the mitochondria from AD brain in the lysosomal compartment, which may suggest increase autophagy to remove dysfunctional mitochondria (Hirai et al., 2001). Oxidative stress in conjunction with Aβ-heme peroxidative activity may render the ApoE4 genotypes at higher risk for AD due to the susceptibility of ApoE4 genotypes to oxidative stress.

It is important to note that as the level of Aβ increases, heme deficiency may be established in brain cells over long period of time and the level of the complex IV decrease, if heme synthesis compensatory response (demonstrated by an increase in ferrochelatase (Atamna and Frey, 2004)), is blunted e.g., by TCA dysfunction. The consequent mitochondrial dysfunction by decline in complex IV results in a decrease in ATP production and an increase production of H_2O_2. Since the peroxidase activity of Aβ-heme utilizes H_2O_2, a cycle of increasing oxidative damage could be created, which would be the basis for much of the oxidative damage observed in AD.

Fig. 1 summarizes the binding of excess Aβ by heme (specifically, regulatory heme) to form Aβ-heme as the primary event. This reduces the availability of heme, thus creating a "functional" heme deficiency. This is the molecular link between Aβ, heme metabolism, and key cytopathologies in AD. Fig. 1 also shows heme binds to APP, which was deduced from our studies and others and suggests that heme may alter the processing of APP and contributes to the increased production of Aβ in neuronal cells (Atamna et al., 2001a, 2002).

The reduced availability of regulatory heme triggers compensatory increase in heme synthesis and iron content as is indeed seen in AD brain autopsy. However, such a compensatory response would be insufficient when excessively high levels of Aβ are continuously present in the AD brain, together with high HO, and a decline in TCA, thus leading to a functional heme deficiency.

7. Mitochondria as a potential therapeutic target for AD

A number of strategies have been proposed for developing therapeutics to treat and prevent AD such as cholinergic and glutaminergic therapies, neurotrophic strategies (e.g., intranasal insulin delivery), anti-Aβ strategies, or therapies targeted at Aβ production or aggregation. Any or all of these approaches may prove valuable. Our intent in focusing on the role of mitochondrial dysfunction in AD is to draw the reader's attention to the mitochondrial abnormalities associated with the disease and provide a rationale for treating these to improve the health of AD patients, and ultimately prevent AD.

Mitochondrial defense mechanisms successfully counteract most of the insults that the mitochondria encounter during their life span, thus cellular stresses do not always result in cell death and disease. Knowledge of the known defense mechanisms of the mitochondria may assist in designing pharmacological or nutriceutical approaches to protect the mitochondrial from Aβ. For example, protecting the mitochondria from free radicals produced by radiation could be accomplished by overexpressing mitochondrial Mn–SOD (Epperly et al., 2003; Epperly et al., 2002). Thus, it might be fruitful to search for ways to increase the level of Mn–SOD in mitochondria, either by gene therapy or by using compounds that induce the expression of MnSOD (e.g., WR-1065). However, the practicality of overexpressing Mn–SOD is questionable, and a pharmacological approach should be carefully considered when applied *in vivo*, where the metabolic activity of the mitochondria exceeds that of cells in tissue culture. A further limitation of the pharmacological approach is that mitochondria can be a target for

several drugs. The electron transfer complexes can participate in redox cycling with nonphysiologic compounds or drugs enhancing their toxicity, while the membrane potential of the mitochondria can concentrates lipophilic cations in the mitochondrial matrix to toxic levels. Therefore, agents that intended to protect the mitochondria may unintentionally damage them when administered *in vivo*.

A nutriceutical approach to protect the mitochondria might be more feasible. It is likely that physiological, nutritional, and environmental factors play important roles in causing mitochondrial dysfunction with age and age-related diseases. Since their optimal metabolic function depends on the availability of many essential vitamins, minerals, and other metabolites (Ames, 2004, 2006; Atamna, 2004), mitochondria are susceptible to micronutrients deficiencies. These micronutrients function as critical cofactors that support basic metabolic pathways of the mitochondria including ATP synthesis, heme synthesis, iron-sulfur-cluster assembly, building electron transport complexes, and the detoxification of oxygen (Atamna, 2004, 2006). Inadequate amounts of some of these micronutrients lead to a decline in critical enzymatic activities, thus increasing the production of reactive oxidants and decreasing the energy charge of the cell, which when combined with Aβ may accelerate mitochondrial dysfunction and AD. Promising therapeutic potential of mitochondria-specific nutriceuticals and vitamins have been demonstrated in cultured cells, animal models, and clinical trials. Such studies were conducted for coenzyme Q for Parkinson's Disease and Freidrech Ataxia (Beal, 2004; Dhanasekaran and Ren, 2005; Young et al., 2007); lipoic acid and acetyl-carnitine in the aging rat brain (Hagen et al., 2002); lipoic acid in a mouse model for AD (Quinn et al., 2007); and diverse metabolites and vitamins affecting mitochondria in clinical trials and case reports (Przyrembel, 1987). Mitochondria-specific nutriceuticals, e.g., coenzyme Q, and vitamins, e.g., tetrahydrobiopterin, reviewed in (Foxton et al., 2007), have been proposed to be beneficial for AD patients. Probably, mitochondria-specific nutriceuticals are most effective for the prevention of age-related neurodegenerative diseases.

8. Summary

Early cytopatholgies of AD suggest a key role for the mitochondria in the development of AD. The interactions of Aβ with mitochondria provide further support for the role of mitochondria. Transforming dysfunctional mitochondria to adequately functioning mitochondria is likely to improve the metabolism of the cells and tissues even in pathological conditions. Therapeutic strategies targeted at preventing, delaying, or treating mitochondrial dysfunction should contribute to the prevention and/or treatment of age-related neurodegenerative diseases.

Although, several potential approaches exist to protect the mitochondria during aging, new strategies that target specific pathologies of each disease are very much needed. The current research on the mechanism of mitochondrial dysfunction related to Aβ may lead to new strategies to prevent or delay AD such as by inhibiting the Aβ-heme peroxidase; blocking the formation of Aβ-heme to counteract heme deficiency; or using Aβ-heme for immunotherapy (targeted at extracellular Aβ-heme). Similarly, preventing the interactions of Aβ with ABAD or the protein import machinery may be other ways to protect the mitochondria. More research is needed on the interactions of Aβ with key metabolites of the mitochondria before designing therapies targeted at these interactions. The level of Aβ-heme in blood may also serve as a biomarker for AD.

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