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Mitochondrial Dysfunction—A Pharmacological Target in Alzheimer's Disease

Gunter P. Eckert · Kathrin Renner · Schamim H. Eckert · Janett Eckmann · Stephanie Hagl · Reham M. Abdel-Kader · Christopher Kurz · Kristina Leuner · Walter E. Muller

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Abstract Increasing evidences suggest that mitochondrial dysfunction plays an important role in the pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD). Alterations of mitochondrial efficiency and function are mainly related to alterations in mitochondrial content, amount of respiratory enzymes, or changes in enzyme activities leading to oxidative stress, mitochondrial permeability transition pore opening, and enhanced apoptosis. More recently, structural changes of the network are related to bioenergetic function, and its consequences are a matter of intensive research. Several mitochondria-targeting compounds with potential efficacy in AD including dimebon, methylene blue, piracetam, simvastatin, *Ginkgo biloba*, curcumin, and omega-3 polyunsaturated fatty acids have been identified. The majority of preclinical data indicate beneficial effects, whereas most controlled clinical trials did not meet the expectations. Since mitochondrial dysfunction represents an early event in disease progression, one reason for the disappointing clinical results could be that pharmacological interventions might came too late. Thus, more studies are needed that focus on therapeutic strategies starting before severe disease progress.

Keywords Mitochondrial dysfunction · Alzheimer's disease · Neurodegenerative disease

G. P. Eckert (✉) · S. H. Eckert · J. Eckmann · S. Hagl · R. M. Abdel-Kader · C. Kurz · K. Leuner · W. E. Muller
Department of Pharmacology, Biocenter, Campus Riedberg, Goethe-University,
Biocentre Geb. N260, R.1.09, Max-von-Laue Str. 9,
60438, Frankfurt, Germany
e-mail: g.p.eckert@em.uni-frankfurt.de
URL: www.eckert-science.com

K. Renner
Department of Haematology and Oncology
University Hospital Regensburg,
University Hospital of Regensburg,
Regensburg, Germany

K. Leuner
Molecular and Clinical Pharmacy,
University of Erlangen-Nuremberg,
Erlangen, Germany

Present Address:
R. M. Abdel-Kader
Department of Pharmacology, German University of Cairo,
Cairo, Egypt

Introduction

Increasing evidence suggests that mitochondrial dysfunction plays an important role in brain aging and the pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD) [1]. Mitochondria are complex, network-forming organelles, involved in different metabolic pathways, e.g., citric acid cycle (TCA), energy transformation, amino acid metabolism, and urea cycle [2]. Mitochondria consist of inner and outer membranes composed of phospholipid bilayers and proteins. The outer mitochondrial membrane, which encloses the entire organelle, contains numerous integral proteins. These porins are responsible for the high permeability of the outer membrane to all molecules up to 5,000 Da, whereas the inner mitochondrial membrane is quite tight. Mitochondrial membranes contain translocases for protein import [3] and various specific mitochondrial carriers in the inner membrane for the import of hydrophilic compounds [4]. The inner mitochondrial membrane harbors the proteins of the electron transfer system (ETS), responsible for oxidative phosphorylation. The mitochondrial oxidative phosphorylation (OXPHOS) system is the final biochemical pathway producing energy in the form of ATP

by consuming oxygen. From complex I and II, electrons are transferred to complex III by Coenzyme Q, the glycerophosphate dehydrogenase, and the electron transferring flavoprotein. From complex III, the electrons are transferred to oxygen via cytochrome *c* and complex IV. Simultaneously, an electrochemical proton gradient is built across the inner mitochondrial membrane (by complex I, III, and IV), and the generated proton motive force is used by complex V to produce ATP (Fig. 1) [5, 6].

Alterations of mitochondrial efficiency and function are mainly related to alterations in mitochondrial content, amount of respiratory enzymes, or changes in enzyme activities [7–10]. Growing evidences indicate changes of the network are related to bioenergetic function, and the consequences are a matter of intensive research [11–13]. A reduction in mitochondrial content or lowered ETS capacity results in a general limitation of energy production. Dysfunction of single complexes of the respiratory system are frequently accompanied by deleterious side effects like loss of mitochondrial membrane potential (MMP) and consequently decreased ATP levels, but also production of reactive oxygen species (ROS) [14]. Dysfunction of single enzyme complexes, ROS production, mitochondrial permeability transition pore opening (mPTP), elevated apoptosis, but also structural alterations, and a diminished mitochondrial content are believed to be crucial for the onset and progression of neurodegenerative diseases [15–17].

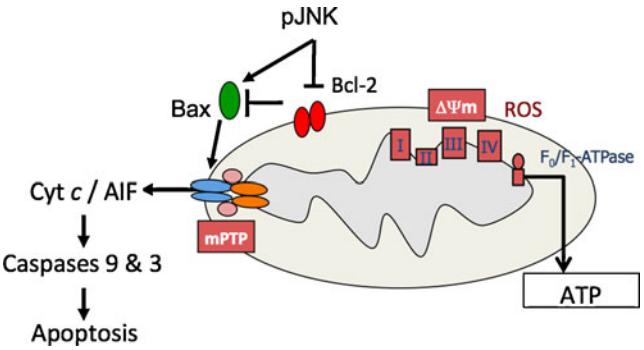

Fig. 1 Mitochondria—energy supply and apoptosis. Mitochondria are complex, network-forming organelles, consisting of inner and outer membranes composed of phospholipid bilayers and proteins. The inner mitochondrial membrane harbors the proteins of the ETS (I–IV). These complexes of the respiration chain are responsible for oxidative phosphorylation. Electrons are transferred from the matrix to the intermembrane space. Simultaneously, an electrochemical proton gradient is built across the inner mitochondrial membrane that creates a membrane potential (Δψm), which represents the driving force for complex V (F0/F1-ATPase) to produce adenine triphosphate (ATP). Electrons are transferred to oxygen to produce water. Failure in this respiration chain, e.g., caused by complex dysfunction, leads to incomplete reduction of oxygen, and reactive oxygen species (ROS) are produced. Mitochondrial permeability transition pore (mPTP) opening, induced by mitochondrial damage or by apoptotic factors like Bax induced by activated c-Jun N-terminal kinases (pJNK), results in the release of pro-apoptotic proteins like cytochrome *c* (Cyt *c*) or the apoptosis-inducing factor (AIF), which in turn activates caspases (9 and 3) and further down-stream apoptotic cell death mechanisms (see Fig. 2). The anti-apoptotic protein Bcl-2 inhibits apoptosis by binding pro-apoptotic proteins including Bax, inhibiting their translocation from the cytosol to the outer mitochondrial membrane and pore formation

Oxidative Stress

Besides enzymatically produced ROS by NADPH oxidases, cytochrome P450-dependent oxygenases, and xanthine dehydrogenases, mitochondria are regarded as the primary site of ROS production within cells. The ETS constantly generates low physiological levels of ROS, which exaggerate in consequence of mitochondrial dysfunction [18].

The major source of superoxide anions are the redox centers of complex I and III of the ETS and different mitochondrial flavoproteins. Superoxide is a rather weak radical, but it is the precursor of most ROS [5, 19, 20]. Superoxide anions from complex I are released into the mitochondrial matrix space and need to be transformed into hydrogen peroxide to be transferred to the cytosol. Complex III-generated superoxide is released into the intermembrane space, where it is transferred into the cytosol through the voltage-dependent anion channel (VDAC). Its transformation into hydrogen peroxide, hydroxyl anion, and formation of peroxynitrate creates strong oxidants [21]. The hydroxyl anion is extremely instable and can react with nearly all cellular macromolecules including DNA, protein, and membrane lipids [22].

The level of ROS production is controlled by oxygen donor concentration and the redox state of the enzymes of the ETS. The higher the oxygen levels and donor concentrations, the more ROS are produced. This holds true in general, although increased ROS production under hypoxic conditions has been observed [23, 24]. Higher reduction of the respiratory complexes, increased amounts of donor concentrations, and a concomitant high membrane potential, e.g., in the resting state, result in increased ROS formation. Both can be diminished through activation of electron transport. Beyond a certain threshold, mitochondrial ROS production can reinforce itself. Thus, mitochondria are both the initiator and the first target of oxidative stress. Proteins of the OXPHOS system are key targets of ROS's deleterious effects leading to membrane depolarization and subsequently impaired mitochondrial function [1, 18].

Cells have evolved a number of defense mechanisms consistent of antioxidative molecules, such as glutathione or vitamin E and antioxidant enzymes such as superoxide dismutase (SOD), catalase, or glutathione peroxidase and glutathione reductase. Furthermore, slight uncoupling, e.g., by uncoupling proteins, is one possibility to achieve a reduction in ROS production. Functional failure of this system leads to deleterious effects, and evidences obtained over the past two decades show that ROS are involved in aging and neurodegenerative disorders [1].

Mitochondria and Apoptosis

Oxidative damage, either of OXPHOS proteins or of omega-3 polyunsaturated fatty acids (PUFA) in the inner mitochondrial membrane, results in loss of MMP, representing one early hallmark of apoptosis [25].

Mitochondria are the signal integrating organelles in the onset of the intrinsic apoptotic pathway. Mitochondrial outer membrane permeabilization and permeability transition result both in the release of pro-apoptotic proteins like cytochrome *c*, the apoptosis-inducing factor (AIF) or Smac/DIABLO, which in turn activate caspases and further down-stream cell death mechanisms (Fig. 1) [26, 27].

The proteins of the Bcl-2 family are important regulators of the intrinsic apoptotic pathway and consist of pro- and anti-apoptotic proteins, controlling directly the outer membrane integrity and, therefore, translocation of pro-apoptotic proteins to the cytosol. The anti-apoptotic protein Bcl-2 itself is mainly localized in the outer mitochondrial membrane, and Bcl-2 and Bcl-x_L inhibit apoptosis by binding pro-apoptotic proteins, inhibiting their translocation from the cytosol to the outer mitochondrial membrane and pore formation by insertion and oligomerization [28]. Bax and Bak are essential pro-apoptotic effectors, whereas the BH3-only proteins (e.g., Bad, Bid, Bik, and Bim) are believed to be regulators that act upstream of Bax and Bak. They exert their regulating function by binding to anti-apoptotic Bcl-2 proteins. The rheostat of pro- and anti-apoptotic family members is crucial for cell survival. Decreasing the Bax/Bcl-2 ratio is protective against mitochondrial damage induced by the NO inducer sodium nitroprusside (SNP), as Bax is required for SNP-mediated neurotoxicity [29]. Complexing Bcl-2 leads to a reduction of MMP, enhances ROS generation, increases cytochrome *c* release, activates caspase-9 and -3, and diminishes respiratory capacity and ATP synthesis [30–32]. A downregulation of Bcl-2 expression and an upregulation of Bax expression are involved in NO-mediated neurotoxic mechanisms [33, 34].

Decreased Bcl-2 expression and increased expression of Bax have been associated with activation of c-Jun N-terminal kinase (JNK). Phosphorylation of Bcl-2 by JNK antagonizes its anti-apoptotic effect (Fig. 1) [35, 36]. Moreover, a negative modulation of pyruvate dehydrogenase (PDH) by JNK has been described. Mitochondrial-localized phosphorylated JNK, found in aged rats, reduces pyruvate dehydrogenase activity [37, 38] and, therefore, limits the citric acid cycle and consequently substrate delivery to mitochondria. Thus, JNK has multiple effects on mitochondrial function [22, 39].

Mitochondrial Permeability Transition Pore

Despite their involvement in outer membrane permeabilization, Bcl-2 proteins can be involved in the permeability transition of the inner mitochondrial membrane [40]. The opening of the mPTP was first described by Hunter and Haworth [41]. It is a decisive stage of apoptosis [42]. Opening of the mPTP is followed by a sudden increase of permeability of mitochondrial membranes, which allows solutes up to 1.5 kDa to equilibrate between mitochondrial matrix and cytosol [43, 44]. This leads to uncoupling of oxidative phosphorylation system, mitochondrial matrix swelling, loss of MMP, increased ROS production, and release of apoptotic proteins (see above) [41, 42]. Numerous effectors can open the mPTP, in particular, calcium plus phosphate and ROS. In addition, small amphipathic peptides like Alzheimer-related amyloid β-peptide (Aβ) can induce mitochondrial permeability transition, activated by calcium [44]. On the other hand, there are numerous endogenous and exogenous inhibitors of mPTP, including high negative potential, low matrix pH, ADP, magnesium, and strontium and as pharmacological relevant agent cyclosporine A [45].

The mPTP represents a dynamic multiprotein complex, which spans the inner and outer mitochondrial membranes at special contact sites [42]. Although the structure of the mPTP is not yet fully elucidated, there are several identified components or modulators of the mPTP. The most common proposed structure of mPTP includes the VDAC and the 18 kDa translocator protein (TSPO; formerly known as the peripheral benzodiazepine receptor) in the outer membrane, the adenine nucleotide translocator (ANT) in the inner membrane, cyclophilin D (Cyc D) from the matrix, and possibly other proteins such as creatine kinase (CK) from the intermembrane space, hexokinase (HK) at the outer surface of the outer membrane, and pro-apoptotic proteins of the Bcl-2 family such as Bax (Fig. 2a) [43, 44, 46].

Recent gene knockout experiments raised questions about the above-described model of the mPTP, and the authors state that the pore-forming core components are not yet identified and proposed a new model of mPTP (Fig. 2b) [42, 43, 46].

Nevertheless, there is strong evidence that VDAC, TSPO, ANT, and Cyp D are involved in mPTP modulation and/or pore forming [42, 43, 47–52].

Furthermore, evidences indicate that differences in mPTP properties might be partly due to the fact that mitochondria from different tissues exhibit different behavior relating to mPTP, which may result from different proteins/isoforms that participate according to the tissue in function and regulation of the pore [42, 53]. Additionally, there are indications that membrane fluidity influences mPTP. Ricchelli et al. showed that during the assembly of mPTP, the membrane fluidity significantly decreased presumably due to conformational protein changes [54]. Consequently, Colell et al. investigated effects of cholesterol on mPTP induction. Cholesterol reduced mitochondrial membrane fluidity and weakened the ANT-mediated mPTP opening [55].

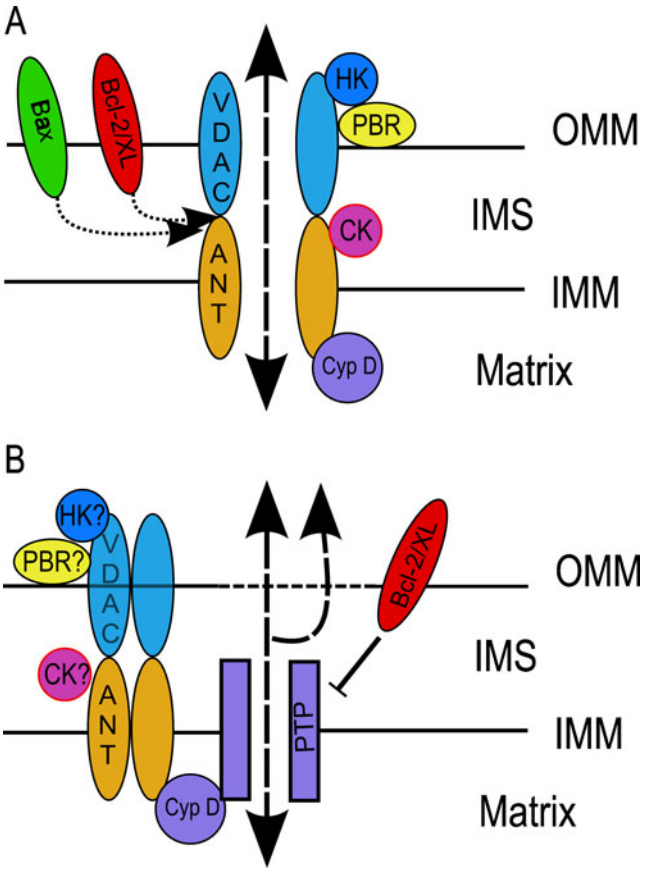

Fig. 2 Proposed structures of mPTP complex. The opening of the mPTP is a decisive stage of apoptosis and is followed by a sudden increase of permeability of mitochondrial membranes, which allows solutes up to 1.5 kDa to equilibrate between mitochondrial matrix and cytosol. This leads to uncoupling of oxidative phosphorylation system, mitochondrial matrix swelling, dissipation of MMP, increased ROS production, and release of apoptotic proteins (refer to Fig. 1). The mPTP represents a dynamic multiprotein complex of the inner (IMM) and outer mitochondrial membrane (OMM). It spans the intermembrane space (IMS), connecting the mitochondrial matrix with the cytosol. There are several identified components or modulators of the mPTP: the most common proposed structure of mPTP (**a**) includes the voltage-dependent anion channel (VDAC) and the 18-kDa translocator protein (PBR; formerly known as the peripheral benzodiazepine receptor), in the outer membrane, the adenine nucleotide translocator (ANT) in the inner membrane, cyclophilin D (Cyc D) from the matrix, and possibly other proteins such as creatine kinase (CK) from the intermembrane space, hexokinase (HK) at the outer surface of the outer membrane, and pro-apoptotic proteins of the Bcl-2 family such as Bax. Anti-apoptotic Bcl-2 or Bcl-XL is able to inhibit mPTP formation. In recent years, experimental data indicate an alternative structure (**b**). However, more studies are needed to clarify mPTP's real structure. **a** Classical view: the pore is built up by VDAC, ANT, and Cyp D. Hexokinase II (HK II), mitochondrial creatine kinase (CK), benzodiazepine receptor (PBR), and Bcl-2-family members (Bcl-2, Bcl-xL, and Bax) are considered as possible regulatory components. **b** Alternative view: the core elements of permeability transition pore (PTP) are not yet identified, but are probably regulated by the adjacent elements as indicated. Especially, the role of VDAC is discussed. For further details, please refer to the text. Figure was adopted from [46]

Mitochondrial Dysfunction in Alzheimer's Disease

AD is a progressive neurodegenerative disorder that leads to dementia and affects approximately 10 % of the population older than 65 years of age. An estimated 5.4 million Americans of all ages have AD in 2012. AD is the sixth-leading cause of death in the USA. Symptoms include memory loss that disrupts daily life, challenges in planning or solving problems, difficulty completing familiar tasks at home, at work, or at leisure, confusion with time or place, trouble understanding visual images and spatial relationships, misplacing things and losing the ability to retrace steps, decreased or poor judgment, withdrawal from work or social activities, and changes in mood and personality [56]. Severe neurodegenerative alterations occur in AD brains including 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 [57, 58]. The cause or causes of AD are not yet known. However, most experts agree that Alzheimer's, like other common chronic diseases, develops as a result of multiple factors rather than a single cause [56].

Defective energy metabolism is a fundamental component of AD [59–61] (see also [62], this issue). Increasing evidence suggests an important role of mitochondrial dysfunction and oxidative stress in AD [63–65]. Early defects in the expression of several subunits of respiration chain complexes [66], decreased mitochondrial respiration mainly mediated by a decline in complex I and complex IV function, and reduced MMP and ATP levels were detected in several AD cell and animal models [65–68]. Moreover, recent data indicate that superoxide-dismutase-2 (SOD2) deficiency induces oxidative stress in an AD mouse model [69].

Familiar forms of AD are associated with mutations in the genes of presenilin-1 (PS1) and presenilin-2 (PS2). Presenilins are components of the γ-secretase complex, which together with β-secretase processes the amyloid precursor protein (APP) to Aβ. Even though the role of PSs in AD is still controversial, there are implications of changed subcellular distribution of PS-1 and PS-2 in mitochondrial-associated membranes (MAM). MAMs are a subcompartment of the endoplasmic reticulum and are considered to be involved in lipid metabolism and calcium homeostasis. Changes in presenilin distribution in MAMs may lead to the increased cholesterol, changed fatty acid composition, and disturbed calcium homeostasis [70].

Direct effects of APP and Aβ on mitochondrial function might induce this early dysfunction. Accumulation of APP in mitochondria, which has been found in both transgenic cell lines and animals, correlates with mitochondrial dysfunction. This might provide one causal link for explaining

the impaired energy metabolism and subsequent rise in ROS/RNS in characterizing models of AD [71–73]. Not only APP but also Aβ itself has been suggested to affect mitochondrial function. Data show that the presence of one of the key enzymes in Aβ release, namely γ-secretase, pinpoints to a direct production of Aβ in these organelles [74].

Early deficits in synaptic mitochondria in an AD mouse model were reported recently [75]. Compared with non-synaptic mitochondria, synaptic mitochondria showed a greater degree of age-dependent accumulation of Aβ and mitochondrial alterations. The synaptic mitochondrial pool of Aβ was detected at an age as young as 4 months, well before the onset of non-synaptic mitochondrial and extensive extracellular Aβ accumulation [75]. Aβ triggers mitochondrial dysfunction through a number of pathways, such as impairment of oxidative phosphorylation, elevation of ROS production, alterations of mitochondrial dynamics, and interaction with mitochondrial proteins. Aβ interaction with different mitochondrial targets including the outer mitochondrial membrane, intermembrane space, inner mitochondrial membrane, and the matrix has been identified [76]. Accordingly, Lustbader et al. reported an interaction of Aβ with mitochondrial β-binding alcohol dehydrogenase (ABAD) in a transgenic mouse model [77]. Noteworthy, neurons cultured from these mice displayed reduced MMP and ATP levels as well as an increase in RNS and ROS production and cytochrome *c* release [78]. Moreover, changes in ABAD gene expression in brain cortex, following Aβ accumulation within mitochondria, have been reported [79].

Interaction of Aβ with Cyp D also caused disturbances of mitochondrial function, increasing ROS production or deregulation of mPTP [80, 81]. Thus, it has been proposed that Cyp D-mediated mitochondrial membrane permeability transition pore formation contributes to mitochondrial and neuronal failure in an Aβ-rich environment [82]. Blockade of Cyp D protects mitochondria from Aβ toxicity [81], and Aβ decreased the threshold of mPTP formation by interacting with Cyp D [82].

Mitochondria-derived ROS are sufficient to trigger amyloidogenic APP-processing in vitro and in vivo, and Aβ itself leads to mitochondrial dysfunction and increased ROS levels. Based on recent findings, it was proposed that starting from mitochondrial dysfunction, a vicious cycle is triggered that contribute to the pathogenesis of sporadic AD [68].

In contrast to ROS, such as superoxide, the importance of nitric oxide (NO) and its derived metabolites, especially peroxynitrite ($ONOO^-$), in AD pathology just started to gain momentum. NO production is regulated by the activity of constitutively expressed (eNOS and nNOS) and inducible NO synthases [83]. In addition, the presence of a mitochondrial NO synthase (mtNOS) has recently been described [84]. NO and $ONOO^-$, by damaging complexes of the respiratory chain, cause severe impairment of mitochondrial function [83]. Keil et al. detected elevated NO production in APP-transgenic PC12 cells [73]. The authors suggested NO to act as an important mediator of Aβ-induced neurotoxicity based on the observation that reduction of Aβ load resulted in reduced NO production and concomitantly partly restored MMP and ATP levels.

Also, addition of an unspecific NOS inhibitor protected primary neurons from $Aβ_{25–35}$-induced cytotoxicity [85]. NO protective properties in AD have been suggested in APP-transgenic, iNOS knockout mice [86]. The lack of iNOS led to elevated levels of Aβ as well as hyperphosphorylated tau protein, indicating that fluctuating NO differently affects developmental and disease states [86].

Increasing evidence suggests that mitochondrial dysfunction in AD originates not only from the deleterious impact of APP/Aβ but also from its interplay with hyperphosphorylated Tau protein on the mitochondrial level [67].

Mitochondria-Directed Drugs and Natural Compounds

While the concept of mitochondrial dysfunction as a major functionally relevant pathomechanism in AD has received substantial support over the last decade, improving mitochondrial function as a target for new drug development has rather not, as most interest has been directed to drugs leading to reduced Aβ load [87]. Molecules that target mitochondria should scavenge free radials and decrease mitochondrial dysfunction and promote healthy mitochondrial biogenesis, enhance axonal transport of organelles including mitochondria, and enhance synapse formation and synaptic branches in AD neurons [88]. Several mitochondria-targeting compounds with potential efficacy in AD including dimebon, methylene blue, piracetam, simvastatin, *Ginkgo biloba*, and omega-3 polyunsaturated fatty acids have been recognized. Although for most of the compound the exact mechanism of mitochondrial interaction is not fully discovered yet, different targets such as MMP, anti-apoptotic proteins (Bcl-2), ROS, beta-amyloid protein (Aβ), fusion and fission (f&f), and mitochondrial membranes have been identified (Fig. 3).

Compounds presented herein were tested in different in vitro and in vivo models as well as in clinical trials. For a discussion of the usefulness of in vitro and in vivo models in AD research, the reader is referred to Schaffer et al., this issue [89].

Dimebon

Dimebon (latrepirdine) represents an old anti-allergic drug, originally developed in Russia as an H_1-antihistaminicum [90, 91]. Based on preliminary findings about cognition-enhancing properties in a small group of AD patients [91], a large placebo-controlled phase II trial was carried out in

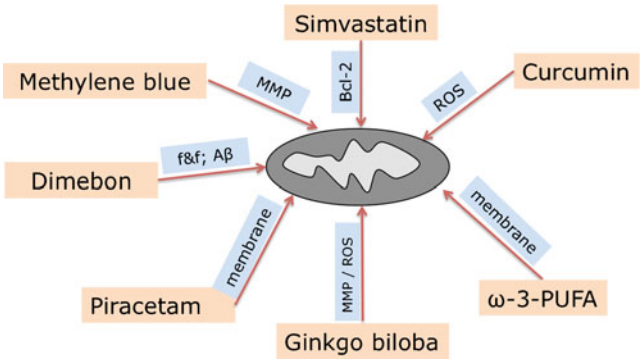

Fig. 3 Mitochondria as pharmacological targets. Several drugs and natural compounds with potential efficacy in Alzheimer's disease have been identified to impact mitochondria. The compounds discussed herein have different mitochondrial targets such as mitochondrial membrane potential (MMP), anti-apoptotic proteins (Bcl-2), reactive oxygen species (ROS), beta-amyloid protein (Aβ), fusion and fission (f&f), and mitochondrial membranes. However, for most of the compound, the exact mechanism of mitochondrial interaction is not fully discovered yet

nearly 200 AD patients indicating substantial therapeutically benefit over placebo after 24 weeks [92]. Dimebon's potential use in geriatric memory disorders was also supported by reports about similar effects in Huntington disease patients [93] and cognition-improving properties in several animal models, including young and adult mice [repeated (0.1 mg/kg) and acute (0.5 mg/kg) i.p. treatment] [94], mice transgenic for mutant human APP (12 mg/kg b.w. Dimebon for 4 months delivered through their drinking water) [95], rats (0.1–30 mg/kg b.w. i.p.) [96, 97], and most recently, rhesus monkeys (3.9–118 μg/kg b.w.) [98]. However, most of the beneficial effects in AD patients [92] could not be reproduced in a subsequent large-scale multicenter phase III trial [99]. Regardless the final proof of Dimebon's clinical efficacy, Dimebon might specifically interfere with mechanisms relevant for the cognitive decline, especially by improving impaired mitochondrial function and/or dynamics in AD [100, 101]. This mechanism represent the most relevant driving force in the vicious cycle between Aβ production, mitochondrial dysfunction, and neurodegeneration, including loss of synapses, neuritis, and nerve cells [68, 102, 103].

Findings that Dimebon (25 μmol/l) protects against the neurotoxic effects of Aβ [91, 104] together with many observations of mitochondria as major target for the cell toxicity of Aβ [68, 102] led to the assumption that mitochondrial protection might be a major mechanism for the beneficial effects of Dimebon in neurodegenerative diseases. A few recent publications reported additional evidence for mitochondrial protection by Dimebon. In micromolar concentrations (1–50 μmol/l), Dimebon protects against L-glutamate neurotoxicity in a cellular model of Huntington's disease [105] and inhibits calcium-induced swelling of rat brain mitochondria, without affecting cytochrome *c* release or calcium retention [106]. More importantly, Dimebon at nanomolar concentrations (0.1–10 nmol/l) improved several measured parameters of mitochondrial function, such as MMP, ATP production, MTT reduction, and apoptosis in human SH-SY5Y neuroblastoma cells and primary rat cortical neurons [106]. Protective effects were observed in both cell lines after treatment with Dimebon alone, but were more pronounced when the cells were additionally stressed, e.g., by serum deprivation [106]. Recent data from our laboratory showed that nanomolar concentrations (100 nmol/l) of Dimebon restored morphologic changes and function of mitochondria, mainly by increasing the amount of ETS in a cellular AD model (HEK-APP_{sw} cells) that produces excess of Aβ [107].

Methylene Blue

The FDA-approved drug methylene blue (MB) is used for more than 100 years for the treatment of various diseases, e.g., as an antidote for different poisonings [108, 109], against malaria [110], and in the treatment of some psychiatric disorder because of its anxiolytic and antidepressant properties [111–113]. MB has well-known pharmacokinetic properties, is readily absorbed, and quickly distributed to various organs, including the brain [114, 115].

Lindahl and Oberg described [116] the cognition-enhancing properties of low doses MB and its oxygen consumption increasing effect in isolated mitochondria [116]. Besides the overall enhancing properties of MB on respiratory function [116–118], MB in low doses (1 mg/kg b.w. i.p.) also increases cytochrome c oxidase (COX) activity and thereby further improves brain energy production [119]. This finding is of importance, since COX activity declines during the progression of AD [61, 120, 121]. Within the complexes of the mitochondrial electron transport chain, complex I is the largest and most susceptible one to oxidative stress [122]. Complex I dysfunction is also involved in aging and thereby in many age-related neurodegenerative diseases [123]. Complex I inhibitors such as rotenone are commonly used to mimic complex I dysfunction [68, 122]. MB (up to 0.07 mg/kg intravitreally microinjected in eyes of mice) showed broad efficacy in reversing the effects of rotenone [116, 122]. Beneficial effects were also demonstrated in anticholinergic models of memory dysfunction (0.15–4.0 mg/kg b.w. i.p.) [124].

Thus, oxidative stress and mitochondrial dysfunction are characteristic and early events in AD [123, 125]. Any disturbances in the electron transport chain lead to elevated ROS level, ROS in turn increases Aβ generation, Aβ is further impairing mitochondrial function, and finally a vicious cycle is initiated [68]. MB as a redox compound is interfering in this pathology; it avoids the reduction of molecular oxygen to superoxide by acting as alternative

electron acceptor [126, 127]. Like an electron shuttle, it transfers electrons in between the electron transport chain to finally reduce oxygen to water [128, 129].

Besides the described low-dose effects, MB in high doses inhibits AD-like tau generation (IC_{50}, 3.4 μmol/l) in a cell-free in vitro binding assay [130] and Aβ formation (250 mg/kg diet) in a 3xTg AD mouse model of Alzheimer's disease [131]. However, the use of MB at micromolar range is critical debated, since it exhibits cellular toxicity, and thus neurotoxic side effects could not be excluded [129, 132]. Modulation of α7-nicotine acetylcholine receptors (α7-nAchR) has been suggested to play a role in neurodegenerative diseases [133]. Recent in vitro data indicate that MB inhibits as a non-competitive inhibitor (IC_{50}, 3.4 μmol/l in vitro) the function of human expressed in *Xenopus ooxytes* and of α7-nAchR-mediated responses in rat hippocampal neurons [134].

Piracetam

Piracetam, a nootropic drug which was approved in the early 1970s, is used since many years to treat cognitive impairment in aging, brain injuries, as well as dementia [135, 136]. A comprehensive meta-analysis including all published and not published clinical studies provided compelling evidence for the global clinical efficacy of Piracetam in a diverse group of older subjects with cognitive impairment [137]. However, its clinical use for the treatment of AD is controversially discussed because clinical trials with current evidence-based requirements are missing.

Although its mode of action is not yet finally known [136], Piracetam improves disturbed membrane fluidity following oxidative stress or aging and ameliorates functional deficits of neurotransmission associated with reduced fluidity of neuronal membranes [135]. Initial evidence that Piracetam's beneficial effects on the fluidity of aged mitochondrial membranes (1 mmol/l in vitro) could contribute to its therapeutic efficacy originated from observations that Piracetam could improve glucose uptake and utilization, MMP levels, as well as ATP production [138–141]. This hypothesis has been supported by more recent studies using a variety of cellular (PC12-APPsw cells; 0.1–1 mmol/l) and animal models (C57BL/6J-Thy1-APP751$_{SL}$ mice; 100–500 mg/kg b.w.) of AD, indicating that improving mitochondrial function, such as improved MMP, ATP levels, mitochondrial respiration, and neuritic outgrowth following a variety of situations associated with oxidative stress, seems to be a major mechanism of action of Piracetam [142–144].

Our initial findings of Piracetam enhancing mitochondrial membrane fluidity [141] and observations that membrane fluidity regulates mitochondrial function probably by enhancing the mobility and function of the complexes of the respiratory chain [55, 145–147] are strongly supporting this assumption.

Simvastatin

Statins are selective inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis [148]. Besides their broad use as lipid-lowering drugs, statins are currently discussed for having potential efficacy in treatment of certain neurologic disorders, including AD [149]. Most epidemiological studies concluded that statins decrease the risk of AD, although the majority of the prospective studies were inconclusive [150, 151]. Although statins reduce Aβ levels in cellular and animal models of AD, the effects on cognition and memory are partly independent from APP processing. Li et al. demonstrated that simvastatin treatment (50 mg/kg b.w. p.o.) for 3 weeks enhances learning and memory independent of amyloid load in TG2676-AD mice [152]. These results were confirmed recently [153, 154].

The Mevalonat pathway produces several biologically active molecules, which play an important role in cell function. One of those molecules is cholesterol, which has certainly garnered intensive study. Upstream of cholesterol in that pathway are two isoprenoids, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which are receiving increasing attention both with respect to normal function and their potential contributions to pathophysiology associated with neurodegenerative diseases. We have recently shown that FPP and GGPP levels are significantly increased in postmortem frontal cortex tissue from AD patients and that simvastatin (50 mg/kg b.w. p.o.) significantly decreases levels of both isoprenoids in brains of C57BL/6J mice [155]. It has been suggested that the neuroprotective effects of statins may be due in part to a reduction in FPP and/or GGPP levels which in turn lessens the abundance of prenylated proteins and which may or may not be cholesterol independent [149, 156]. For further reading, please refer to Li et al., this issue [157].

However, we recently reported that chronic statin administration altered gene expression patterns in cerebral cortex of C57BL/6J mice (50 mg/kg b.w. p.o. for 21 days) [158]. Most notably, expression levels of genes associated with apoptosis were significantly altered including upregulation of the major anti-apoptotic gene Bcl-2 [158]. Subsequent studies demonstrated that nanomolar concentrations (100 nmol/l) of simvastatin protected primary cortical neurons and SH-SY5Y cells by upregulation of Bcl-2 mRNA levels [159] and involve endothelin-1 (ET-1) protein and the transcription factor NFATc3 [160]. In vivo studies confirmed that simvastatin (50 mg/kg b.w. p.o. for 21 days) provides neuroprotective properties by upregulation of Bcl-2 protein (Fig. 1) [161]. Moreover, protein levels of pro-apoptotic Bax, the major opponent of Bcl-2, were significantly reduced. The Bax/Bcl-2 ratio, a crucial parameter for regulating apoptosis, was significantly decreased in brain tissue of simvastatin-

treated animals. In agreement with earlier findings, overexpression of Bcl-2 prevented mitochondria from SNP-induced MMP loss [161–163]. Subsequently, dissociated brain cells isolated from simvastatin-treated animals were protected against activation of caspase-9 and -3 [161]. Bcl-2-mediated neuroprotective properties of simvastatin (1 mg/kg b.w. i.p. for 2–8 weeks) were recently confirmed in a rat model of Huntington's disease [164] and in retinal ganglion cells following ischemia reperfusion injury [165].

Ginkgo biloba

Standardized extracts of *G. biloba*, particularly EGb761®, are successfully used as herbal drug for the improvement of cognitive and memory impairments; EGb761® contains 24 % of flavonoids and 6 % of terpenens [166–168]. The clinical usefulness of EGb761 in dementia has been shown in many clinical trials (see recent meta-analyses of short-term trials [169, 170]). Accordingly, EGb761 has recently been included in the guidelines for treatment of AD of the World Federation of Societies of Biological Psychiatry [171]. Its possible usefulness in long-term treatment to prevent dementia is more controversial. While the GEM study in cognitively very healthy elderly participants did not show any preventive effect [172], the very recent GUIDAGE study reported a significant protection of conversion to dementia in elderly with memory complains [173]. Besides its effects on monoaminergic neurotransmission [174], several terpene lactones (Ginkgolides, Bilobalide) show substantial mitochondria-protecting properties [167].

EGb761® given shortly after initiating mitochondrial damage by sodium nitroprusside (nitric oxide donor) improved the MMP of PC12 cells significantly and dose dependently. Under these conditions, EGb761® also reversed the decrease in ATP production. In addition, similar protection against oxidative damage was found in dissociated brain cells and isolated brain mitochondria after in vitro or in vivo treatment with EGb761®. Moreover, PC12 cells bearing an AD-related mutation in APP, which leads to enhanced Aβ production, showed greater benefit from treatment with EGb761® than did control cells [175]. These findings were confirmed recently: EGb761® alleviated mitochondrial functions in PC12 cells at concentrations as low as 0.01 mg/ml [167]. Treating two different age groups of mice with EGb761® (100 mg/kg body weight for 14 days) showed beneficial effects on complexes I, IV, and V of the mitochondrial respiratory chain and against nitrosative stress. Interestingly, these effects were only observed in the aged mice group, proving higher efficacy of EGb761® during aging [167].

While the terpene lactones mainly protect mitochondrial properties, the flavonoid fraction seems to be mainly responsible for the free radical scavenging characteristics. The effects of oxidative stress were reduced in lymphocytes and brain cells derived of EGb761®-treated AD-transgenic and non-transgenic mice (100 mg/kg b.w. p.o. for 14 days) [167, 176, 177]. A recent review summarized that EGb761® also has been shown to improve all aspects of impaired neuroplasticity following oxidative stress including reduced long-term potentiation, reduced spine density, impaired neuritogenesis, and even reduced neurogenesis [178].

Recent data, however, indicate that EGb761® also affects APP processing, for example, by upregulating α-secretase activity in hippocampal slices (5–200 μg/ml) and in brains of Sprague–Dawley rats (80 and 150 mg/kg b.w. p.o.) for 5 days [179]. Moreover, in a neuroblastoma cell line stably expressing an AD-associated double mutation, incubation with EGb761® (100 μg/ml) led to a suppression of Aβ fibril formation and subsequent reduction in caspase-3 activation. This observation indicates that, in addition to the inhibition of Aβ fibrillogenesis—possibly due to a direct interaction with Aβ—*Ginkgo* extract may act on intracellular signaling pathways [180]. In aged and/or AD transgenic mice, EGb761® treatment (100 mg/kg b.w. p.o. for 21 days) resulted in improved memory compared to control animals [181, 182]. The mechanisms responsible for latter observation are still a matter of debate. Whereas Luo et al. [183] reported changes in APP load in rats treated with Ginkgo extract (100 mg/kg b.w. p.o.) for 15 days, Garcia-Alloza et al. [184] suggested changes in the extent of oxidative stress to account for the neuroprotection in EGb761®-fed APPswe/PS1d9 transgenic mice (100 mg/kg b.w. p.o. for 15 days). Interestingly, the EGb761®-associated reduction in Aβ plaque-linked oxidative stress in mice brain was unaffected by plaque size or number. Similarly, Tg2576 transgenic mice benefited from repeated EGb761® oral intake (70 mg/kg b.w. in water for 6 months), evident by improved spatial memory, although soluble and Aβ plaque burden was unaffected [185].

Curcumin

Curcumin (diferuloylmethane) is the yellow pigment derived from the rhizome of the plant turmeric (*Curcuma longa*), a major component of the spice curry, and frequently used as a natural colorant by the food industry. The lipophilic phenolic diferuloylmethane has a large number of biological functions, including antioxidative, anti-inflammatory, cholesterol-lowering, anti-proliferative, and neuroprotective activity [186–190].

Curcumin targets pathways involved in the pathophysiology of AD, such as processing of APP, tau phosphorylation, neuroinflammation, or oxidative stress. These findings suggest that curcumin might be a promising compound for the development in AD therapy.

Several in vitro findings identified mitochondria as a promising target for curcumin. In neuronal PC12 cells, curcumin (25 μmol/l for 2 h) maintained mitochondrial redox and respiratory functions after hydroxynonenal (4-HNE) treatment without a marked effect on ROS production and cell viability [191]. In rat cortical neurons challenged with tert-butyl hydroperoxide (t-BHP) to induce oxidative damage, curcumin (2.5–20 μmol/l) compensated the loss of MMP and cytochrome *c* release, blocked the activation of caspase-3, and altered the expression of Bcl-2 family proteins. Further, curcumin treatment also prevented cellular glutathione levels and decreased intracellular ROS generation [189].

In vivo, curcumin treatment (5, 15, or 45 mg/kg i.p. for 10 days) decreased MDA and superoxide anion levels significantly, rescued hippocampal cells, and improved learning and memory in a homocystein-induced rat aging model [192]. Another study confirms curcumin's antioxidative effects in aged mice [193]. A significant reduction of ROS level and protein carbonylation was observed after administration of dimethyl sulfoxide-dissolved curcumin (90 mg/kg i.p. for 3 days) [193]. In vivo findings identified mitochondria as promising target for curcumin [189]. Chronic administration of D-galactose significantly impaired cognitive function, locomotor activity, oxidative defense, and activities of mitochondrial enzyme complexes I, II, and III. Curcumin treatment (15 and 30 mg/kg b.w. p.o.,) for 6 weeks significantly improved cognitive tasks, locomotor activity, oxidative defense, and restored mitochondrial enzyme complex activity as compared to control [194].

In a diabetic rat model, streptozotocin induced downregulation of mitochondrial complex I and IV activity and loss of ATP level in the brain, which were counteracted after oral administration of curcuminoids (120 mg/kg p.o. for 4 weeks) [195]. Moreover, curcumin (60 mg/kg b.w. i.p. for 2 days) prevented the isoprenaline-induced increase in mPTP calcium susceptibility in isolated rat cardiomyocytes ex vivo without affecting mitochondrial biogenesis and mitochondrial network dynamic [196].

At present, four clinical trials concerning the effects of curcumin on AD have been conducted with negative outcome [197]. Otherwise, insolubility in water and poor bioavailability of curcumin may have limited clinical trials and their outcome [198]. Thus, to be effective, new delivery strategies need to be developed for curcumin [198].

Besides curcumin, other antioxidant approaches including vitamins C and E in treating AD patients are also thus far disappointing. Besides insufficient blood–brain barrier permeability of naturally occurring antioxidants, a not well-thought-out experimental design of clinical trials may have limited the success of antioxidant clinical trials [88]. Moreover, most clinical trials were conducted thus far in late-stage AD patients, and thus treatment may occur not in a favorable therapeutic window. However, mitochondria-targeted molecules such as MitoQ appear to be promising to treat AD (for extensive review and discussion, refer to [88]).

Omega-3 Polyunsaturated Fatty Acids

More than a dozen epidemiological studies have reported that reduced levels or intake of omega-3 polyunsaturated fatty acids (PUFA) or fish consumption is associated with increased risk for age-related cognitive decline or dementia such as AD [199].

Recent in vitro data indicate that the beneficial effects of docosahexaenoic acid (DHA), an omega-3 long-chain fatty acid, abundant in fish oil are related to mitochondria. In HEK-APP cells, DHA (20 μmol/l) significantly increased membrane fluidity and non-amyloidogenic processing of APP, leading to enhanced secretion of sAPPα. This enhanced secretion of sAPPα was associated with substantial protection against mitochondrial dysfunction and apoptosis by thapsigargin-induced ER-Ca^{2+} store depletion [200]. These in vitro data support the growing evidence that dietary omega-3 PUFA, particularly DHA, has profound effects on mitochondrial membrane phospholipid composition and mitochondrial function. Supplementations with n-3 PUFA increase membrane phospholipid DHA and deplete AA. Moreover, increased cardiolipin levels, a tetra-acyl phospholipid that is unique in mitochondrial inner membrane and essential for optimal mitochondrial function, were detected [201].

Aging is one of the most important risk factors for AD, and activities of respiratory chain complexes I and IV are significantly decreased in mitochondria isolated from brains of aged rodents [202–206]. Treatment of dissociated brain cells (DBC) with low concentrations of SNP, which inhibits mitochondrial respiratory chain complexes I and IV at low concentrations, was used as an ex vivo model for brain aging [207]. Guinea pigs were treated with herbal omega-3 PUFA-rich *Perilla* frutescence seed oil (PFSO; 0.5 and 1.0 g/kg b.w. p.o. for 21 days), which contains 60 % of alpha-linolenic acid. Isolated DBC were less vulnerable against SNP-induced loss of MMP ex vivo, but were not prevented from SNP-induced ATP loss [207]. PFSO modulated genes related to energy household, lipid metabolism, and mitochondrial respiration, further indicating mitochondria-stabilizing effects [208]. Moreover, diets enriched in PFSO affect the learning ability in mice and rats, providing evidence for functional beneficial activities in the brain [209, 210].

Conclusion and Future Prospect

Mitochondrial dysfunction represents a common pathological event in AD, but also in brain aging, which is the most important risk factor for neurodegeneration. The majority of preclinical data indicate beneficial effects of diverse drugs

including Piracetam, Dimebon, methylene blue, simvastatin, curcumin, and omega-3 polyunsaturated fatty acids, whereas most controlled clinical trials did not meet the expectations. One exception is EGb761, a standardized *Ginkgo biloba* extract with proved mitochondrial-improving properties and clinical usefulness in dementia. Most clinical trials were conducted thus far in late-stage AD patients, and thus treatment may occur not in a favorable therapeutic window. Although mitochondria-targeted compounds appear to be potential efficacious to treat AD, more studies are needed that focus on therapeutic strategies starting before severe disease progress.

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