Alzheimer’s Disease-Associated Neurotoxic Mechanisms and Neuroprotective Strategies

C. Pereira*, P. Agostinho*, P.I. Moreira, S.M. Cardoso and C.R. Oliveira*

Center for Neuroscience and Cell Biology and Institute of Biochemistry, Faculty of Medicine of Coimbra, 3004-504 Coimbra, Portugal

Abstract: The characteristic hallmarks of Alzheimer’s disease (AD), the most common form of dementia in the elderly, include senile plaques, mainly composed of beta-amyloid (Aβ) peptide, neurofibrillary tangles and selective synaptic and neuronal loss in brain regions involved in learning and memory. Genetic studies, together with the demonstration of Aβ neurotoxicity, led to the development of the amyloid cascade hypothesis to explain the AD-associated neurodegenerative process. However, a modified version of this hypothesis has emerged, the Aβ cascade hypothesis, which takes into account the fact that soluble oligomeric forms and protofibrils of Aβ and its intraneuronal accumulation also play a key role in the pathogenesis of the disease. Recent evidence posit that synaptic dysfunction triggered by non fibrillar Aβ species is an early event involved in memory decline in AD. The current understanding of the molecular mechanisms responsible for impaired synaptic function and cognitive deficits is outlined in this review, focusing on oxidative stress and disturbed metal ion homeostasis, 
Ca2+ dysregulation, mitochondria and endoplasmic reticulum dysfunction, cholesterol dyshomeostasis and impaired neurotransmission. The activation of apoptotic cell death as a mechanism of neuronal loss in AD, and the prominent role of neuroinflammation in this neurodegenerative disorder, are also reviewed herein. Furthermore, we will focus on the more relevant therapeutic strategies currently used, namely those involving antioxidants, drugs for neurotransmission improvement, hormonal replacement, γ- and β- secretase inhibitors, Aβ clearance agents (Aβ immunization, disruption of Aβ fibrils, modulation of the cholesterol-mediated Aβ transport), non-steroidal anti-inflammatory drugs (NSAIDs), microtubules stabilizing drugs and kinase inhibitors.

Keywords: Beta-amyloid, tau, neuroinflammation, synaptic dysfunction, oxidative stress, neurotransmission, cholesterol, therapeutics.

1. INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly. The risk of AD dramatically increases with aging, affecting 7-10% of individuals over age 65, and about 40% of persons over 80 years of age, and it is predicted that the incidence of AD will increase threefold within the next 50 years if no therapy intervenes [1]. In developed societies where life expectancy has been considerably extended, this devastating disease actually represents a major public health concern, being estimated that 22 million people worldwide will develop this progressive neurodegenerative disorder by 2025 [2]. AD is characterized clinically by global cognitive dysfunction, especially memory loss, behavior and personality changes, and impairments in the performance of activities of daily living that leaves end-stage patients bedridden, incontinent and dependent on custodial care. Patient’s death occurs on average, 9 years after diagnosis. Despite the strong progresses made in AD research in the last decades, no treatment with a strong disease-modifying effect is currently available.

1.1. Neuropathological Hallmarks

The neuropathological hallmarks of AD are neuritic (senile) plaques, which are extracellular deposits predominantly composed of fibrillar beta-amyloid (Aβ) peptide, usually surrounded by reactive astrocytes, activated microglia and dystrophic neurites (altered axons and dendrites), and intracellular neurofibrillary tangles (NFT) composed of filamentous aggregates called paired helical filaments (PHF) of hyperphosphorylated protein tau, frequently conjugated to ubiquitin [3]. These histopathological lesions, prerequisites for a confirmed clinical diagnosis of AD after death, are restricted to selective regions, particularly the hippocampus, a centre for memory, and the cerebral cortex, which is involved in reasoning, memory, language and other important thought processes. These brain regions are reduced in size in AD patients as the result of degeneration of synapses and death of neurons.

Most cases of AD are sporadic, and probably result from the synergistic action of genetic and environmental factors. Advanced age and inheritance of the ε4 allele of the polymorphic apolipoprotein E gene (APOE) are the major risk factors, although neither is sufficient to cause the disease. A small percentage of cases, referred as familiar AD, are inherited in an autosomal dominant fashion (reviewed in [4]). Sporadic and familiar cases of the disease are pathologically and clinically indistinguishable, but the familiar forms generally have an earlier age of onset.

*Address correspondence to the author at the Center for Neuroscience and Cell Biology and Institute of Biochemistry, Faculty of Medicine of Coimbra, University of Coimbra, 3004-504 Coimbra, Portugal; Tel: 351-239-820190; Fax: 351-239-822776; E-mail: catarina@cnc.cj.uc.pt

*The authors contributed equally to this review.
1.2. βAPP Processing

Glenner and Wang [5] isolated and purified neuritic plaques from the brain of AD patients, and determined that the major constituent was a very short protein fragment of ~4 KDa made up of either 40 or 42 amino acids, the Aβ peptide. Aβ is derived from the proteolytic processing of the β-amyloid precursor protein (βAPP), a type I integral membrane glycoprotein with a large ectodomain and a short cytoplasmic tail, with a broad tissue distribution including neurons [6]. Aβ region is located at the cell surface or in the luminal side of endoplasmic reticulum (ER) and Golgi membranes, with part of the peptide embedded in the membrane. The normal functions of βAPP are not fully understood, but increasing evidence suggests that it has important roles in regulating neuronal survival, neurite outgrowth, synaptic plasticity and cell adhesion (reviewed in [7]). Axonal transport of βAPP to presynaptic terminals, where it accumulates at relatively high levels, can result in Aβ deposition at synapses [8]. βAPP is proteolytically processed by three different proteases, named secretases, to generate biologically active peptides (reviewed in [9]). The predominant proteolytic pathway involves cleavage of the extracellular domain of βAPP within the Aβ domain, by the membrane-associated α-secretase, leading to the release of the soluble N-terminal fragment APPsα into the culture medium (in vitro) or the cerebrospinal fluid (in vivo), and retention of a 83-residue C-terminal fragment (C83) in the membrane. C83 can undergo cleavage by the γ-secretase to release the p3 peptides and a shorter C-terminal derivative (AICD) into the cytoplasm. This pathway is non-amyloidogenic, because the cleavage precludes the generation of Aβ. The α-secretase activity seems to be exerted by TACE (an enzyme responsible for cleavage of members of the TNF receptor family at the cell surface) and two related proteases, ADAM9 and ADM10 [10]. An alternative cleavage pathway, usually called amyloidogenic pathway, involves two sequential cleavages, the β- and γ-secretase enzyme activities, and gives rise to a series of β-sheet-containing peptides known as Aβ. The first cleavage occurs at the amino side of Aβ by β-secretase, producing a shorter secreted amino-terminal derivative, APPsβ, and an Aβ-bearing membrane-associated C-terminal of βAPP derivative containing 99-residues (C-99). This is subsequently cleaved by γ-secretase almost in the centre of the transmembrane domain to produce Aβ, which is secreted, and AICD, which is released into the cytoplasm and translocates into the nucleus to regulate gene expression [11]. Two highly homologous enzymes with β-secretase activity have been identified: BACE1 (β-site APP cleaving enzyme), thought to be the main β-secretase enzyme in the brain, and BACE2, which seems to be located predominantly in peripheral tissues [12]. Accumulating evidence now supports the concept that presenilin 1 (PS1), a transmembrane domain aspartyl protease that cleaves its substrates in the membrane-spanning region, might be the γ-secretase responsible for the generation of Aβ fragments and that it requires at least three other membrane proteins (nicastrin, APH-1 and PEN-2) as co-factors to confer proteolytic activity (reviewed in [13, 14]). In addition to βAPP processing, γ-secretase plays an important role in the cleavage of the transmembrane domain of a growing number of membrane-bound substrates present locally in synapses, including the Notch family of cell-surface receptors [15], which regulates cell-fate determination [16].

The βAPP-processing events summarized above occur in varying degrees in virtually all neuronal and non-neuronal cells, making Aβ a normal product of cells [17], with the isoforms ending at residue 40 (Aβ40) corresponding to around 90% of secreted Aβ peptides.

1.3. Aβ Clearance and Degradation

Recent data indicate that Aβ is degraded by several pathways (reviewed in [18]), which seem to mainly involve degradation through cleavage by zinc metalloendopeptidases, either neprilysin, a neutral endopeptidase, or insulin-degrading enzyme (IDE), an enzyme responsible for insulin degradation. Presynaptic neprilysin has been demonstrated to degrade Aβ efficiently, particularly the soluble forms of Aβ, and to retard the development of amyloid plaques in mouse brain [19]. However, the fact that expression levels of neprilysin are particularly low in vulnerable regions with senile plaques [20], together with the observation that the endogenous Aβ levels are significantly enhanced in the brain of neprilysin gene-disrupted mice [21], suggest that even a subtle reduction in neprilysin activity could promote Aβ deposition, possibly contributing to AD development. In AD brain, the activity, levels and mRNA of IDE (an enzyme demonstrated to play a key role in Aβ degradation in vitro and in vivo and to be selective for Aβ monomers) has been shown to be decreased, an event that might be associated with increased Aβ levels [22].

1.4. Genetics of AD and Risk Factors

Despite the genetic determinants that have been associated with AD that are considered to represent a minor contribution to the general risk of developing this disorder, their discovery improved our knowledge of the molecular and cellular mechanisms responsible for neuronal degeneration and cognitive dysfunction in AD. Certain genetic defects that cause autosomal dominant AD, such as mutations in the gene encoding for βAPP (localized on chromosome 21 [15]) or mutations in the PS1 and PS2 genes (on chromosome 14 and chromosome 1, respectively [23, 24]) augment the amyloidogenic pathway of βAPP processing in cells in a way that favors production of the Aβ42 variant. Aβ42, which is selectively deposited in affected brain regions both in humans and in transgenic mouse models, is more prone to oligomerization and fibril formation than the slightly shorter and less hydrophobic Aβ40 form [25]. Zou and colleagues have suggested that Aβ40 protects neurons from damage induced by Aβ42, both in vitro and in vivo, by inhibiting the β-sheet transformation and fibril formation of Aβ42, suggesting a mechanism by which elevated Aβ42/Aβ40 ratio accelerates the development of familiar AD [26]. Mutations in βAPP that cause familiar AD result in one or two amino acid changes within or immediately adjacent to Aβ that enhance its cleavage by β- and γ-secretase, whereas PS mutations alter γ-secretase activity [15]. Aβ42 normally comprises only about 5-10% of total secreted Aβ peptides, but this fraction rises to about 15-40% when either βAPP or PS is mutated.

The major genetic risk factor for the more common, late-onset, sporadic AD is the presence of the ε4 allele of APOE...
gene on chromosome 19 [27]. The increased risk conferred by the presence of the ε4 allele possibly involves enhanced Aβ aggregation and reduced Aβ clearance, enhanced amyloidogenic processing of βAPP, oxidative stress, neuroinflammation and impaired synaptic plasticity (reviewed in [28]). About 30 candidate genes other than APOE have been considered to contribute to the risk of sporadic AD (reviewed in [29]). Polymorphisms or mutations in alpha-macroglobulin, low-density lipoprotein receptor-related protein (LRP), LRP-associated protein, very-low-density lipoprotein receptor and cathepsin D genes are considered AD risk factors. Genes associated with oxidative stress, mitochondrial function, apoptosis and inflammation have also been investigated as AD risk factors, and AD has been associated with chromosome 10, which harbours the IDE gene.

In addition to genetic abnormalities, several environmental factors may affect the risk of AD. Epidemiological findings supported by in vivo studies suggest that a low education level, history of head trauma, consumption of high-caloric/high-fat diets and a sedentary life may each increase the risk of occurrence of AD [30-34]. Insulin resistance may also be a risk factor for the development of AD [35]. Insulin appears important for learning and memory [36], and it was shown to inhibit the phosphorylation of tau [37], to stimulate the secretion of APPsα [38], and also to reduce the intracellular pool of Aβ [39]. A very recent report demonstrated that insulin can induce the upregulation of IDE, both in vitro and in vivo [40].

The causes of altered βAPP metabolism and Aβ deposition in sporadic cases of AD are not understood, but may include age-related increases in oxidative stress, impaired energy metabolism and perturbed cellular ion homeostasis.

2. NEUROTOXIC MECHANISMS

2.1. Hypothesis for the Pathogenesis of AD

2.1.1. The Amyloid Cascade Hypothesis

The fact that the genes that either directly cause AD or increase the risk for developing AD modulate some aspect of the formation or stability of Aβ, together with the demonstration that the accumulation of Aβ aggregates is neurotoxic (reviewed in [41, 42]), constitute the most compelling evidence in support of the amyloid cascade hypothesis for the pathogenesis of AD. According to this hypothesis, the accumulation of Aβ fibrils (especially the amyloidogenic isofoms that end at residue 42), resulting from an imbalance between Aβ production and Aβ clearance mechanisms, is the initiating molecular event that triggers neurodegeneration in sporadic and familiar AD (reviewed in [43]). Several observations suggest that altered βAPP processing occurs before tau tangle formation, and that tau pathology is an important aspect of the pathogenic cascade of AD induced by Aβ. First, mutations in the genes encoding the tau protein cause frontotemporal dementia characterized by cell death and neurofibrillary pathology in the absence of amyloid deposition [44], and Aβ42 induces the formation of NFT in mutant tau transgenic mice [45]. Second, in transgenic mice overexpressing both mutant human βAPP and mutant human tau, enhanced formation of tau-positive tangles occurs without any significant alteration in the structure and number of amyloid plaques [46]. Third, studies of patients dying from Down’s syndrome, who carry an extra copy of the βAPP locus on chromosome 21, have consistently shown that Aβ deposition occurs before NFT formation. Finally, in mouse hippocampal primary neuronal cultures, Aβ toxicity is tau dependent [47].

2.1.2. The Aβ Cascade Hypothesis

Recent data from several groups gradually led to the development of the Aβ cascade hypothesis, a modified version of the amyloid cascade hypothesis, (reviewed in [43, 48]), which includes the possibility that some other less well characterized soluble, non-fibrillar assembly forms of Aβ, including small oligomers, also referred to as Aβ-derived diffusible ligands (ADDLs) [49], and protofibrils (PF) [50], may be responsible for some of the pathological alterations that underlie memory impairment in AD and in AD animal models. Aβ42 oligomers were shown to be potent neurotoxins in organotypic cultures at nanomolar concentrations [51] and to inhibit hippocampal long-term potentiation (LTP), which is required for memory formation, and to disrupt synaptic plasticity [51, 52]. The levels of Aβ oligomers were shown to be increased in vulnerable brain regions of AD patients [53] within abnormal processes and synaptic compartments [54], in the absence of amyloid plaques and, in many cases, in the absence of NFT as well [55]. These results suggest that accumulation of Aβ oligomers occurs very early in the disease process in humans. Young βAPP transgenic mice showed altered synaptic morphology and electrophysiological changes well before the appearance of Aβ deposits [56]. In addition, Aβ42 dimers accumulate as early as memory impairments become obvious, further supporting the notion that Aβ oligomers are tightly linked to memory failure in AD [57]. However, some authors believe that the increased generation of Aβ monomer and oligomeric species in degenerating synapses represent a compensatory response aimed to prevent neuronal degeneration (reviewed in [58]).

The Aβ cascade hypothesis also considers that intracellular Aβ42 accumulation in vulnerable brain regions might be an early event in the pathological process in AD (reviewed in [59]), and it was suggested that amyloid plaques are formed as a consequence of the lysis of Aβ42-burdened neurons [60]. This view is supported by several observations. First, an insoluble pool of intracellular Aβ42 increases with time in culture, and the ratio Aβ42/Aβ40 within neurons is higher than that of secreted Aβ [61, 62]. Second, Aβ42 levels are selectively increased within AD-vulnerable neurons prior to the appearance of PHF, and seem to be attenuated with increasing cognitive dysfunction and plaque deposition [63, 64]. Third, in several AD animal models, intraneuronal Aβ accumulation in vulnerable brain regions is not correlated with the amount of extracellular deposited Aβ, but is associated with early synaptic dysfunction prior to plaque or tangle pathology and declines with increased plaque accumulation [65-68]. Fourth, cells expressing mutations in the AD-related genes for βAPP, PS1 or PS2 increase the ratio of intracellular Aβ42/Aβ40 levels [69, 70]. Fifth, studies of rat organotypic hippocampal slices were shown to preferentially internalize
exoenogenous Aβ42 [71]. The selective intracellular accumulation of Aβ42, due to uptake from extracellular space was suggested to result from the high affinity binding of the carboxy-terminally extended species that contain 42 residues to α7 nicotinic acetylcholine receptors, leading to the subsequent internalization of the complex [72]. Finally, neurons in the hippocampus and entorhinal cortex from the brain of Down’s syndrome patients show strong intraneuronal Aβ42 immunoreactivity, which declined with the deposition of extracellular Aβ plaques [73], supporting the hypothesis that the amyloid pathology in AD occurs as a consequence of increased intracellular Aβ levels.

2.2. Tau Phosphorylation

Tau is a microtubule-associated protein that plays a role in the maintenance of neuronal morphology. Aggregation of hyperphosphorylated tau (p-tau) is a common feature of AD, as well as of other neurodegenerative disorders. The phosphorylation of tau is a key post-translational modification that regulates tau’s function regarding microtubule stability and polymerization. Tau has about 30 potential phosphorylation sites, most of which are putative targets of proline directed serine (Ser)/threonine (Thr) kinases (reviewed in [74]). Hyperphosphorylation of tau causes conformational changes and aggregation of this protein, leading to impairment of microtubule stability and cytoskeletal dynamics. In AD brain, three p-tau epitopes have been identified on residues Thr212/Ser214, Thr231 and Ser422 [75]. The abnormal phosphorylation of tau that occurs in AD can be related to alterations in kinases and/or phosphatases activities. In vitro, glycogen-synthase kinase-3β (GSK-3β), extracellular signal-regulated kinase -1 and -2, and mitogen-activated protein kinases, p38 and c-Jun, and cyclin-dependent kinase 5 (Cdk5) can phosphorylate tau protein [76-78]. In AD and in frontotemporal dementia, the insoluble PHF contain tau hyperphosphorylated on residues that overlap those phosphorylated by GSK-3β and Cdk5 in vitro [74]. Recent studies have demonstrated that GSK-3β and Cdk5 phosphorylate tau in vivo [79, 80], and an intense Cdk5 immunoreactivity in neurons in the early stages of NFT formation was observed [81]. In AD brain, both GSK-3β and Cdk5 are associated with pre-NFT or tangle-bearing neurons. These findings provide evidence that these kinases precede NFT formation, and are probably involved in AD progression [75, 82].

Cdk5 is a Ser/Thr kinase, whose activity in neuronal cells is regulated by its binding to specific activators p35, p39 or p25 (the truncated carboxy-terminus of p35). The proteolytic cleavage of p35 into p25 occurs in response to several insults, for instance inflammation, oxidative stress and excitotoxicity, which lead to calpains activation [83]. The p25/Cdk5 complex is more active and stable than p35/Cdk5, so it is thought that the p25/Cdk5 complex causes tau hyperphosphorylation and neurotoxicity [75, 82]. The divergent results obtained by different groups regarding the effect of Cdk5 on tau phosphorylation in vivo may be partially explained if it is considered that it can regulate tau phosphorylation through direct and indirect pathways. Indeed, Cdk5 can regulate the activity of protein phosphatase 1 (PP1), which dephosphorylates tau by phosphorylating the PP1 inhibitors [82]. Moreover, it was also reported that Cdk5 could act upstream of GSK-3β [80]. It should be pointed out that outside the role of Cdk5 and GSK-3β as tau kinases, they may have also important roles in neurodegeneration, as well as in neuronal survival (reviewed in [82]). Recently, it was proposed that microtubule affinity regulating kinase (MARK) activation triggers phosphorylation of tau and causes its dissociation from microtubules, priming tau for further hyperphosphorylation by Cdk5 and then by GSK-3β [84]. The development of pharmacological inhibitors of tau phosphorylation and aggregation would certainly be useful to prevent neurodegeneration.

2.3. Synaptic Dysfunction

Synaptic dysfunction and loss, without an associated loss of neurons are early events in AD, which occur before amyloid plaque formation and seem to contribute to memory loss and cognitive deficits in AD (reviewed in [85, 86]). The memory and cognitive decline observed in AD patients correlates better with the synaptic pathology than either plaques or tangles [87]. Synaptic activity regulates βAPP processing, and release of Aβ oligomers from synaptic terminals may, in turn, modify synaptic plasticity [88]. Therefore, the biochemical targets of Aβ that produce selective toxic effects on synapses could be important for early treatment [89].

Post mortem morphological studies have shown a significant decrease in synaptic density and in the number of synapses per neuron in the association cortices and hippocampus of AD brain [90]. The degree of cognitive decline in patients has been correlated with changes in the presynaptic vesicle protein synaptophysin in vulnerable brain regions [91]. Several proteins that regulate clathrin-mediated vesicle recycling are reduced in AD, suggesting that synaptic function may be affected due to the impairment of synaptic vesicle recycling (reviewed in [92]).

In transgenic mouse models, synaptic dysfunction and memory impairment can occur in the absence of any overt evidence of amyloid deposition. The occurrence of progressive learning deficits, along with declines in synaptic transmission and in the number of presynaptic terminals and neurons in the hippocampus of βAPP transgenic mice before any amyloid plaques are observed [56, 93], supports a role of soluble Aβ species in AD synaptotoxicity. This is further supported by the observation of a significant decrease in dendrite length of dentate granule cells many months before amyloid deposition [94]. Dendritic dystrophy seems to be sufficient to result in major abnormalities in synaptic function and disrupted propagation of information in cortical networks [95]. Very recent reports state that fibrillar Aβ deposition is also detrimental to neuronal circuitry in vivo, leading to synaptic dysfunction and reducing the ability of neurons to successfully integrate and propagate information [95, 96].

Analysis of human brains demonstrated significant correlations between cortical levels of soluble Aβ, which include soluble oligomers, and the extent of synaptic loss and severity of cognitive decline [97, 98]. In βAPP transgenic mice, the presynaptic terminals are also critically dependent on cortical Aβ levels, their number being significantly decreased as their soluble Aβ levels rise, but
before Aβ deposition in plaques begins [56]. The observation that deficits of memory function in βAPP transgenic mice were reversed by a single intraperitoneal injection of anti-Aβ antibodies, which did not decrease brain amyloid burden [99], strongly supports the notion that synaptic dysfunction is triggered by Aβ species other than fibrillar plaques.

The nature of the synaptotoxic species in the brain of transgenic mouse models of AD is very difficult to define, because the animals accumulate several Aβ forms, including monomers, soluble oligomers, PFs and insoluble amyloid fibrils that are likely to exist in dynamic equilibrium. Several recent reports provided evidence that oligomers of human Aβ, in the complete absence of Aβ monomers, PF, or fibrils, confer synaptotoxicity [100]. In AD brain sections, Aβ oligomers were shown to target and disrupt synapses [101]. Synthetic Aβ peptides can induce similar changes, decreasing cell viability, causing neuronal death and blocking LTP [51, 52, 102]. In cultured hippocampal neurons, Aβ oligomers extracted from AD brain or made in vitro, bind particular synaptic terminals [101]. The expression of Arc and of other synaptic plasticity-related genes was demonstrated to be decreased in a transgenic mouse model of AD in association with amyloid deposition and at the age when the animals developed cognitive dysfunction. Additionally, these changes occurred without changes in synaptic structure, suggesting that they occur before the degeneration of synapses [103].

Aβ PFs can also induce subtle synaptic alterations by altering membrane excitability [104] by a mechanism involving inhibition of specific K+ currents and glutamate receptors [105, 106]. The ‘Artic’ βAPP mutation was shown to cause AD by enhanced Aβ PFs formation [107]. One cause of synapse loss is deficiency in axonal transport, which is impaired in the brain of AD patients [108]. Additionally, Aβ and PS1 mutations impair axonal transport [109, 110].

Synaptic accumulation of oligomers of Aβ may be an early event in AD, leading to multiple adverse effects including induction of oxidative stress, impairment of Ca2+ homeostasis and perturbation of mitochondria and ER function, which are involved in functional and structural abnormalities in synapses, including the activation of apoptotic cascades.

2.4. Oxidative Stress and Metal Ion Homeostasis

Currently, it is believed that oxidative stress plays a significant role in AD pathogenesis, and Aβ peptides have been proposed as a source, but also as a consequence, of oxidative stress in this disorder.

A considerable number of oxidative stress markers are found in the brain of AD patients associated with neuritic plaques and NFT (reviewed in [111]). Indirect evidence of oxidative stress in AD brain comes from studies showing that treatment of AD patients with antioxidants delays the progression of the disease [112]. The finding that increased levels of isoprostanes, products of lipid peroxidation, were found in urine, plasma and brain of βAPP transgenic mice [113] and also in urine and plasma of AD patients [114], indicates that oxidative stress is important in AD amyloidogenesis. In a transgenic animal model of AD, it was demonstrated that the increased load of reactive oxygen species (ROS) in the brain is specifically associated with neuritic plaques [115].

Aβ peptides have been proposed as a source of oxidative stress in AD. Antisense directed at the Aβ region of βAPP decreases brain oxidative stress in a senescence accelerated mouse strain which exhibits Aβ accumulation, increased oxidative stress markers and memory and learning deficits [116]. Aβ-induced oxidative damage and neurotoxicity have been demonstrated in hippocampal neuronal cells, in cortical synaptosomal membranes (reviewed in [117]), and in cultured cell lines [118]. H2O2 is directly generated during the process of Aβ aggregation [119] and Aβ induces lipid peroxidation and subsequent production of the cytotoxic aldehyde 4-hydroxynonenal (4-HNE) [120], which seems to increase neuronal vulnerability to apoptosis [121]. 4-HNE can also lead to the oxidative modification of tau, promoting its aggregation and formation of NFT [7]. The methionine residue 35 of Aβ42 has been demonstrated to be critical for the oxidative stress and neurotoxic properties of this peptide, being involved in the formation of its intermediary aggregation forms, namely PF (reviewed in [122]).

Several lines of evidence suggest that synaptic dysfunction induced by Aβ occurs through oxidative stress-mediated mechanisms. First, synaptosomes isolated from human APOE4 knock-in mice were shown to be significantly vulnerable to oxidative stress upon Aβ42 treatment [123]. Second, exposure of synaptosomes to Aβ generates 4-HNE, which impairs glucose and glutamate transport and induces mitochondrial oxidative stress and dysfunction [124]. Third, 4-HNE impairs glutamate transport and mitochondrial function in synaptosomes [125]. Finally, docosahexaenoic acid, an essential omega-3 polyunsaturated fatty acid, whose consumption has been associated with reduced AD risk, was recently shown to protect from dendritic pathology in an AD mouse model through a mechanism involving oxidation of synaptic proteins [126].

Oxidative stress can induce amyloidogenic processing of βAPP, resulting in accumulation of potentially neurotoxic Aβ species [127]. Recent data in mutant βAPP transgenic mice demonstrated that aging-induced alterations in brain oxidative status triggers enhanced production and deposition of Aβ as a result of the increased activity of β-secretase [128].

Metal ion homeostasis is severely dysregulated and may be related to oxidative damage in AD brain (reviewed in [129]). Increased concentrations of copper, iron and zinc are detected in affected brain regions within amyloid plaques [130]. These metals bind to Aβ and were shown to accelerate its aggregation and enhance metal-catalysed oxidative stress associated with amyloid plaque formation [131]. In addition, copper, iron and zinc have been reported to increase the toxicity of Aβ [132]. It was recently described that upon coinjection of human Aβ42 with iron, copper or zinc (at the concentrations found in plaques) into rat cerebral cortex, Aβ complexed with either iron or zinc, but not with copper, was more toxic than Aβ alone [133]. Selective metal chelators enhance solubility of Aβ from post mortem brain tissue of AD patients and transgenic mouse brains [134] and were
shown to attenuate formation of soluble Aβ oligomers in a cell-free culture medium [135]. The accumulation of the redox-active metals, iron and copper, may be a major source of ROS, which are in turn responsible for oxidative stress observed in AD. Zinc, despite being a redox-inactive metal, has also been shown to induce the generation of ROS [136]. In APOE4 transgenic mice, zinc treatment was shown to increase tau phosphorylation, suggesting that APOE4 and zinc act in concert to contribute to the pathogenesis of AD [137].

2.5. Cholesterol Homeostasis

Cholesterol is largely present in neurons and glial cells, and is an essential component for the formation and maintenance of cell membranes. Nerve cells preserve cholesterol homeostasis mainly through de novo biosynthesis in the ER and by the receptor-mediated internalization of lipoproteins that are responsible for cholesterol fluxes [138]. At the steady state in the adult brain, cholesterol clearance is facilitated by the formation of 24-hydroxycholesterol, an oxysterol that passes the blood-brain-barrier more easily than cholesterol [139]. Accumulating data suggest that alterations in cholesterol levels affect AD progression, by affecting Aβ peptide formation and deposition. Since the proteolytic βAPP processing occurs at or in close proximity to cholesterol-rich plasma membrane, modifications in cholesterol levels can influence Aβ production. In fact, a positive correlation between membrane cholesterol levels and Aβ formation was shown [139, 140]. In accordance, it was reported in a mouse model of AD that a high cholesterol diet causes an increase in brain Aβ levels [141]. Recent data have shown that cholesterol is essential for Aβ binding to membranes and cytotoxicity [142]. Moreover, it was also reported that Aβ could oxidize cholesterol into 7β-hydroxycholesterol, a proapoptotic oxysterol that might be neurotoxic [143], whereas 22R-hydroxycholesterol, other oxysterol formed during cholesterol metabolism, was found to protect against Aβ neurotoxicity [144]. On the other hand, Aβ peptides were described to affect cholesterol metabolism, since oligomeric Aβ promotes cholesterol release and the consequent formation of complex Aβ–HDL (high density lipoprotein), which could not be internalized, leading to a decline in cellular cholesterol levels [145].

The principal cholesterol carrier in the brain is the APOE. This glycoprotein has an important role in maintaining cholesterol homeostasis in nervous cells and can also bind Aβ peptides contributing to Aβ aggregation and internalization. The isoform APOE4 is more efficient in binding Aβ than APOE3, and this may account for AD risk associated with APOE ε4 alleles [140]. APOE and cholesterol are co-localized in the core of mature senile plaques, further supporting their involvement in fibrillar plaque formation or maintenance [146].

2.6. Ca²⁺ Homeostasis

The compromise of cellular Ca²⁺ homeostasis appears to be involved in synaptic dysfunction and neuronal death in AD (reviewed in [147]), and possibly in cognitive impairment observed in AD patients, since Ca²⁺ ions play fundamental roles in learning and memory. This hypothesis is supported by several studies of AD patients, of cultured neurons and transgenic mice overexpressing AD-causing βAPP and PS1 mutations, and of synaptosomes or neuronal cell cultures exposed to βAPP-derived peptides (reviewed in [148]).

The levels of the calcium-binding protein calneslin, were shown to be elevated in the cerebral cortex of AD patients, in the neocortex and hippocampus of mutant βAPP transgenic mice and also in cortical and hippocampal cultured neurons exposed to Aβ42 [149]. Tangle-bearing neurons exhibit high amounts of Ca²⁺ and increased levels of Ca²⁺-dependent proteases and Ca²⁺-activated kinases [150, 151]. Recently, calpains were shown to mediate Ca²⁺-induced hyperphosphorylation of tau in the brain of AD patients [152]. Studies of lymphocytes from patients with familial or sporadic AD, and from βAPP and PS1 mutant mice have demonstrated disruption of Ca²⁺ homeostasis [153], suggesting that this is a common feature in both familial and sporadic forms of AD. Alterations in Ca²⁺ signaling have also been reported in mitochondrially transformed cells (cybrids) prepared from platelets of AD patients [154].

The AD-related PS mutations increase neuronal vulnerability to Aβ, excitotoxicity and apoptosis [155-157], by a mechanism involving ER perturbed Ca²⁺ homeostasis and activation of calpains and the ER-associated caspase-12 [9], alterations in ryanodine receptor channels [158], and the ER protein Herp, which stabilizes neuronal Ca²⁺ homeostasis during ER stress [156]. Recent data demonstrate that IP₃-mediated ER Ca²⁺ liberation in cortical neurons is substantially enhanced in transgenic mice expressing a PS1 mutation [159].

Disrupted intraneuronal Ca²⁺ levels in AD may result from the increased production of Aβ42 and/or from the decreased levels of APPsΔCT [127], as a consequence of the altered proteolytic processing of βAPP. Aβ may perturb Ca²⁺ regulation by inducing oxidative stress, leading to an increase of membrane lipid peroxidation and generation of the aldehyde 4-HNE, which impairs membrane Ca²⁺ pumps and enhances Ca²⁺ influx through voltage-dependent channels and ionotropic glutamate receptors (reviewed in [148]). The formation of channels in membranes, the activation of cell surface receptors coupled to Ca²⁺ influx and the enhanced Ca²⁺ release from ER have also been described to occur in the presence of Aβ peptides (reviewed in [148]). The potent antioxidant and free radical scavenger melatonin, which alleviates the learning and memory deficits in a transgenic mouse model of AD, was shown to prevent Aβ-induced intracellular Ca²⁺ overload [160]. Dysregulation of Ca²⁺ homeostasis and the activation of calpains and caspases were shown to be involved in Aβ-induced impaired cell proliferation, survival and neuronal differentiation of neural progenitor cells, which have been reported to play important roles in learning and memory processes [161]. Reduced levels of βAPPΔCT have also been shown to promote disruption of synaptic Ca²⁺ homeostasis [162], and this βAPP fragment protects neurons against cellular insults such as Aβ, excitotoxicity and PS1 mutations by stabilization of intracellular Ca²⁺ [162-164]. On the other hand, Aβ secretion can be increased as a consequence of the
elevation of cytosolic Ca\(^{2+}\) from extracellular or intracellular sources in βAPP overexpressing cells [165].

Compelling evidence suggests that synapses are the primary sites of Ca\(^{2+}\) dysregulation in AD (reviewed in [148]). Exposure of synaptosomes to Aβ peptides results in impairment of the plasma membrane Ca\(^{2+}\) ATPase [120]. Enhanced levels of presynaptic Ca\(^{2+}\) were shown to be involved in the alteration of synaptic plasticity in the hippocampus of PS1 mutant transgenic mice [166], and Ca\(^{2+}\) responses to depolarization were larger in the hippocampus as compared to other cerebral areas [167].

### 2.7. Mitochondria and Endoplasmic Reticulum Dysfunction

Energy metabolism is severely compromised in AD (reviewed in [168]). Brain imaging studies have clearly demonstrated deficits in glucose consumption in living AD patients, and this metabolic compromise seems to occur before the onset of clinical symptoms [169]. Furthermore, it was demonstrated that declines in brain synaptic activity and energy consumption are coupled during the evolution of AD [170]. Decreased activities of cytochrome c oxidase, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH) complexes in the brain of AD patients have been reported [169]. Recently, it was described that oxidative phosphorylation genes are differentially expressed in patients with AD [171]. Mitochondrial degeneration was shown to be an early sign of AD pathology appearing before NFT are evident [172], and is particularly prominent in neurons which show loss of dendritic spines and reduction of dendritic arborization [173].

Several studies suggest that altered proteolytic processing of βAPP is synergistically related with impaired energy metabolism. First, brain glucose metabolism is decreased in cognition-related brain regions of βAPP mutant mice in association with increased amounts of Aβ [174]. Second, hypoxic tolerance is significantly decreased in presymptomatic βAPP mutant mice [175]. Third, dietary restriction protects neurons in experimental models relevant to AD [34]. Finally, impaired energy metabolism can induce amyloidogenic processing of βAPP, resulting in accumulation of potentially neurotoxic forms of Aβ [127].

A pivotal role for mitochondrial dysfunction in AD pathogenesis is supported by recent studies demonstrating that PS1 and the active γ-secretase complex formed by nicastrin, PS, APH-1 and PEN-2, can be located in mitochondria [176]. In addition, it has also been shown that βAPP may be targeted to mitochondria, potentially leading to mitochondrial dysfunction [177]. Taken together, these data suggest that Aβ peptides generated directly in the mitochondria may be responsible for the impairment of mitochondrial function that occurs in AD. The mechanism by which Aβ impairs mitochondrial function seems to involve enhanced ROS production [178], since several enzyme complexes of the respiratory chain are particularly vulnerable to damage by both Aβ and ROS [118, 179, 180].

Acrolein, a lipid peroxidation product which is increased in the AD brain, was found to decrease PDH and KGDH activities [181]. Aβ also has multiple direct effects on isolated mitochondria, causing alterations in enzyme activity, damage to the respiratory chain, and opening of the mitochondrial permeability transition pore, which promotes cytochrome c release and triggers apoptotic cell death [182, 183]. An intact respiratory chain was shown to be required for Aβ-induced apoptosis, because Aβ did not cause apoptosis in mtDNA-depleted cells [184]. In AD cybrids, Aβ toxicity is enhanced by a mechanism involving mitochondrial dysfunction [185]. In mutant βAPP-transfected cells, the increased Aβ production was suggested to trigger the dysfunction of respiratory chain complexes, enhancing cell death upon oxidative stress [186]. Additional evidence for the correlation between mitochondrial dysfunction, oxidative stress and Aβ generation comes from studies that recently demonstrated that mitochondrial ABAD directly interacts with Aβ, leading to oxidative stress and mitochondrial dysfunction [187]. Increased levels of amyloid plaque burden as a result of increased levels of mitochondrial oxidative stress were observed in mice obtained through the genetic cross of MnSOD heterozygous knockout mice with mice overexpressing mutant βAPP [188].

Mitochondria were shown to have close physical contact to the ER and the interaction between both organelles is essential for normal cell functioning [189]. Coupling of ER and mitochondria may also play a role in the pathogenesis of neuronal cell death, since apoptotic cross-talk between both organelles has been identified [190]. Agents that induce ER stress increase the release cytochrome c from mitochondria and subsequently activate caspase-3, these effects being blocked by Bel-2 specifically targeted to the ER compartment [190]. These observations, together with recent results from different groups [191, 192] suggest that in pathological states of the brain, associated with mitochondrial dysfunction and induction of apoptosis, ER dysfunction may be an upstream process. Several evidences suggest that neuronal death is induced by ER stress in familiar and sporadic forms of AD (reviewed in [193, 194]). PS1 mutations associated with familiar AD and an alternatively spliced form of PS2 (PS2V) expressed in sporadic AD, increase the production of Aβ and downregulate the unfolded protein response (UPR), which is a defense mechanism against ER stress caused by inhibition of protein glycosylation, perturbation of Ca\(^{2+}\) homeostasis or reduction of disulfide bonds leading to accumulation of unfolded proteins in the ER. Nakagawa and colleagues reported that knock-out mice for the ER-resident caspase-12 are resistant to ER stress and death caused by Aβ, suggesting that ER stress and caspase-12 are involved in the process of neuronal death in AD [195]. Caspase-4 (which is considered the human caspase-12) was demonstrated to play a key role in ER stress-induced apoptosis and seems to act upstream of the Aβ-induced ER stress pathway, suggesting that it might mediate neuronal death in AD [196]. Disrupted ER Ca\(^{2+}\) homeostasis has been suggested to have a key role in the pathogenesis of AD (reviewed in [197]). In particular, the ability of Aβ to evoke Ca\(^{2+}\) leakage from IP\(_3\) and ryanodine receptors in ER has been shown to be involved in the activation of apoptotic death in cells stressed with Aβ peptides [198-200].

### 2.8. Alterations in Cholinergic and Glutamatergic Systems

One of the earliest pathological events in AD is thought to be the dysfunction and loss of basal forebrain cholinergic
neurons and their cortical projections. In addition to a large neuronal loss within the brain regions, the evidences implicating the cholinergic system in AD neuropathology come from studies that report a decline in choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities, acetylcholine (ACh) release, high affinity choline uptake and in the levels and/or binding of both nicotinic and muscarinic cholinergic receptors in the brain of AD patients, as compared with non-dementia control brains (reviewed in [201]). These evidences have contributed to the development of the so-called “cholinergic hypothesis”, which essentially postulates that cholinergic dysfunction contributes to the cognitive deficit associated with AD and elderly [202, 203]. Consequently, cholinergic replacement therapeutic strategies have been used to ameliorate the cognitive deficit associated with AD. However, these therapeutic strategies, have limited success (reviewed in [204]). The cholinergic hypothesis as it applied to AD, has been questioned, because most of studies that report alterations in cholinergic markers are performed in brains of end stage AD patients [205, 206]. Several in vitro studies have been carried out in order to establish how cholinergic system dysfunction is involved in AD symptomatology and disease progression. It has been stated that Aβ peptides depress ACh release and synthesis and affect ACh degradation in the synaptic cleft (reviewed in [201]). AChE, a key enzyme in cholinergic transmission, has become the target for the development of therapeutic strategies for AD, because AChE inhibitors blocking the ACh hydrolysis promote cholinergic function, thus improving the cognitive deficit [207]. Although AChE levels are reduced in the brain of AD patients, the activity of this enzyme is increased around amyloid plaques and in neurofibrillary tangle-bearing neurons [205, 208]. The increases in AChE activity are likely to be due to a direct effect of Aβ on enzyme. Accordingly, we and other authors have reported that Aβ synthetic peptides increase the activity of AChE [209, 210]. Furthermore, it was also reported that this enzyme promotes Aβ aggregation [211]. Therefore, AChE inhibitors, besides having the capacity to ameliorate cholinergic levels, may also act as inhibitors of amyloid fibrillogenesis [212]. Butryrylcholinesterase (BuChE) is also involved in ACh breakdown in the brain, however, it has a lower affinity for ACh than AChE. It was reported that BuChE activity is increased in AD brain, but the role of this enzyme in cognitive dysfunction associated with AD remains to be clarified (reviewed in [213]).

A large body of evidence identified the selective loss of nicotinic ACh receptors (nAChRs) as the biochemical parameter most closely associated with the severity of AD. These receptors are ligand-gated ion channels, and the subtypes α4β2 and α7 are the most abundant in basal forebrain cholinergic neurons and target regions, such as hippocampus. The α7-nAChRs are more Ca^{2+}-permeable than α4β2-nAChRs, nevertheless, both are involved in a number of functional processes, including cognition, learning and memory (reviewed in [214]). It has been reported that Aβ peptides bind to nAChRs, mainly to α7 subtype, and that this interaction affects the nicotinic currents [215] and the mitogen activated protein kinase (MAPK) signaling [216], and this may contribute to the cognitive impairment in AD. Recently, it was also reported that α7-nAChR activation by Aβ peptides increases AChE expression [209], providing an explanation for the increased AChE activity observed close to amyloid deposits. The α7-nAChRs are also involved in microglia activation [217], suggesting a role of this receptor in the neuroinflammatory process associated with AD. Although the number of muscarinic ACh receptors seems not be affected in AD brain, it was reported in vitro that muscarinic signaling pathways are affected by Aβ peptides [218], and the activation of these receptors protects against Aβ-toxicity, via mitogenic Wnt signal transduction pathway [219].

The brain of AD patients exhibits a massive loss of cortical and hippocampal pyramidal neurons and synapses, and most of the remaining neurons show neurofibrillary tangles. Glutamate is the main neurotransmitter of these neurons, and this excitatory amino acid is considered to be involved in cognitive and memory functions (reviewed in [220]). Since glutamatergic neurons are influenced by the cholinergic system, which is dysfunctional in AD, these two neurotransmitter systems can have a synergistic effect in cognitive impairment [221]. Although biochemical evidence suggests alterations in the pre- and post-synaptic glutamatergic markers in AD brain [222], the alterations in the levels of gluta mine transporters and receptors could be due to post mortem proteolytic degradation. Currently, it is widely accepted that an excitotoxic component is involved in AD pathogenesis [223, 224]. Indeed, it was reported that Aβ peptides sensitize neurons to excitotoxic (L-Glutamate) damage, and increase synaptic transmission via N-methyl-D-aspartate (NMDA) receptors [225, 226]. Excessive activation of NMDA receptors has been implicated in neurodegeneration caused by Aβ peptides, and antagonists of these receptors may provide neuroprotection [227]. Among AD brains, different degrees of decline in the concentration of other neurotransmitters, such as GABA, serotonin and neuropeptide Y were observed [228]. Thus, the replacement therapies that aimed at just one neurotransmitter have met with very limited success in terms of cognitive improvement.

2.9. Apoptosis

Increasing evidence suggests that apoptosis may be one of the mechanisms leading to neuronal death in AD. Lymphocytes bearing genetic or sporadic risk factors of AD, share an increased susceptibility to apoptotic cell death, suggesting that apoptosis is a common feature shared by both sporadic and familiar forms of AD [229].

Several reports supporting the view that at least a subset of neurons in AD brain die by apoptosis has been provided by the demonstration, of DNA fragmentation in the AD brain [230], by the presence of activated forms of caspases -3, -8 and -9 [231-233] and altered expression of cell death-related genes such as Bcl-2 family members (reviewed in [234]). Recently, Zhu and colleagues [235] demonstrated that the expression of Bcl-w, a novel member of the Bcl-2 family that promotes cell survival, is increased in AD, suggesting an early attempt of neurons to protect against apoptosis.

Aβ peptides may be one trigger of apoptotic cell death in AD, since proapoptotic proteins are associated with Aβ deposits in the brain. Synapses are likely to be the sites at
which neuronal death is initiated, because Aβ can induce apoptotic cascades in synaptic terminals [9]. Studies from different laboratories provided evidence that the so-called intrinsic and extrinsic apoptotic pathways that often involve alterations in mitochondria or ER or signaling through cell membrane death receptors, respectively, are associated with Aβ-mediated apoptotic processes [187, 195, 236, 237]. Studies in mutant βAPP transgenic mice demonstrated elevated caspase-3 activity upon enhanced activation of both intrinsic and extrinsic apoptotic pathways [238]. The involvement of caspase-3 apoptotic cascade in Aβ-induced neuronal loss was recently demonstrated in vivo, as well as in vitro, using mice deficient in caspase-3 [239].

Several mechanisms have been proposed to mediate Aβ-induced neuronal apoptosis. One of the consequences of the Aβ-induced increase in intracellular Ca2+ concentration is the activation of calpains, which were shown to be involved in Aβ-induced apoptosis [240]. Oxidative stress induced by Aβ peptides leads to the activation of the JNK stress-activated protein kinase that mediates the regulation of gene expression and induction of apoptosis [241, 242]. Aβ induces the expression of immediate early genes, which is a prerequisite for induction of apoptosis [243]. The binding of Aβ to the extracellular domain of p75NTR (p75 neurotrophin receptor) induces activation of caspase-3 [244]. In primary cultures of cerebral endothelial cells, Aβ activates an apoptotic cascade involving AP-1 DNA binding, subsequent Bim induction, followed by Smac release and binding to X-linked inhibitor of apoptosis (XIAP) [245]. Aβ stimulation of rat microglia specifically leads to the increase in the chloride ion channel CLIC1 and to the functional expression of CLIC1 chloride conductance, which was shown to be involved in the activation of neuronal apoptosis [246]. Aβ treatment leads to the expression of the proapoptotic Bcl2 family protein DP5, which interacts with Bax initiating the downstream apoptotic cascade [247]. Recent data demonstrate that induction of Dickkopf-1, a negative modulator of the Wnt pathway, is associated with neuronal apoptosis in primary cultures of cortical neurons challenged with Aβ and in degenerating neurons in the brain from AD patients, where it co-localized with NFT and dystrophic neurites [248]. Induction of apoptotic neuronal death upon exposure to Aβ has also been shown to require the action of cell cycle regulators [249], whose expression was documented in populations of vulnerable neurons in the AD brain [250], suggesting that the neurons are stimulated to re-enter the cell cycle, but that this process may be aborted and thus trigger apoptosis. It is possible that PS mutations trigger the abortive re-entry of the neurons into the cell cycle, leading to apoptosis [251]. In addition, PS mutations were shown to render neurons susceptible to apoptosis induced by Aβ by altering Ca2+ regulation [147] and down regulating the anti-apoptotic Bcl-2 protein [252].

Several reports show that intraneuronal Aβ may lead to neuronal apoptosis. First, the presence of intracellular Aβ deposits correlates with apoptotic cell death in brains of AD patients [253]. Second, transgenic mice expressing intraneuronal Aβ42 show substantial evidence for apoptosis [254] and βAPP/PS1 double-transgenic mice exhibit an increased number of apoptotic neurons in hippocampus [67]. Third, overexpression of human βAPP in rat cortical neurons leads to the accumulation of intracellular Aβ42, associated with the appearance of apoptotic nuclei [255] and microinjections of Aβ42 into human primary neurons induced cell death [256]. Fourth, it was shown that Aβ interacts with the proapoptotic serine protease Htra2/Omi [257], which also mediates PS1-induced cell death [258]. Finally, agents that induce apoptosis decrease secreted Aβ levels, but led to an increase in cellular Aβ42 levels in damaged neurons [259].

Activation of apoptosis-related caspases was demonstrated to be involved in the development of the pathological lesions characteristic of AD. Executioner caspases were shown to be involved in the in vivo cleavage of βAPP, leading to the formation of amyloidogenic Aβ peptides [260]. In transgenic mice and AD brains, it was suggested that Aβ accumulation triggers caspase activation, leading to caspase-cleavage of tau, and that this is an early event that may precede NFT formation and cognitive decline in AD [261]. The cleavage of tau by caspase-3 has previously been shown to convert tau into an effector of apoptosis [262].

Taken together, these data support the view that enhanced Aβ formation that occurs as a consequence of genetic predisposition or other physiological factors, leads to activation of endogenous cell death pathways in susceptible neurons, which in turn, produce elevated levels of Aβ peptides, and this vicious cycle culminates in neuronal dysfunction and cognitive impairment.

2.10. Neuroinflammation

The involvement of inflammatory processes in the pathogenesis of AD received strong support from epidemiological studies indicating that chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) significantly reduces the risk of disease [263]. The inflammatory reaction is the first line of protection against injury, but an excessive inflammatory response can be harmful. The neuroinflammatory process involves microglia cells, astrocytes and neurons (to a lesser extent), as well as the complement system [264]. Factors released by injured or lysed neurons, such as cytokines, chemokines, acute phase proteins and complement proteins, may be one of the primary activators of microglia and astrocytes (reviewed in [265]). Given the heterogeneity of neuronal populations within distinct brain regions, it has been proposed that the effects of neurochemical environment on microglia and astrocytes are site-specific, and this could account for variations in the extent of inflammatory reactions in different CNS regions [266].

Microglia, the immune effector cells of brain, are sensitive to minor disturbances in brain homeostasis, and turn into an activated state during stressful and neuropathological conditions. In vivo, microglia activation occurs as a graded response involving a stereotypical pattern of changes that include proliferation and migration to sites of injury and transformation into a macrophage-like phenotype. Microglia activation is accompanied by the expression of a number of cell surface molecules and by the production of neurotoxic factors and/or neurotrophic factors (reviewed in [267]). A large number of reactive microglia are present in AD brains, and it is thought that activated
microglia might contribute to neurodegeneration through the production of high levels of proinflammatory cytokines, reactive oxygen and nitrogen species (ROS and RNS, respectively), proteases and acute phase proteins [268, 269]. Pro-inflammatory cytokines, such as tumor necrosis factor-\( \alpha \), (TNF-\( \alpha \)), interferon-\( \gamma \) (IFN\( \gamma \)) and interleukin (IL)-1\( \beta \) and IL-6, are key regulators of the inflammatory process. These cytokines regulate the pro-inflammatory gene expression in different cell types and, acting alone or in concert with other microglia-derived factors, can exert neurotoxic or neuroprotective effects or no major function [270]. Indeed, the precise role of pro-inflammatory cytokines in the pathogenesis of AD is under discussion [271, 272]. The increased production of superoxide anion (O\(_2^\cdot\)) due to microglial NADPH oxidase activation, and the subsequent formation of ROS derived from O\(_2^\cdot\), contributes to the oxidative damage that occurs in AD brain. Moreover, the upregulation of inducible nitric oxide synthase (iNOS) and the consequent production and secretion of NO and other RNS are likely to contribute to oxidative neuronal damage [268, 269]. Although the role of astrocytes in the inflammatory process associated with neurodegenerative disorders is more difficult to establish, it is known that activated astrocytes produce high levels of pro-inflammatory cytokines, ROS and RNS, similar to and overlapping with those produced by microglia [273].

Senile plaques are known to be associated with activated microglia, as well as with reactive astrocytes. It has been demonstrated that the interaction of microglia with amyloid deposits triggers the phenotypic activation of these cells. A number of pro-inflammatory immune receptors and cell surface proteins are overexpressed in plaque-associated microglia, such as the leukocyte antigen CD45, complement receptors (CR3, CR4 and LFA-1) MHC II surface antigens, and the immunoglobulin receptors Fc\( \gamma \)RI, RII and RIII. The acute phase proteins (amyloid P and C-reactive protein) and the protease inhibitors (\( \alpha \)-1-antichymotrypsin and \( \alpha \)-l-antitrypsin) are also elevated during the inflammatory process and present within the senile plaques in AD brain (reviewed in [265, 268]). In addition, there is a large body of evidence reporting that fibrillar A\( \beta \) peptides induce cytokines (IL-1, IL-6 and TNF-\( \alpha \)) and chemokines (macrophage inflammatory protein-1 and IL-8) synthesis and are released by cultured microglia [274, 275]. These pro-inflammatory factors released by microglia can stimulate astrocytes to produce cytokines, chemokines and acute phase proteins that in turn activate microglia cells. Secretion of cytokines and chemokines by reactive microglia contributes to a feed-forward mechanism of microglia activation. The presence of elevated cytokines levels in the AD brain strongly supports the idea that gliosis (activation and proliferation of microglia and astrocytes) associated to senile plaques contributes to neuronal death (reviewed in [265, 268]).

The presence of complement proteins in AD brain has been well-documented. These proteins are associated with senile plaques, and it is known that A\( \beta \) fibrils can activate the classical complement cascade via Clq (complement protein) binding [264, 276]. The acute phase proteins, amyloid P and C-reactive protein [277], as well as the tau protein [278], can also act as activators of the classical complement pathway. The activation of complement cascade results in the production of opsonizing components, which increase the susceptibility of antigens to phagocytosis. In addition, increases of membrane attack complex (MAC) can compromise the plasma membrane integrity of adjacent cells inducing cell death [277].

The presence of activated microglia surrounding A\( \beta \) deposits supports the postulated role of these cells in the clearance of amyloid deposits [279]. Indeed, it has been reported that A\( \beta \) fibrils trigger a phagocytic response in cultured microglia in a dose- and time-dependent manner [280]. Several studies have tried to identify the cell surface receptor(s) that mediate microglia interaction with A\( \beta \) fibrils. Recently, it was reported that microglia interacts with A\( \beta \) fibrils through a cell surface complex of receptors that include the B-class scavenger receptor CD36, \( \alpha \)_\( \beta \)_1 integrin (integrin-associated protein) and the CD47 [281]. The phagocytosis of aggregated A\( \beta \) by microglia may occur by scavenger receptors of class A and B, whereas the opsonized A\( \beta \) fibrils are phagocytosed by complement receptors and Fc receptors [279, 281]. It was reported that antibodies against A\( \beta \), administrated directly to the brain or formed after active or passive immunization, foment the phagocytic clearance of A\( \beta \) deposits [282]. Estrogen is another substance that can enhance microglial phagocytosis of A\( \beta \) [283]. Moreover, astrocytes activation can also regulate microglial phagocytic activity [285]. In AD brain, A\( \beta \) was found within astrocytes in the form of lysosomal granules, suggesting that these cells may have phagocytic activity [284]. Thus, activated microglia and the reactive astrocytes can act in concert to perpetuate the inflammatory reaction. Since one of the major functions of microglia is the clearance of protein aggregates and cell debris, the non-specific blocking of its functions, even causing disruption of inflammatory process, could aggravate the amyloid plaque formation and consequently the AD progression (reviewed in [285]).

### 3. THERAPEUTICAL STRATEGIES

#### 3.1. Antioxidant Therapies

Evidence from both in vitro and animal studies suggests that treatment with antioxidants may be useful in neurodegenerative disorders (reviewed in [286]). However, only a small number has been formally studied in clinical trials in AD patients.

The AD Cooperative Study [112] indicated that vitamin E (a chain-breaking lipid soluble antioxidant) and selegiline (a selective monoamine oxidase type B inhibitor with antioxidant properties) appeared to be beneficial in patients with moderately severe AD by delaying the time of progression to severe dementia, loss of ability to perform activities of daily living, institutionalization or death. However, it was observed that both treatments given together had no additional benefit over either alone. A recent Cochrane review [292] concluded that after adjusting for differences between patient groups in the AD Cooperative Study, there was insufficient evidence to recommend vitamin E. Recently, a cross-sectional and prospective study indicated that the use of vitamins E and C supplements in combination is associated with reduced prevalence and incidence of AD [293]. In the same line, a study aimed to determine if dietary antioxidants such as vitamins C, E, \( \beta \)-carotene and flavonoids could help to prevent AD by
reducing oxidative stress, demonstrated that, after adjusting for age, sex, alcohol intake, education, smoking status, body mass index, total energy intake, and mental examination score at baseline, a high intake of vitamins C and E was associated with a lower risk of AD [294]. However, Laurin and colleagues [295] observed that midlife intake of β-carotene, flavonoids, and vitamins E and C were not associated with the risk of dementia or its subtypes.

α-Lipoic acid is a co-enzyme for mitochondrial PDH and KGDH. It is a powerful antioxidant and can recycle other antioxidants such as vitamins C, E and glutathione (GSH) [287]. It was reported that old rats supplemented with (R)-α-lipoic acid, showed an improvement of mitochondrial function, decreased oxidative damage, and increased metabolic rate [288]. Accordingly, old rats injected with (R)-α-lipoic acid presented an improvement in GSH redox status of both cerebral and myocardial tissues when compared with control rats [289]. The administration of 600 mg α-lipoic acid/day to nine patients with AD for an average of 337 days, promoted the stabilization of cognitive evaluations [290]. However, it was reported that (R)-α-lipoic acid stimulated deficient brain PDH complex in vascular dementia, but not in AD [291].

Recent years have witnessed a renewed interest in plants as pharmaceuticals. This interest has been focused not only on the discovery of new biologically active molecules by the pharmaceutical industry, but also on the adoption of crude extracts of plants, such as infusions, for self-medication by the general public [357]. The use of plant extracts in dementia therapy varies according to the different cultural traditions. In orthodox Western medicine, contrasting with that in China medicine, pharmacological properties of traditional cognitive- or memory-enhancing plants have not been widely investigated in the context of current models of AD. An exception is *Gingko biloba*, which contains a variety of compounds, including flavonoids and terpenoids that have antioxidant, neuroprotective and cholinergic activities relevant to AD mechanisms (reviewed [358]). Recently, the ability of *Gingko biloba* to antagonize the age-related behavioral impairment and neuropathology exhibited by Tg2576, a transgenic mouse model for AD, was tested [296]. It was observed that transgenic mice treated with *Gingko biloba* exhibited spatial memory retention comparable to wild-type mice. However, there were no differences in soluble Aβ and hippocampal Aβ plaque burden between treated and untreated TG2576 mice. In a randomized controlled trial, EGB 716, an extract of *Gingko biloba*, was examined in 327 patients, 45 years or older, with mild to severe dementia resulting from either AD or vascular dementia [297]. In this study, 150 AD patients were included and half received placebo for 1 year. Patients in the active treatment group had a significantly higher score on the Alzheimer’s Assessment Scale-Cognitive Subscale (ADAS-Cog), a measure of cognitive impairment, and in improved Geriatric Evaluation by Relative’s Rating Instrument (GERRI) score, a measure of daily living and social behavior. The review article by Gertz and Kiefer based on literature about older people with AD or vascular dementia or age-associated memory impairment treated with EGB 761 extract, revealed that this extract has reproducible effects on cognitive functions in AD [359]. Ahlemeyer and Krieglstein tested the neuroprotective and anti-apoptotic ability of the main constituents of the non-flavone fraction (ginkolides A, B, C, J and bilobalide) of EGB 761 extract in focal cerebral ischemia rat models, primary cultures of hippocampal neurons and astrocytes exposed to glutamate and chick embryonic neurons exposed to cyanide, staurosporine and serum deprivation [360]. The authors observed that the constituents of the non-flavone fraction tested possess neuroprotective and anti-apoptotic capacity, with bilobalide being the most potent one. They also observed that only high concentrations (100-500 µM) of ginkgolic acids induce neuronal death, however, these concentrations are much higher than those that appear in the EGB 761 extract. Taking together, these results suggest a neuroprotective effect of EGB 761 that agrees with clinical studies showing the efficacy of an oral treatment in patients with mild and moderate dementia.

### 3.2. Drugs Involved in Neurotransmission Improvement

**Acetylcholine:** In the past decade, treatment for AD has largely involved replacement of neurotransmitters that are known to be lacking in AD, mostly based on the cholinergic hypothesis of AD [203, 298, 299]. To improve cholinergic neurotransmission, different strategies have been investigated including the increase of ACh synthesis and presynaptic release, the stimulation of cholinergic postsynaptic muscarinic and nicotinic receptors, and the reduction of ACh synaptic degradation with cholinesterase inhibitors. However, current data does not support the use of precursors of acetylcholine, presynaptic releasing agents or muscarinic agonists, because of lack of efficacy and unacceptable side effects.

The only Federal Drug Administration (FDA)-approved drugs for symptomatic treatment of AD are the inhibitors of acetylcholinesterase: tacrine, donepezil, rivastigmine and galantamine [340]. These agents do not stop disease progression, but clinical studies have shown that they temporally stabilize cognitive impairment and help to maintain global function, often delaying the need for patient placement in nursing homes by several months [300-302]. The Cochrane Dementia Group published three systematic reviews focused on the efficacy and safety of donepezil, rivastigmine [303, 304] and galantamine [305]. For these meta-analyses, data was extracted from randomized, double-blind, parallel-group trials in which response to the treatment with each of the inhibitors was compared with placebo in patients with mild to moderate AD. Each drug had a similar effect at 6 months on global and cognitive rating scales. These findings were supported by previous randomized, double-blind, placebo-controlled trials reporting benefits in a 1 year trial with donepezil [306] and efficacy of donepezil in the moderate to severe AD stages [307]. A recent multicenter, randomized, double-blind, 24-week, placebo-controlled study that enrolled patients with early-stage AD showed significant treatment benefits of donepezil in this stage of the disease [308]. Furthermore, Grossberg and colleagues reported that rivastigmine treatment had a beneficial effect on cognitive performance for up to 2 years in patients with AD, versus no treatment or placebo treatment in historical-control subjects [309].

Galantamine has also been shown to activate some subtypes of nicotinic ACh receptor-ion channels, apparently
acting as a positive allosteric modulator, enhancing the receptor response to available ACh and increasing the frequency of ion channel opening [310]. This dual action may be responsible for the promising outcomes of numerous phase III clinical trials reported recently [311]. Most patients in these trials had mild to moderate AD, and approximately 20% maintained cognitive function at baseline levels for 36 months, with good tolerance for the drug. Thus, therapies that enhance cholinergic function are likely to remain in the multidrug regimens that will one day have a significant impact on this debilitating disease.

Glutamate: The main excitatory neurotransmitter in the CNS is thought to be involved in the excessive activation of NMDA receptors, with consequent intracellular accumulation of Ca\(^{2+}\), leading to a cascade of events that results in neuronal death. Memantine is a non-competitive, moderate affinity, phencyclidine-site, NMDA antagonist that might protect neurons from glutamate-mediated excitotoxicity without preventing physiological activation of NMDA receptors [361]. This drug was approved in Europe for the treatment of moderately severe to severe AD. In a randomized, double-blind, placebo-controlled parallel study, Winblad and Portits [312] randomly assigned 166 patients with severe dementia to receive either memantine or placebo. Patients treated with memantine presented an improvement in both primary outcome measures (the Clinical Global Impression of Change and the Behavioral Rating Scale for Geriatric Patients) when compared with patients that received placebo. Another randomized, placebo-controlled trial of 252 patients with advanced AD showed that memantine treatment was associated with significantly less deterioration in cognitive and functional measures compared with placebo [313]. Recently, preliminary results from a 6 month, multicenter, randomized controlled trial of memantine combined with donepezil, compared with donepezil and placebo in 400 patients with moderate to severe AD were reported [314]. The memantine-donepezil combination therapy led to improvement from baseline and significant benefit over the donepezil-placebo combination. Li and co-workers [315] reported that memantine inhibits and reverses the PP-2A inhibition-induced abnormal hyperphosphorylation and accumulation of tau in organotypic culture of rat hippocampal slices. Despite these results, more studies are needed on memantine as a monotherapy, in combination therapy in mild-to-moderate AD, and in early stage dementia when it might, on the basis of its pharmacological properties, be most effective.

3.3. Hormonal Replacement Therapy

The sex steroid hormones (estrogens and androgens) have been shown to influence brain differentiation, neuronal plasticity and neurotransmission. Estrogens play a pleiotropic role in many neurodegenerative disorders. Estrogens exert its effects by: i) binding to intracellular receptors, that in turn lead to transcription and translation of proteins and ii) acting on cell membrane receptors, affecting the same secondary messenger systems activated by neurotransmitters and growth factors [316]. It has been shown that estrogens increase NMDA currents, reduce seizure thresholds, enhance long-term potentiation, exert effects on learning and memory and regulate synaptogenesis in the hippocampus (reviewed in [362]). Moreover, they may act as antioxidants by themselves or in synergistic interaction with GSH [317, 318].

It has been shown that post-menopausal women have increased risk to develop AD when compared to men. Several descriptive studies have shown that post-menopausal women who take estrogen have a lower incidence of AD [319, 320]. In addition, neuroimaging studies demonstrated improved cerebral metabolism in women taking estrogen [reviewed in 321]. Although estrogen may have a neuroprotective effect [322], it does not appear to improve cognition or function in patients with AD [323], and the combination of estrogen and progestin may actually increase the risk for dementia and stroke [324, 325]. Due to conflicting data, more studies should be performed to clarify the real role of estrogens in AD pathophysiology.

3.4. γ - and β-Secretase Inhibitors

Identification of the protein responsible for γ-secretase activity, the enzyme that cleaves βAPP within the membrane, has been very challenging. The use of γ-secretase inhibitors has provided insights into the proteolytic activity and suggested that such inhibition might be a useful therapeutic strategy. Some compounds are currently in phase I clinical trials (reviewed in [361]). Characterization of the γ-secretase revealed a similarity with Notch1, a protein required for the transcriptional regulation during development [326]. Deletion of the PS1 gene in mice is lethal in utero, with a phenotype similar to that observed in Notch-1-null mutants [327]. Furthermore, inhibitors of γ-secretase blocked proteolysis of Notch1 by a γ-secretase-like activity designated “S3”, raising significant concerns about the potential in vivo effects of drugs targeting γ-secretase in AD. Recently, a γ-secretase inhibitor was developed that is able to reduce Aβ production without affecting Notch signalling [328]. However, due to the potential deleterious side-effects promoted by γ-secretase inhibitors more studies should be performed in order to elucidate the mechanism of action of these agents.

β-secretase also termed memapsin (membrane aspartyl protease of the statin family), ASP-2 or BACE (β-site βAPP cleaving enzyme), was initially discovered through an expression cloning strategy to identify genes that altered Aβ production. The properties of BACE as a membrane-bound Asp protease have been further characterized, along with discovery of the very similar BACE2 [12]. Although many structures are possible because of non-stringent specificity of β-secretase, little information about β-secretase inhibitors has been published.

Based on substrate specificity information on β-secretase, two peptidomimetic inhibitors were designed: OM99-1 and OM99-2 [365]. Moreover the crystal structure of memapsin 2 in complex with OM99-2 has been presented [366], leading to important structural information. Other peptidomimetic inhibitors were synthesized such as OM00-3 and a cyclohexyl hydroxyethylene isopropanol dipeptide isostere [367]. On the other hand, several non-peptidic compounds with an expected better oral bioavailability and penetration into the CNS but with poor inhibitory activity can be found in the literature [367]. Finally, it has been shown that MG132, a peptide aldehyde known to inhibit γ-secretase, is also active on β-secretase [368].
Recently, it has been shown that Aβ production can be inhibited by means of antibodies against the β-secretase cleavage site of BAPP. These antibodies were found to bind human BAPP overexpressed by CHO cells, and the formed immunocomplex was visualized in the early endosomes. Indeed, the authors showed that blocking of the β-secretase site by these antibodies interferes with BACE activity and inhibits both intracellular and extracellular Aβ formation in these cells [369].

3.5. Amyloid β-Peptide Clearance

In addition to the substantial efforts devoted to inhibition of Aβ generation as a therapeutic strategy, several approaches are being developed to reduce the presence of Aβ in the brain. There are three main strategies aimed to clear Aβ from the brain: Aβ immunization, disruption of Aβ fibrils or aggregates and modulation of the cholesterol-mediated Aβ transport (reviewed in [361]).

Aβ immunization: In 1999, Schenk and co-workers [329] reported a striking reduction of cortical Aβ deposits in transgenic BAPP mice (PDAPP) after vaccination with human fibrillar Aβ42. The investigators reported an almost complete prevention of Aβ deposition in vaccinated 6-week-old mice and a slowing of the progression of AD pathology in older mice. These findings were associated with evidence of serum antibody titres against Aβ42. Moreover, both neuritic dystrophy and astroglialosis were reduced in treated animals suggesting a benefit beyond the reduction of Aβ burden in the brain [329]. Similarly, Weiner and co-workers [330] showed a 50-60% reduction in amyloid burden in the brains of PDAPP mice immunized intranasally with freshly solubilized Aβ40. In addition, it has been shown that passive immunization with monoclonal and polyclonal antibodies against Aβ reduced AD pathology in the brain in a mouse model of the disease [282, 331]. Several authors have described a partial reversal of memory deficits in an animal model of AD, without a clear reduction in amyloid burden in the brain [99, 332]. Recently, it has been hypothesized that soluble Aβ might be involved in cognitive impairment in AD [98, 333], and also that the improvement of memory deficits induced by passive immunization can be related to the binding and sequestration of soluble Aβ [99].

The application of this therapy to humans led to the development of severe side effects [334]. Elan Corporation stopped a phase II clinical trial of their AD vaccine when 17 of 360 patients developed signs and symptoms consistent with meningoccephalitis [334]. During autopsy of one of the trial participants given the Aβ vaccine, leptomeningeal infiltrates primarily around amyloid-laden blood vessels with T lymphocytes were noted [335]. Hock and colleagues [336] have recently published the first aggregate data from this study. They reported that vaccinated patients had serum antibodies that stained Aβ in diffuse deposits and plaques as well as vascular amyloid, but did not react with BAPP or soluble Aβ. Similarly, an increase of microhemorrhages associated with cerebral amyloid angiopathy in a mouse model of passive immunization in AD was shown [337]. Recently, several other immunization protocols have been proposed [338, 339].

Disruption of Aβ fibrils: The possibility of disrupting the process of fibrillogenesis has attracted much attention and is leading to the development of novel strategies to intervene at this step. Small molecules as Congo Red, some antibiotics and antineoplastics such as doxycrubicin, prevent Aβ toxicity, possibly by stabilizing the monomers and preventing oligomerization.

Clioquinol is a hydrophobic chelator of copper and zinc that freely crosses the blood-brain barrier. In 1999, Cherny and collaborators [134] reported that copper and zinc chelators solubilized Aβ plaques from brains of AD patients after death. Furthermore, it has been shown in BAPP transgenic mice that oral administration of clioquinol increased brain levels of soluble Aβ and decreased immunohistochemical amyloid plaque burden. Clioquinol was withdrawn from use as an antibiotic due to induction of a vitamin B12 deficiency, but this agent is now being tested in a phase II clinical trial that includes administration of vitamin B12 supplements [340].

A novel approach to destabilizing existing amyloid deposits consists in a small compound that dimerizes serum amyloid P (SAP) and reduces its concentration in plasma (probably via metabolism in the liver) [341]. SAP is a normal plasma glycoprotein that can bind to all types of amyloid fibrils avoiding their degradation and clearance. Deletion of SAP gene in an animal model of systemic amyloidosis induced a beneficial effect [342]. The data above mentioned raised the possibility that therapeutic removal of a barrier to degradation of Aβ through targeted depletion and deactivation of SAP could destabilize Aβ deposits in vivo. However, more long-term studies should be preformed to elucidate the possible occurrence of side effects.

Modulation of the cholesterol-mediated Aβ transport: The isoform APOE4 is associated with a slight increase of serum cholesterol [363] and might have a role in the deposition of amyloid fibrils and Aβ aggregation [364]. It has been shown that animals fed a cholesterol-rich diet and treated with statins [343] or BM15766 (a compound that inhibits the last step of cholesterol synthesis) [141] presented a reduction of the Aβ burden. Although the exact mechanisms by which cholesterol regulation alters Aβ production are not known, it is apparent that cholesterol can have numerous effects on the BAPP secretases. Cholesterol has been reported to negatively regulate α-secretase, whereas β- and γ-secretase activities are positively regulated by cholesterol [344]. Furthermore, the amount of cholesterol in the membrane appears to directly affect γ-secretase activity. Thus, the net effect of decreasing cholesterol is to increase α-secretase cleavage of BAPP and decrease both β- and γ-secretase cleavage, resulting in a net decrease in Aβ production. Conversely, increasing cholesterol appears to have the opposite effect on these combined activities, resulting in increased Aβ production [344].

Several retrospective epidemiological studies have provided some preliminary clinical evidence that the chronic use of statins was associated with a significant decreased risk of developing dementia [345]. However, the potential beneficial role of statins has recently been challenged by a randomized controlled trial of pravastatin, in which, after 3 years, no significant effect on cognitive function was observed in elderly individuals at risk of vascular disease [346].
3.6. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs could reduce the inflammatory responses in the plaques through cyclo-oxygenase inhibition and direct effects on amyloid processing. In transfected cells and mutant βAPP transgenic mice, some NSAIDs (ibuprofen, indomethacin and sulindac) decreased the production of Aβ42 and led to an increase in Aβ1-38 independently of COX inhibition and Notch signaling pathway, suggesting that NSAIDs exert an effect on γ-secretase activity [347]. Over the past decade, particularly in the past 5 years, several placebo-controlled, randomized trials have been done to investigate the efficacy of various anti-inflammatory approaches to the treatment of AD. In a short-term study, nimesulide had no beneficial effect on cognition in AD, but the drug was well-tolerated for up to 2 years in elderly patients [348]. Trials of naproxen [349] and of the selective COX2 inhibitor, rofecoxib [350], have not found any efficacy in AD symptoms. However, a recent meta-analysis that involved 11 prospective and non-prospective studies showed that NSAID intake was associated with decreased risk of AD [351].

The effects of compounds from every chemical class of NSAIDs on Aβ40 and Aβ42 secretion were recently investigated, using both Neuro-2a cells and rat primary cortical neurons [352]. Among non-selective NSAIDs, flurbiprofen and sulindac sulfide reduced the secretion of both Aβ peptides. Surprisingly, both COX-2 (celecoxib; sc-125) or COX-1 (sc-560) selective inhibitors significantly increased Aβ42, and either of the two did not alter (sc-560; sc-125) or reduce (celecoxib) Aβ40 levels [352]. The levels of βAPP C-terminal fragments and Notch cleavage were not altered by any of the NSAIDs, indicating that these drugs did not change γ-secretase activity [352]. The present findings show that only a few non-selective NSAIDs possess Aβ lowering properties, i.e., the capacity to decrease Aβ production.

3.7. Microtubules Stabilizing Drugs and Kinase Inhibitors

Drugs targeted to prevent neurofibrillary pathology may help to slow progression of cell death. Identification of such agents is still in very early stages, but some efforts are...
focused on agents that might prevent the loss of microtubule (MT) structure and/or decrease abnormal phosphorylation of tau. Identification of the kinases involved in tau phosphorylation is being actively pursued, since such enzymes are potential therapeutic targets. GSK3β and cdk5 are the primary targets for drug discovery efforts due to their association with MT, phosphorylation of tau at AD-relevant epitopes, and involvement in apoptotic cascades in various models [353]. A large number of compounds such as indirubins and paullones have been shown to be potent inhibitors of GSK3β and Cdk5 [340], but their effects on Aβ-induced tau phosphorylation and in vivo toxicity have not yet been reported. The MT-stabilizing drug paclitaxel protects neurons against Aβ toxicity [354]. In addition, in vitro assays showed that MT-stabilizing drugs effectively block Aβ-induced tau phosphorylation by Cdk5 and the calpain-mediated cleavage of p35 to p25 [355, 356].

CONCLUSION

Recent evidence posits that synaptic dysfunction triggered by Aβ species is an early event involved in memory decline in AD. The main pathogenic events that may contribute to synaptic dysfunction in this neurodegenerative disorder including oxidative stress, Ca2+ deregulation, mitochondria and endoplasmic reticulum dysfunction, and impaired cholinergic neurotransmission are depicted in Fig. 1. The activation of apoptotic cell death as a mechanism of neuronal loss in AD, and the prominent role of neuroinflammation are also represented in Fig. 1. Despite the progress made in recent decades in the identification of the molecular mechanisms responsible for impaired synaptic function in AD, no treatment with a strong disease-modifying effect is currently available. The more relevant therapeutical strategies currently used in human studies, namely those involving antioxidants, drugs for neurotransmission improvement, hormonal replacement, γ- and β-secretase inhibitors, Aβ clearance agents (Aβ immunization, disruption of Aβ fibrils, modulation of the cholesterol-mediated Aβ transport), non-steroidal anti-inflammatory drugs (NSAIDs), microtubules stabilizing drugs and kinase inhibitors are represented in Fig. 2. Present knowledge must be expanded to develop more specific, effective and well-tolerated therapies, and more controlled
clinical trials are required to provide conclusive evidence for a protective action of the compounds above discussed. Since AD is a complex multi-faceted disease, it is possible that combined therapies represent the most effective weapon against AD progression and evolution.

REFERENCES


