Mol Neurobiol (2010) 41:159–171
DOI 10.1007/s12035-010-8141-5

Mitochondrial Dysfunction: Common Final Pathway in Brain Aging and Alzheimer's Disease—Therapeutic Aspects

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Received: 28 January 2010 / Accepted: 15 April 2010 / Published online: 12 May 2010

Abstract As a fully differentiated organ, our brain is very sensitive to cumulative oxidative damage of proteins, lipids, and DNA occurring during normal aging because of its high energy metabolism and the relative low activity of antioxidative defense mechanisms. As a major consequence, perturbations of energy metabolism including mitochondrial dysfunction, alterations of signaling mechanisms and of gene expression culminate in functional deficits. With the increasing average life span of humans, age-related cognitive disorders such as Alzheimer's disease (AD) are a major health concern in our society. Age-related mitochondrial dysfunction underlies most neurodegenerative diseases, where it is potentiated by disease-specific factors. AD is characterized by two major histopathological hallmarks, initially intracellular and with the progression of the disease extracellular accumulation of oligomeric and fibrillar β-amyloid peptides and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. In this review, we focus on findings in AD animal and cell models indicating that these histopathological alterations induce functional deficits of the respiratory chain complexes and therefore consecutively result in mitochondrial dysfunction and oxidative stress. These parameters lead synergistically with the alterations of the brain aging process to typical signs of neurodegeneration in the later state of the disease, including synaptic dysfunction, loss of synapses and neurites, and finally neuronal loss. We suggest that mitochondrial protection and subsequent reduction of oxidative stress are important targets for prevention and long-term treatment of early stages of AD.

Keywords Mitochondrial dysfunction · Alzheimer's disease · Aging · Oxidative stress · *Ginkgo biloba* extract · Piracetam · Dimebon

Abbreviations

Aβ	Amyloid beta
AD	Alzheimer's disease
APP	Amyloid precursor protein
COX	Cytochrome *c* oxidase
dG	8-oxo-2′-Deoxyguanosine oxo^8
FAD	Familial Alzheimer's disease
GPx	Glutathione peroxidase
HNE	4-Hydroxynonenal
MDA	Malondialdehyde
Mn-SOD	Manganese superoxide dismutase
mtDNA	Mitochondrial DNA
PS	Presenilin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SNP	Sodium nitroprusside

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Mitochondrial Dysfunction and Oxidative Stress in Brain Aging

Mitochondrial Dysfunction

Like in other differentiated tissues, cells in the central nervous system are affected by aging and react to aging as indicated by a decline of several physiological abilities including sensory, motor, and cognitive functions [1, 2]. Aging cells are affected by increasing amounts of oxidative stress, perturbed energy homeostasis, accumulation of damaged proteins, and lesions in their nucleic acids on the molecular level. Impaired function of signaling mechanisms and altered gene expression occur at the cellular level. These changes are significantly amplified in neurodegenerative disorders. Cell organelles playing a major role in aging itself and in aging-related neurodegenerative disorders are mitochondria, due to their central role in producing adenosine triphosphate (ATP) as the central source of cellular energy, as major source of physiologically produced oxidative stress (free oxygen species, reactive oxygen species—ROS), and as critical regulators of apoptosis during aging [2–6].

Alterations of mitochondrial efficiency and function are mostly related to alterations in concentration and efficiency of the complexes of the respiratory chain. The respiratory chain is located in the inner mitochondrial membrane and consists of five membrane spanning enzyme complexes which transfer electrons through a series of oxidation and reduction reactions, culminating into the reduction of oxygen. This energy of the electrochemical gradient is in turn used to phosphorylate adenosine diphosphate (ADP) via complex V (F1-F0 ATPAse). Complexes II and III of the respiratory chain are almost unaffected by the aging process. In contrast, complexes I and IV show significantly decreased enzymatic activities in mitochondria isolated from aged rat and mouse brain [4, 7–10]. In our own experiments, we could confirm a decrease of complex I activity as a rather early event within the scenario of the aging mouse brain (Fig. 1a).

In addition, it is well established that mitochondrial DNA (mtDNA) accumulates mutations with aging. Although most mitochondrial proteins are encoded by the nuclear genome, mitochondria contain many copies of their own DNA, e.g., encoding for 13 polypeptide complexes of the respiratory chain. Aging-dependent increase in the level of damaged DNA can be detected through biomarkers, e.g., the formation of 8-oxo-2′-deoxyguanosine (oxo^{8}dG). The levels of oxo^{8}dG were found to be significantly higher in mtDNA compared to nuclear DNA [11]. These differences can be explained by the proximity of mtDNA to oxidative stress generated by the mitochondrial respiratory chain itself, the lack of any protective histone covering, and a deficient repair mechanism compared to nuclear DNA. Therefore, mitochondria themselves are extremely sensitive to oxidative stress.

Oxidative Stress

The free radical theories of aging and mitochondrial decline hypothesis are most popular among the different theories of aging. Oxidants consist of ROS and reactive nitrogen species (RNS). In order to prevent oxidative damage, the cell has evolved a number of synergistic defense mechanisms. The antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase act in concert to catabolize ROS or RNS (Fig. 2) [12]. Further antioxidant support comes from endogenous (like GSH or uric acid) and exogenous radical scavengers (e.g., vitamin C or secondary plant metabolites, Fig. 2) (for review, please see [13, 14]). An imbalance in the catabolism of ROS and RNS leads to tissue damage (Fig. 2). ROS are generated in multiple compartments and by multiple enzymes within the cell but mainly as overflow by-products of complexes I, II, and III. Approximately 90% of cellular ROS can be traced back to mitochondria. Mitochondrial membrane lipids are highly susceptible to ROS, especially the long chain polyunsaturated fatty acid components. Furthermore, the inner mitochondrial membrane proteins such as complexes I, II, and III are themselves directly susceptible to effects of oxidative stress leading to membrane depolarization and subsequently impaired mitochondrial function [15] including increased oxidized proteins and mtDNA damage with age which in turn may cause further dysfunction.

Keeping in mind that our brain is especially vulnerable to free radical damage, brain aging seems to be closely associated with ROS [16, 17]. This vulnerability can be explained by its high content of easily peroxidizable unsaturated fatty acids, the high number of neuronal mitochondria implicating high oxygen consumption rate, and relative paucity of antioxidant enzymes in comparison with other organs [1, 18, 19]. The complexity of the different mechanisms of brain aging in aged NMRI mice is depicted in Fig. 1a, b. Brain aging displays substantial cognitive deficits beginning in late adulthood at around 12 months of age as shown in Fig. 1a for passive avoidance learning. At the same time, oxidative damage like enhanced lipid peroxidation and reduced membrane fluidity can be demonstrated (Fig. 1a). Similarly, impairment of complex I activity is detected earlier (Fig. 1b), but is probably compensated by the respiratory chain for some time until the whole system gets disturbed (see RCR, Fig. 1a). In addition, SOD, the major antioxidant defense system of the brain is elevated (Fig. 1b). However, as GPx activity is not altered, oxidative damage by enhanced levels of H_2O_2, the

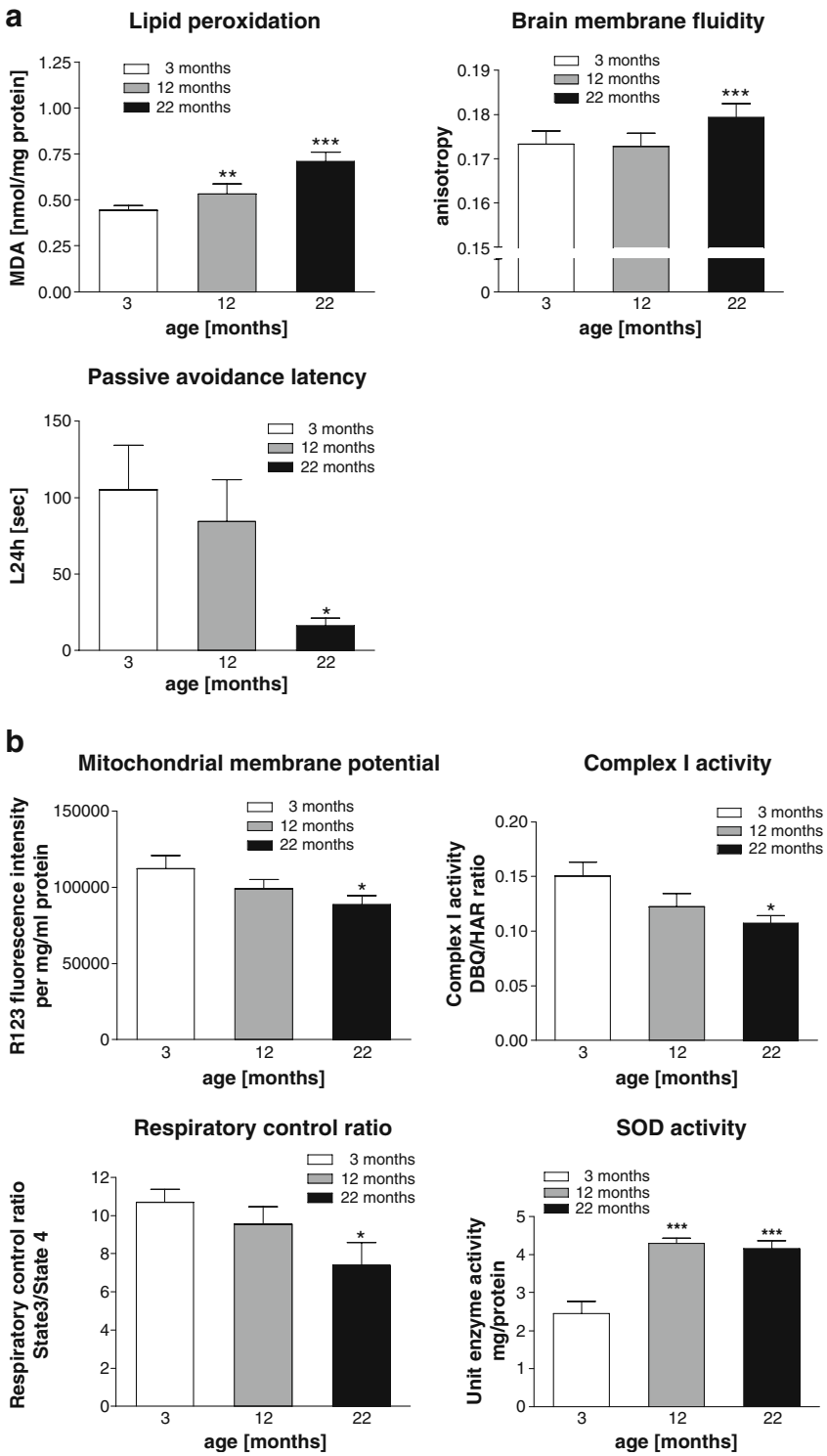

Fig. 1 **a** Biochemical and functional aspects of the aging mouse brain. All experiments were carried out with young (3 months), middle-aged (12 months), and old (24 months) female NMRI mice. Typical changes of the aging mouse brain due to free radical damage like enhanced lipid oxidation measured by the accumulation of malondialdehyde (*MDA*) [20] or by reduced membrane fluidity as indicated by the anisotropy of the fluorescence dye 1,6-diphenyl-1,3,5-hexatriene usually appear mainly after mid-age of the animals around 12 months. At the same time, cognitive deficits begin to start but are present only in old animals as indicated by the 24-h latency in a passive avoidance paradigm [141]. **b** Biochemical and functional aspects of the aging mouse brain. All experiments were carried out with young (3 months), middle-aged (12 months), and old (24 months) female NMRI mice. Indices of mitochondrial dysfunction like the mitochondrial membrane potential as measured by the fluorescence dye rhodamine 123, the activity of complex I normalized to the complex I content and expressed as DBQ/HAR ratio, and the respiratory control ratio also seem to start at an age of 12 months but manifest only in old animals around 24 months of age [9]. By contrast, SOD as a major player of the first antioxidant defense system (Fig. 3) is upregulated already at an age of 12 months explaining why no changes of the ROS concentration were detected in middle-aged and aged mice (modified after Leutner et al. [20])

major product of SOD, might explain elevated LPO during aging [20]. Two major conclusions can be drawn from these experiments: (1) One simple mechanism within this complex scenario might be misleading (e.g., SOD), and (2) the majority of changes start to take place after an age of about 12 months, which is about midlife for the NMRI mouse.

Mitochondrial Dysfunction and Oxidative Stress in Animal and Cell Models of Alzheimer's Disease

Increasing evidence suggests an important role of mitochondrial dysfunction and oxidative stress in the pathogenesis of many aging-related neurodegenerative diseases, especially Alzheimer's disease (AD) [9, 21]. AD is a

progressive disorder that leads to dementia and affects approximately 10% of the population older than 65 years of age. The clinical symptoms of AD include a progressive loss of memory and impairment of cognitive ability. The AD brain is marked by severe neurodegenerative alterations, such as the loss of synapses and neurons, atrophy, and the selective depletion of neurotransmitter systems (e.g., acetylcholine) in the hippocampus and cerebral cortex. Such defects are mainly observed in the late stage of the disease and have also been partially demonstrated using transgenic animal models of AD [22, 23].

Mitochondrial dysfunction is observed in AD brain [24] and has been proposed as an underlying mechanism of disease pathogenesis since defective energy metabolism is a fundamental component of AD [25–28]. Furthermore, early defects in glucose utilization in the brain of AD patients suggest possible abnormalities in mitochondrial function [27]. Interestingly, the activities of those enzymes, which are reduced in the brains of AD patients, such as α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, were inhibited by amyloid beta (Aβ) [29]. The most consistent defect in mitochondrial electron transport enzymes in AD is a deficiency in cytochrome *c* oxidase (COX), which was reported in both AD platelets and postmortem brain samples [30, 31]. Histochemical analyses revealed a significant reduction of cytochrome *c* oxidase activity in the dentate gyrus and other subfields of the hippocampus of AD patients. In situ hybridization studies also showed decreased messenger RNA levels of the mtDNA-encoded subunit II, but not the nuclear DNA-encoded subunit IV, of cytochrome *c* oxidase in AD brain [32]. COX dysfunction increases ROS production, reduces energy stores, and disturbs energy metabolism [33].

Mitochondrial Dysfunction in AD Animal Models

In order to address the effects of Aβ and aging on mitochondrial function in AD, we studied Thy1-APP_{751}SL mice at different ages [34]. These mice express the mutant human APP_{751} with the Swedish double and the London mutation, regulated by the neuronal murine Thy1 promoter resulting in the development of typical Aβ depositions in the form of amyloid plaques at the age of 6 months, whereas only moderately elevated intracellular Aβ load can be detected at an age of 3 months [35]. Using isolated mitochondria from 3- as well as 6-month-old amyloid precursor protein (APP) transgenic (tg) mice and non-tg littermate control animals (non-tg), we found decreased basal levels of mitochondrial membrane potential in tgAPP mice compared to littermate non-tg control mice, similar to the decrease observed in dissociated cells (Fig. 3), suggesting that the increased intracellular Aβ production might trigger the dysfunction of the mitochondrial respiratory quite early and independently of Aβ plaques. When tracing mitochondrial dysfunction at the level of the respiratory chain, we detected decreased complex IV activity in 6-month-old mice. These early detected deficits cumulate in 17-month-old APP transgenic mice which show a significantly reduced state 3 respiration with reduced nicotinamide adenine dinucleotide (NADH) generating (Fig. 3). Therefore, Aβ-related mitochondrial dysfunction may exacerbate during aging and may be one of the mechanisms explaining the pronounced accumulation of AD pathology with aging. In agreement, Caspersen et al. used isolated mitochondria from tg mAPP mice and observed comparable oxygen consumption at 4 months in tg mAPP and non-tg littermates, a trend towards lower levels in tg mAPP

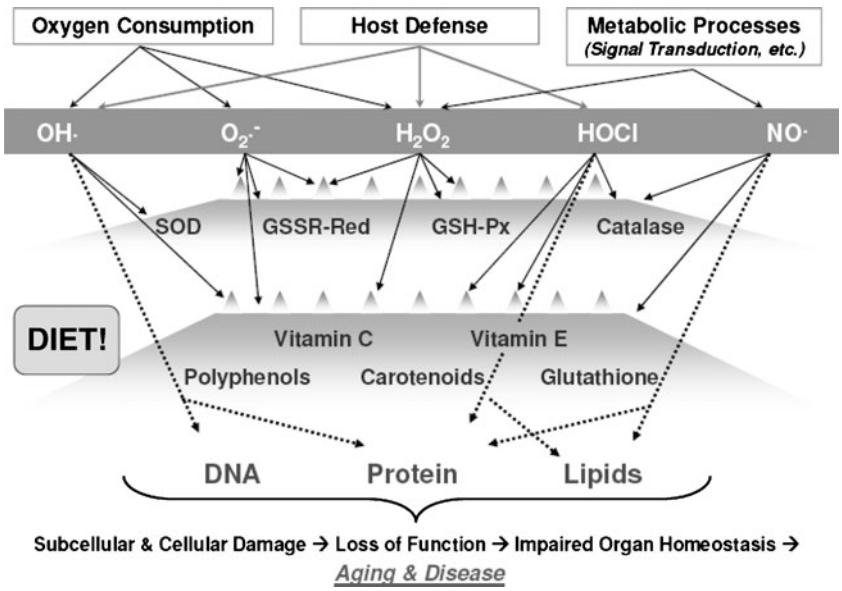

Fig. 2 First and second defense mechanisms against free radicals. Tissue protection against free radicals generated in our body by different mechanism by the first (antioxidant enzymes) and the second (dietary antioxidant) physiological defense systems

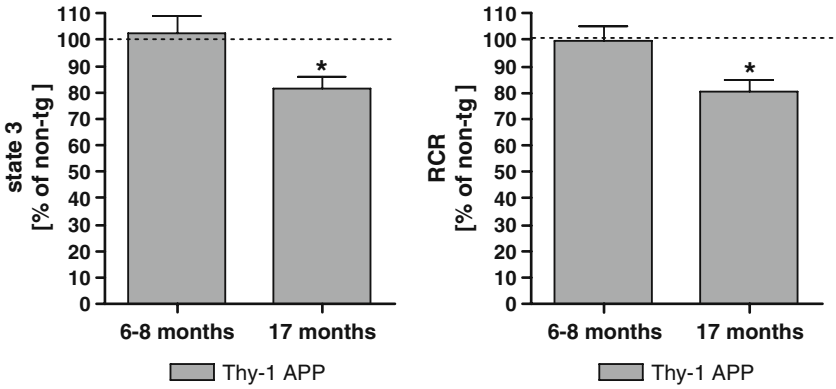

Fig. 3 Mitochondrial dysfunction enhances with aging in Thy1-APP mice. Functional relevant measures of mitochondrial function like state 3 respiration or the respiratory control ratio are decreased in 17-month-old but not in 6-month-old mice overexpressing mutant human amyloid precursor protein (Thy1-APP) relative to control animals of same age (data are adapted from Hauptmann et al. [34])

mice at 8 months that achieved statistical significance at the age of 12 months [36]. Furthermore, Aleardi et al. reported the inhibition of the respiratory chain complexes depending on the Aβ concentration by using isolated rat mitochondria [37]. Furthermore, impairment of mitochondrial oxidative phosphorylation was also extensively reported in the brain of AD patients [38] as well as that the degree of impairment being proportional to the clinical disability [39]. Besides APP mutations, mutations in the presenilins PS1 and PS2 account for the majority of familial Alzheimer's disease (FAD) cases, and clinical onset in some carriers of PS1 mutations is extremely early, occasionally as soon as in the third decade of life [40]. Presenilins are a component of the γ-secretase complex which is involved in the formation of Aβ from APP. Several different PS mutations have been shown to alter γ-secretase processing of APP to yield higher levels of $A\beta_{1\text{-}42}$ isoform and a higher production of $A\beta_{1\text{-}42}$ correlated with an earlier onset of Alzheimer dementia in carriers of PS1 mutations [41]. In human brain sections bearing a PS1 mutation, abnormal morphologic changes in mitochondria have been reported [42]. More importantly, neuronal cells carrying mutant PS1 had decreased mitochondrial membrane potential, reduced permeability transition [43], altered mitochondria function, and enhanced ROS generation [44]. Furthermore, FAD PS mutations sensitize cells to die by apoptosis after mitochondrial failure [43]. Therefore, PS1 mutations may in addition directly impair mitochondrial function independent of the Aβ cascade.

Furthermore, Aβ progressively accumulates in mitochondria, and this colocalization is associated with altered mitochondrial morphology and diminished complex IV activity [36]. Aβ is imported into the mitochondria through the translocase of the outer membrane (TOM) and is mostly localized to mitochondrial cristae and the inner mitochondrial membrane [45]. Lustbader et al. demonstrated that Aβ-binding alcohol dehydrogenase is a direct molecular link between Aβ and mitochondrial toxicity [46]. Besides Aβ, APP itself accumulates in mitochondrial membranes [47] and forms a stable complex with TOM and links TOM and the translocase of the inner membrane together [48]. This mitochondrial accumulation correlates with decreased import of nuclear coded proteins and decreased activity of cytochrome *c* oxidase [49]. Mitochondrial Aβ accumulation might also be in part responsible for impaired mitochondrial dynamics [50]. The tightly regulated balance between mitochondrial fusion and fission is shifted to enhanced fission which is accompanied by increased protein levels of fission proteins such as Fis-1 and reduced protein levels of fusion proteins like OPA-1 and Drp-1[51]. Enhanced nitrosative stress by Aβ is also discussed to be involved in altered mitochondrial dynamics by altering the expression of fusion and fission factors [52]. Finally, these defects in mitochondrial dynamics seem to contribute to mitochondrial depletion from axons and dendrites and thereby induce synaptic failure [53].

The other hallmark of AD, the appearance of neurofibrillary tangles, primarily composed of aggregated hyperphosphorylated tau also seems to be involved in mitochondrial dysfunction. Using transgenic mice overexpressing the P301L mutant human tau protein, we could demonstrate mitochondrial dysfunction by proteomic and functional analyses in these mice [54]. The P301L transgenic mice express the human pathogenic mutation P301L of tau together with the longest human brain tau isoform under control of the neuron-specific mThy1.2 promoter [55] characterized by increased tau hyperphosphorylation already at 3 months and intracellular neurofibrillary tangle formation at 6 months of age [55, 56].

Our functional analysis demonstrated reduced NADH-ubiquinone oxidoreductase (complex I) activity and, with age, impaired mitochondrial respiration and ATP synthesis in P301L tau mice (Fig. 4). In particular, the reduction in state 3 respiration reflects a reduced capacity of mitochon-

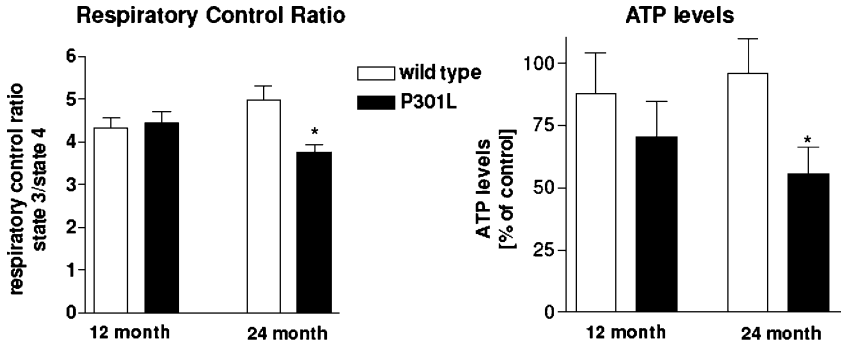

Fig. 4 Mitochondrial dysfunction in P301L tau mice appears with aging. Mitochondrial function in P301L tau mice was detected either by measuring the respiratory control ratio or ATP levels. Again, significant changes in both parameters could be detected only in aged P301L tau mice (24 months) relative to control animals of same age (data are adapted from David et al. [54])

dria to metabolize oxygen and the complex I substrates in the presence of a limited quantity of ADP [54]. Accordingly, higher levels of reactive oxygen species in aged transgenic mice were detected, and P301L tau mitochondria displayed increased vulnerability towards Aβ peptide insult, suggesting a synergistic action of tau and Aβ pathology on the mitochondria. The synergistic interaction of aging with AD-specific alterations like tau pathology, APP as well as PS1 mutations leading to mitochondrial dysfunction has recently been confirmed using double and triple transgenic mice [57, 58]. Yao et al. [58] showed that even in embryonic hippocampal neurons mitochondrial respiration was significantly decreased and increased glycolysis. These defects are markedly exacerbated during aging. Using a different triple transgenic mouse model, Rhein et al. [57] detected a massive deregulation of mitochondrial proteins mainly related to complexes I and IV. Importantly, deregulation of complex I was tau dependent, whereas complex IV dysfunction is mediated by Aβ. The first synergistic deficits were evident at an age of 8 months.

Oxidative Stress in AD

Post-mortem tissue provides strong evidence for increased levels of cellular oxidative stress in vulnerable regions of AD brains compared to aged controls [38, 59–61]. Increased protein oxidation, protein nitration, and lipid peroxidation were detected in brain areas showing neurofibrillary tangles and amyloid plaques [62]. Further evidence comes from studies investigating peroxidation products such as 4-hydroxynonenal (HNE) in cerebrospinal fluid and plasma of AD patients [63, 64]. Elevated HNE levels were detected which are particularly devastating for neuronal function. They impair the function of membrane ion motive ATPases and glucose and glutamate transporters. These changes consecutively lead to a disruption of cellular calcium homeostasis. Additionally, alterations in the levels of antioxidant enzymes such as catalase, Cu/Zn-SOD, and Mn-SOD support the evidence for increased oxidative stress in Alzheimer post-mortem tissue and AD animal models [65, 66]. In other studies, oxidative modified brain proteins were detected in AD patients with redox proteomics [39, 67–69]. For example, in patients carrying PS1 mutations, oxidative modifications of ubiquitin carboxyl-terminal hydrolase, gamma enolase, actin, and dimethylarginin were detected [39]. Importantly, only in the hippocampus and not in the cerebellum where oxidative modifications were found, consistent with the lack of pathology in this brain region in AD [68].

Several recent studies in transgenic animals, post-mortem brains, and biological fluids from subjects with AD or mild cognitive impairment support the strong relationship between mitochondrial dysfunction and oxidative stress in AD and the early involvement of these two parameters in the pathology of AD. Nunomura et al. [17] found that oxidative damage was more pronounced in AD subjects with lesser amount of Aβ plaques. Importantly, individuals with MCI or very mild AD showed elevated levels of lipid peroxidation and nucleic acid oxidation in post-mortem brain tissue [70] and increased levels of lipid peroxidation and nucleic acid oxidation compared to patients suffering from severe AD [25, 71, 72]. Furthermore, decreased levels of plasma antioxidants and total plasma antioxidant activity were observed [73, 74], as well as enhanced ROS levels in lymphocytes isolated from AD patients [75]. These results are supported by studies conducted in AD animal models [72, 76–78]. Schuessel et al. [65, 78] demonstrated that 3-month-old APP transgenic mice showed increased levels of HNE before Aβ plaques can be detected. These increased HNE levels were accompanied by reduced activity of Cu/Zn-superoxide dismutase. This suggests that impaired antioxidants defense is causally responsible for increased formation of HNE.

The mechanism of how oxidative stress increases in AD is still unknown, but several findings suggest a link

between Aβ toxicity and the generation of reactive oxygen species [79, 80]. In AD brain, protein oxidation occurs in Aβ-rich regions, such as the cortex and hippocampus, but not in the cerebellum where Aβ levels are negligible [81]. Furthermore, lipid membrane damage is promoted by Aβ aggregates [65, 82], and enhanced ROS were found as a consequence of Aβ-mediated mitochondrial dysfunction [83, 84]. In addition, we demonstrated in cell culture experiments that extracellular Aβ causes oxidative stress [83] and generates free radicals in vitro [81]. These results are in agreement with the hypothesis that Aβ is a metalloenzyme which is capable of generating hydrogen peroxide through its superoxide dismutase activity [85]. It has been further suggested that oxidative stress may promote the amyloidogenic pathway [86]. A cyclic and vicious pathway of excess Aβ promoting oxidative damage which promotes further Aβ production would result in increased neurotoxicity and subsequently enhance AD progression [85]. Therefore, we propose the following hypothetical sequence of pathogenetic steps linking sporadic AD, oligomeric and fibrillar Aβ, tau, mitochondrial dysfunction, and oxidative stress (Fig. 5). In an early phase of AD, intracellular Aβ and tau cause mitochondrial dysfunction by impairing complex IV activity. Consequently, mitochondrial membrane potential is reduced, ATP levels are decreased, and ROS generation/oxidative stress is enhanced. Mitochondrial dysfunction and increased oxidative stress result via a pathogenic cascade in enhanced apoptosis and cell death [9, 87, 88]. Moreover, by decreasing membrane fluidity, Aβ can additionally stimulate its own production [89].

Pharmacological Strategies to Improve Mitochondrial Function

While the concept of Aβ-induced mitochondrial dysfunction in AD has received substantial support over the last decade, improving mitochondrial function as a target for new drug development has not. Until recently, scientific interest was mostly focused on drugs leading to reduced Aβ load. However, as several disease-modifying compounds failed to show clinical effectiveness in AD trials [90], a report about substantial therapeutic effects of dimebon in a 1-year clinical trial [91] received a lot of interest. Dimebon, an old Russian antihistaminic drug, was later characterized as a mitochondrial stabilizer [92, 93]. The concept of mitochondrial protection as a treatment strategy for dementia has recently been further supported by not yet final published data on substantial clinical improvement in AD patients treated with methylene blue [90]. In several animal studies, methylene blue enhances cognitive functions associated with elevated oxygen consumption which seems to be associated with activating complex I and IV activities at the cellular level [94–96].

Besides these two rather new drugs, several antioxidants have a long history as possible treatments for AD and even have been and are used in this context [59, 97]. Initially, mainly Vit E or Vit C or the combination of both has been investigated. While both at high concentrations definitively show antioxidant properties in vitro and in vivo, their therapeutic benefit to improve or even prevent age-related cognitive impairment in AD is still under discussion [98].

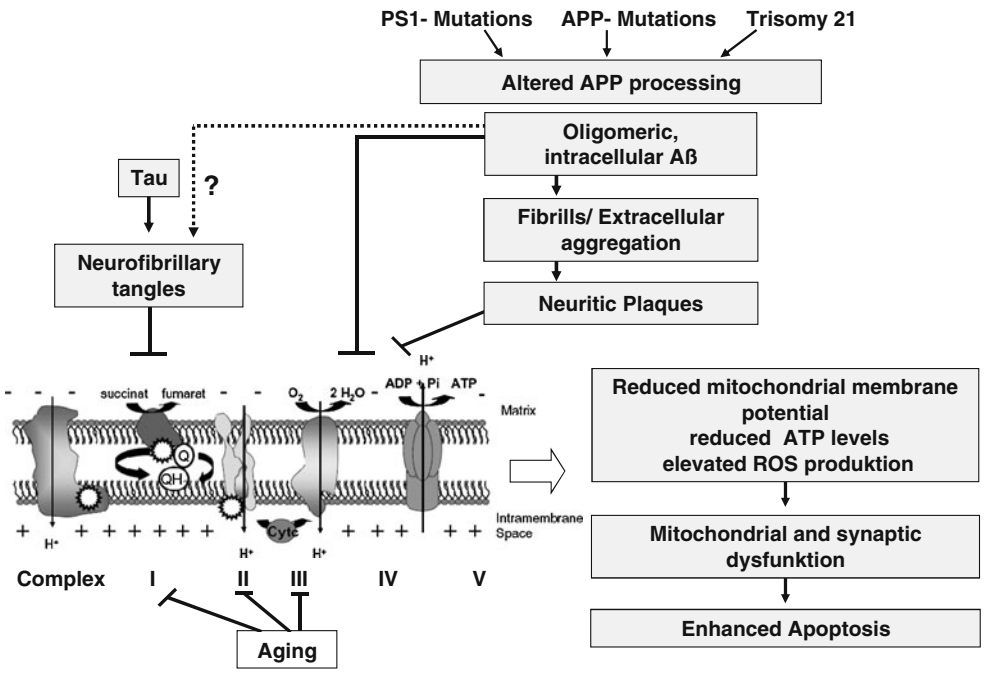

Fig. 5 Role of aging and Aβ in mitochondrial dysfunction. Mitochondrial dysfunction is an early common pathological pathway in aging. Activity of complexes I, III, and IV is decreased. In addition, Aβ pathology results in a decrease of complex IV activity. Furthermore, tau pathology may impair complex I activity. All these pathogenic changes cumulate together with PS1-induced reduction of mitochondrial function independent of the Aβ cascade. Therefore, we conclude that Aβ-related mitochondrial dysfunction is exacerbated by aging and leads to decreased MMP, decreased ATP levels, enhanced ROS production, and finally synaptic failure, apoptosis, and neurodegeneration

Another important class of naturally accruing antioxidants are flavonoids or other polyphenols, which also are fairly good antioxidants reducing oxidative stress in vitro and in vivo. Flavonoids also improve mitochondrial dysfunction and seem to have therapeutic benefit for long-term treatment of age-related cognitive impairment in animals and men [13, 14]. The significant reduction of the risk in getting AD by Mediterranean diet is very likely explained by the high daily intake of flavonoids. In general, even if the effectiveness of those natural occurring antioxidants to protect against AD seems to be limited, they seem to be the major players of diet in reducing oxidative stress and acting as a second, though weaker defense system (Fig. 2) [99–101]. As the tissue level or even brain levels of those dietary antioxidants are rather low, it has been suggested that their protective properties might be associated with the phenomenon of neurohormesis, sensitization of the whole antioxidant system (Fig. 2) by low levels exposure, probably by inactivation of free radicals relevant for cellular signaling [102, 103].

Another case of an herbal drug is the standardized *Ginkgo biloba* extract (EGb 761), which has been used for many years as a prescription or OTC drug in many countries to treat aging-related cognitive disorders including AD. EGb 761 contains 24% of flavonoids and 6% of terpenes. The terpene lactones are represented by the ginkgolides A, B, C, J, and M and bilobalide. The flavonoid fraction is composed of quercetin, kaempferol, and isorhamnetin, which are mainly responsible for the free radical scavenging properties. However, the activity of EGb 761 also includes substantial mitochondria-protecting properties which have been described in several publications. Our group showed protection of mitochondrial function in a neuronal-like cell line and in dissociated brain cells and isolated mitochondria of EGb 761-treated animals [104, 105].

To evaluate the effects of *G. biloba* extract ex vivo, NMRI mice were treated per os with 100 mg/kg for 14 days. In isolated mitochondria and in dissociated brain cells from EGb 761-treated animals, a significant protection against sodium nitroprusside (SNP)-induced decrease of mitochondrial membrane potential could be observed (Fig. 6). Furthermore, EGb 761 showed significant protective effects on complexes I, IV, and V in isolated mitochondria of aged mice (15 months) and no effects in young mice (3 months). In accordance with our data, treatment with EGb 761 prevented age-associated changes in mitochondrial morphology, mitochondrial glutathione levels, and respiratory function of rat brain mitochondria [106].

Janssen et al. investigated the effect of bilobalide on ischemia-induced alterations of the mitochondrial respiratory chain. Bilobalide was found to allow mitochondria to maintain their respiratory activity in ischemic conditions by protecting complex I and probably complex III activities [107].

Several studies provide evidence for the antioxidant properties of EGb 761. It can scavenge ROS, such as hxdroxy, peroxy radicals, superoxide anions as well as nitric oxide [108, 109]. Schindowski et al. showed a significant reduction of ROS-induced apoptosis by EGb 761 in T lymphocytes [109]. Wu et al. found increased resistance to oxidative stress in EGb 761-treated wild-type *Caenorhabditis elegans* worms [110]. Moreover, an increase in catalase and SOD activities in the hippocampus, striatum, and substantia nigra of rats and lipid peroxidation was decreased in the hippocampus of rats after the treatment with EGb 761 [111]. The extract's flavonoid fraction is mainly in the antioxidant properties. Smith and Luo showed a direct attenuation of ROS by the flavonoid fraction of EGb 761 [112]. The extract scavenges directly and preferentially hydroxyl radicals [113]. Additional antioxidant properties of EGb 761 can be explained by

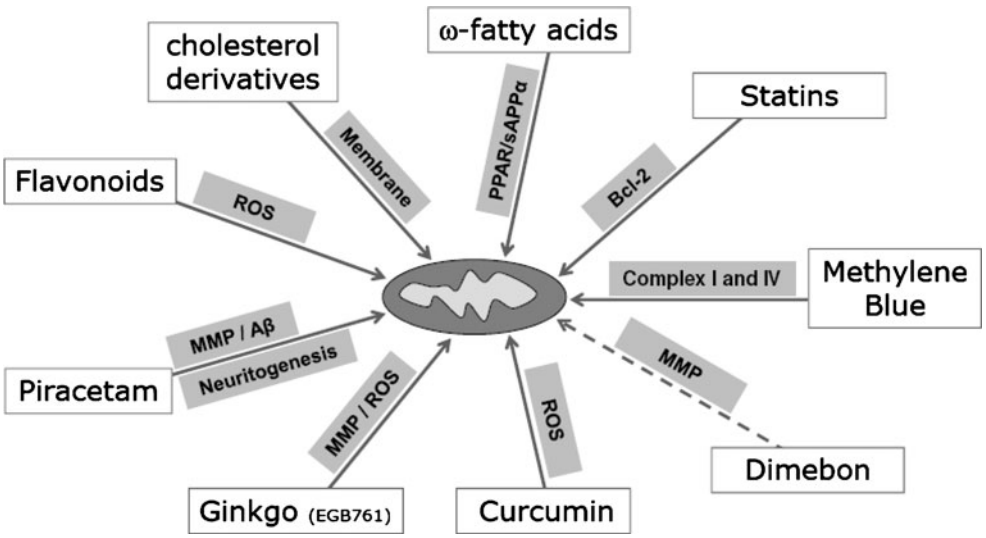

Fig. 6 A variety of different compounds are currently investigated as potential mitochondria protecting agents (for details, see text)

flavonoids chelating prooxidant transitional metal ions, e.g., Fe^{2+} [114] and therefore consequently inhibiting the generation of new hydroxyl radicals.

While in many cases there is a substantial overlap between antioxidant and mitochondria protecting properties, it is important to note that both do not share the same mechanism, and several compounds show significant mitochondrial protection without having antioxidant or radical scavenging properties. A drug which has been extensively characterized in this respect is the metabolic enhancer piracetam [115], which shows no antioxidant properties but exhibits pronounced mitochondrial protection ex vivo as well as in vitro [116–118]

Evidences that piracetam might improve disturbed mitochondrial function originate from observations that piracetam improves glucose uptake and utilization as well as ATP production [119–122]. In line with these data are our previous observations of significant mitochondrial protection and enhanced ATP synthesis by piracetam against experimentally induced oxidative as well as nitrosative stress in vitro and after ex vivo treatment, where again aged animals with well-characterized mitochondrial dysfunction benefited most [116]. In agreement with the assumption of mitochondrial membranes as primary target, the beneficial effect of piracetam was similar after experimental impairment of each of the five respiratory chain complexes [116].

Aβ-induced mitochondrial dysfunction which proceeds the development of Aβ plaques has been previously described by our lab in the brains of mice transgenic for human APP bearing the Swedish double mutation [34]. At the age of 3 months, dissociated brain cells from these animal show pronounced measures of mitochondrial dysfunction [34] including reduced mitochondrial membrane potential (MMP) and decreased ATP synthesis as confirmed in the present data. Comparable to its beneficial effects on mitochondrial dysfunction induced by brain aging, piracetam considerably improved MMP and ATP production in tgAPP mice.

Our observation of significantly reduced brain levels of soluble Aβ ($p<0.01$) in the piracetam-treated tgAPP mice was unexpected. However, as increasing evidence indicates enhanced Aβ production following oxidative stress [123, 124], reduced Aβ brain levels in piracetam-treated tgAPP mice again might be a consequence of improved mitochondrial function [117]. This concept was confirmed by experiments showing protection by piracetam against SNP-elevated Aβ levels in APPwt HEK cells [117]. In addition, piracetam improves mitochondrial membrane potential under similar conditions.

In agreement with our previous findings that piracetam improved mitochondrial dysfunction following oxidative stress [116], piracetam also ameliorated mitochondrial dysfunction following $A\beta_{1\text{-}42}$ and $A\beta_{25\text{-}35}$ exposure in PC12 cells and in acutely dissociated brain cells prepared from young and aged mice. A comparable protection against Aβ-induced mitochondrial dysfunction was reported in dissociated brain cells of young and aged mice treated with piracetam for 2 weeks. The effect was considerably more pronounced in aged animals.

As mitochondria are highly localized at the synaptic level [125], impairment of mitochondrial function is importantly associated with synaptic deficits including reduced synapse formation and impaired neuritogenesis [126, 127]. Accordingly, reduction of synaptic plasticity by low molecular weight (oligomeric) Aβ species has been considered as a key pathomechanism of AD, beginning quite early in the course of the disease [22, 23, 126, 128]. Neuritic outgrowth as an important part of synaptic plasticity has been repeatedly reported to be impaired by β-amyloid peptides in vitro and in vivo [129–131]. In our initial experiments, we confirmed that $A\beta_{1\text{-}42}$ reduces neuritic outgrowth using PC12 cells in vitro under conditions of optimal trophic support. The effect was already present at nanomolar concentrations and was much more pronounced for oligomeric than for fibrillar $A\beta_{1\text{-}42}$ confirming other findings [132]. Piracetam significantly improved neuritic outgrowth under these conditions ($p<0.01$). Piracetam was similarly effective when reduced neuritogenesis was induced by intracellular production of Aβ ($PC12_{wt}$, $PC12_{sw}$ cells) [117]. Piracetam's effectiveness was seen at low up to maximum nerve growth factor (NGF) concentrations, where it still significantly enhanced NGF activity, suggesting that piracetam is not merely acting by shifting the NGF dose–response curve to the left. This is further supported by preliminary findings from our lab that piracetam improves disturbances of mitochondrial dynamics (unpublished data). As all those conditions are associated with mitochondrial dysfunction and metabolic impairment, we propose that improved mitochondrial function underlies the neurotrophic effects of piracetam.

Many other compounds which have been suggested as possible treatments for dementia and AD (Fig. 6) also seem to work at least in part by protecting mitochondria or improve disturbed mitochondrial function including statins [133, 134], omega fatty acids [135–137], cholesterol derivatives [138], and the dopamine metabolite hydroxytyrosol [139].

Conclusion and Further Perspective

Impaired mitochondrial metabolism associated with respiratory chain dysfunction and oxidative stress is considered to be a major pathological mechanism in a number of neurodegenerative diseases including AD. In contrast to Aβ

plaques and tau tangles seen in the late stage of AD, mitochondrial dysfunction and oxidative stress are two early events in the pathology of AD cumulating with aging-associated changes in mitochondrial function, morphology, dynamics, and oxidative stress. Aβ-mediated complex IV impairment together with complex I, III and IV-reduced activities in aging leads to severe defects in mitochondrial energy supply. The changes result in a vicious cycle inducing electrons leakage from the electron transport chain (ETC) leading to enhanced production of ROS. These elevated levels of ROS themselves damage ETC components and mtDNA and further increase mitochondrial dysfunction and oxidative stress. When phenotypic threshold and severe energy deprivation are reached, neuronal and synaptic dysfunction appears which is enhanced by depletion of mitochondria from axons and dendrites. Thus, both mitochondrial dysfunction and oxidative stress clearly play an important role in the pathogenesis of AD. Nevertheless, the precise mechanism of events in AD pathogenesis still remains uncertain.

Currently available drug treatment for AD is symptomatic with no beneficial effect on progressive underlying disease processes. Although antioxidant therapies hold promise for improving mitochondrial function, it is uncertain which types and ratios of antioxidants, and in which forms, will result in the best outcomes. In vitro and animal model studies support the potential beneficial role of various mitochondria-protecting drugs. Our own results have established substantial mitochondrial protection for compounds like piracetam or *G. biloba* extract EGb 761®, but also for compounds becoming of interest only lately like statins [133, 134, 140], omega fatty acids [137], methylene blue, or dimebon. Even if all those drugs show clear benefits in disease-specific experimental models, e.g., cell lines or animals and most also in clinical trials, none alone may probably be active enough for substantial clinical usefulness. However, as each of the compounds seems to work via a different molecular mechanism, a combination of different compounds not only seems to be feasible but also may prove to demonstrate superior efficacy.

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