

# Molecular Pathogenesis of Alzheimer's Disease: An Update

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## Keywords

Alzheimer's disease · Dementia · Amyloid · Neurobiology · Pathogenesis · Review

## Abstract

Dementia is a chronic or progressive syndrome, characterized by impaired cognitive capacity beyond what could be considered a consequence of normal aging. It affects the memory, thinking process, orientation, comprehension, calculation, learning ability, language, and judgment; although awareness is usually unaffected. Alzheimer's disease (AD) is the most common form of dementia; symptoms include memory loss, difficulty solving problems, disorientation in time and space, among others. The disease was first described in 1906 at a conference in Tübingen, Germany by Alois Alzheimer. One hundred and ten years since its first documentation, many aspects of the pathophysiology of AD have been discovered and understood, however gaps of knowledge continue to exist. This literature review summarizes the main underlying neurobiological mechanisms in AD, including the theory with emphasis on amyloid peptide, cholinergic hypothesis, glutamatergic neurotransmission, the role of tau protein, and the involvement of oxidative stress and calcium.

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## Search Strategy and Selection Criteria

A literature review was conducted in January 2016 using PubMed, Ovid, and Science Direct with the following descriptors: Alzheimer's disease (AD), neurobiology, pathology, review. Results obtained ranged between 150 and 2,200 records after the combination of different keywords. Scientific publications between 1996 and 2015 either in English or Spanish, discussing the neuromolecular hypotheses of AD were selected. Papers concerning clinical presentation, diagnostic methods, and treatment were excluded.

Alzheimer's disease (AD) was first described in 1906 at a conference in Tübingen, Germany by Alois Alzheimer [1], as a "peculiar severe disease process of the cerebral cortex." In recent times, AD is considered a chronic or progressive syndrome, characterized by impaired cognitive capacity beyond what could be considered a consequence of normal aging; that affects the memory, thinking, orientation, comprehension, learning, language, and judgment. More than one hundred years have passed since the first pathophysiological aspects of AD were described. Neurobiological mechanisms underlying AD have been a key element in the understanding of the pathology, currently the most important alterations identi-

fied can be explained through: the amyloid peptide theory, the cholinergic hypothesis that includes glutamatergic neurotransmission alterations, the role of tau protein, and the involvement of oxidative stress (OS) and calcium.

### The Cholinergic Hypothesis

At a molecular level, the cholinergic hypothesis is the first and most studied approach that describes AD pathophysiology. It was defined more than 30 years ago as a primary degenerative process capable of selectively damaging groups of cholinergic neurons in the hippocampus, frontal cortex, amygdala, nucleus basalis, and medial septum, regions and structures that serve important functional roles in conscious awareness, attention, learning, memory, and other mnemonic processes [2]. This selective alteration generates a downregulation of cholinergic markers such as acetyltransferase and acetylcholinesterase that associate with the onset of cognitive impairment [3, 4]; through the existence of proportionality between the decrease in cholinergic markers, the density of neurofibrillary alterations, and the severity of the pathology.

The main findings supporting this premise are the fact that non-selective muscarinic antagonists such as scopolamine-induced cognitive impairment, favor the production of beta-amyloid peptide and decrease the activity of  $\alpha$ -secretase [5]. Some triterpenoid saponins have shown to reduce scopolamine-induced amnesia [6–8] and non-selective and selective muscarinic agonists have shown to improve learning and memory. Selective M1 muscarinic agonists are a pivotal target that link major hallmarks of AD. The exact molecular mechanisms of the effect of cholinergic drugs in learning and memory, and their clinical treatment viability are still being studied [9–12].

This hypothesis was reinforced through immunohistochemical, neuroimaging, and other analyses that revealed: a decrease in the number and density of nicotinic receptors in AD patients (mainly  $\alpha 4\beta 2$  subtype), a reduced expression of  $\alpha 3$ ,  $\alpha 4$ , and  $\alpha 7$  subunits at cortex and hippocampus, and a decline in the binding ability of  $\alpha 7$  hippocampal and  $\alpha 4$  cortical receptors [13–15].

The main alterations in cholinergic neurons considered in this hypothesis are: choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, dysfunctional neurotrophin support, and deficits in axonal transport [16–18]. Recent studies have shown that amyloid  $\beta$  interacts with cholinergic receptors affecting their function [19].

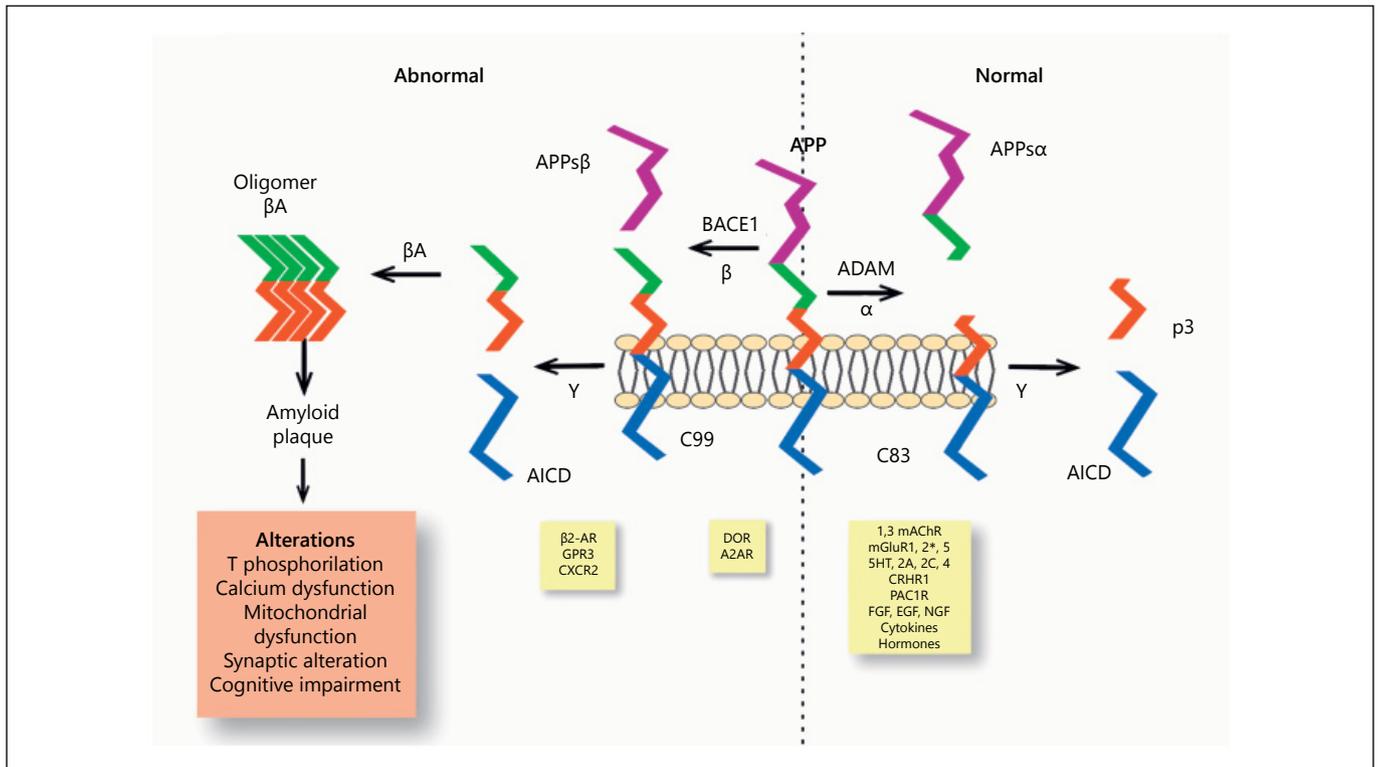
Because the cholinergic and glutamatergic systems significantly interact during neurotransmission alterations in the glutamatergic, signaling has been associated with cholinergic disruptions found in AD [20], an aspect that enhances cholinergic hypothesis. This postulate dictates that acetylcholine and its receptors, especially ( $\alpha 7$ )<sub>5</sub> are considered as neuroprotective by modulating glutamate-mediated neuronal excitability [21, 22]. In AD abnormalities in glutamatergic, neurotransmission is initially observed at the entorhinal cortex (EC), which is followed by further neurotransmission defects in the hippocampus, amygdala, frontal cortex, and parietal cortex [23].

The physiological glutamatergic neurotransmission in the hippocampus produces a cytosolic calcium signal, which mediates synaptic plasticity phenomena such as long-term potentiation (LTP), encouraging learning and memory consolidation [24]. However, a sustained increase in calcium, sodium, and chloride ions as a result of the hyperactivation of NMDA glutamate receptors has been associated with excessive depolarization of the postsynaptic membrane, onset neurodegenerative processes and cell death [25–27]. Likewise, an increase in intraneuronal calcium as a consequence of a dysfunctional glutamatergic neurotransmission can generate a long-lasting depression in the cerebellum (LTD), with calcium overload in mitochondria, activation of nitric oxide synthesis, generation of free radicals, OS, initiation apoptosis and neuronal death [28–31].

Experimentally incubating neurons with glutamate promotes the deposit of filaments similar to neurofibrillary tangles observed in AD. Also, the exposure of neuronal cultures to amyloid  $\beta$  promotes glutamate-induced neurotoxicity and regulates the expression of NMDA receptors on the membrane [32, 33].

At the synaptic level, the lack of enzymes responsible for the degradation of glutamate causes neuronal transporters at the neuronal and glial levels to be responsible for the reuptake of the excess of neurotransmitter [34]. In AD, the inhibition of presynaptic and glial glutamate transport [35], the reduced activity of glutamine synthetase (converts glutamate to glutamine) [36], the discrete depolarization of neurons, and stimulation of nitric oxide production by amyloid  $\beta$  [23] favor the prolonged presence of extra neuronal glutamate, and hence the continued receptor stimulation and excitotoxicity [16, 34].

The cholinergic hypothesis has served as a basis for the majority of treatment strategies and drug development approaches (acetylcholinesterase inhibitors, cholinergic precursors, cholinergic receptor agonists, allosteric cho-



**Fig. 1.** Amyloidogenic and non-amyloidogenic processing of the APP. The top part of the figure shows the 2 main paths in the processing of APP, the most important elements involved, and the main alterations associated with the amyloidogenic pathway of APP. The bottom part of the figure shows potential modulators of the secretase activity (activation of receptors, growth factors, cytokines, hormones).  $\alpha$ ,  $\alpha$ -secretase activity;  $\beta$ ,  $\beta$ -secretase activity;  $\gamma$ ,  $\gamma$ -secretase activity; BACE1,  $\beta$ -site – APP – cleaving enzyme 1;  $\beta$ A, amyloid  $\beta$  peptide; ADAM,  $\alpha$ -secretase; APPs $\alpha$  and APPs $\beta$ , soluble portions produced by the effect of  $\alpha$ - and  $\beta$ -secretase; C83, fragment of 83 amino acids of the carboxyl terminal portion produced by the effect of  $\alpha$ -secretase; C99, frag-

ment of 99 amino acids of the carboxyl terminal portion produced by effect of  $\beta$ -secretase; p3, peptide resulting from  $\gamma$ -secretase; AICD, carboxy-terminal fragment referred to as the intracellular domain of APP; T, Tau protein; mAChRs, muscarinic acetylcholine receptors; mGluR, metabotropic glutamate receptors; 5HT, serotonin receptors; CRHR1, receptor 1 of the corticotropin-releasing hormone; PAC1R, receptor 1 of pituitary adenylate cyclase; FGF, EGF, NGF, growth factors of fibroblasts, epidermis and the nervous system respectively; DOR, opioid receptor  $\delta$ ; A2AR, adenosine receptor 2A;  $\beta$ 2-AR,  $\beta$ 2 adrenergic receptor; GPR3, receptor 3 coupled to protein G; CXCR2, chemokine receptor 2.

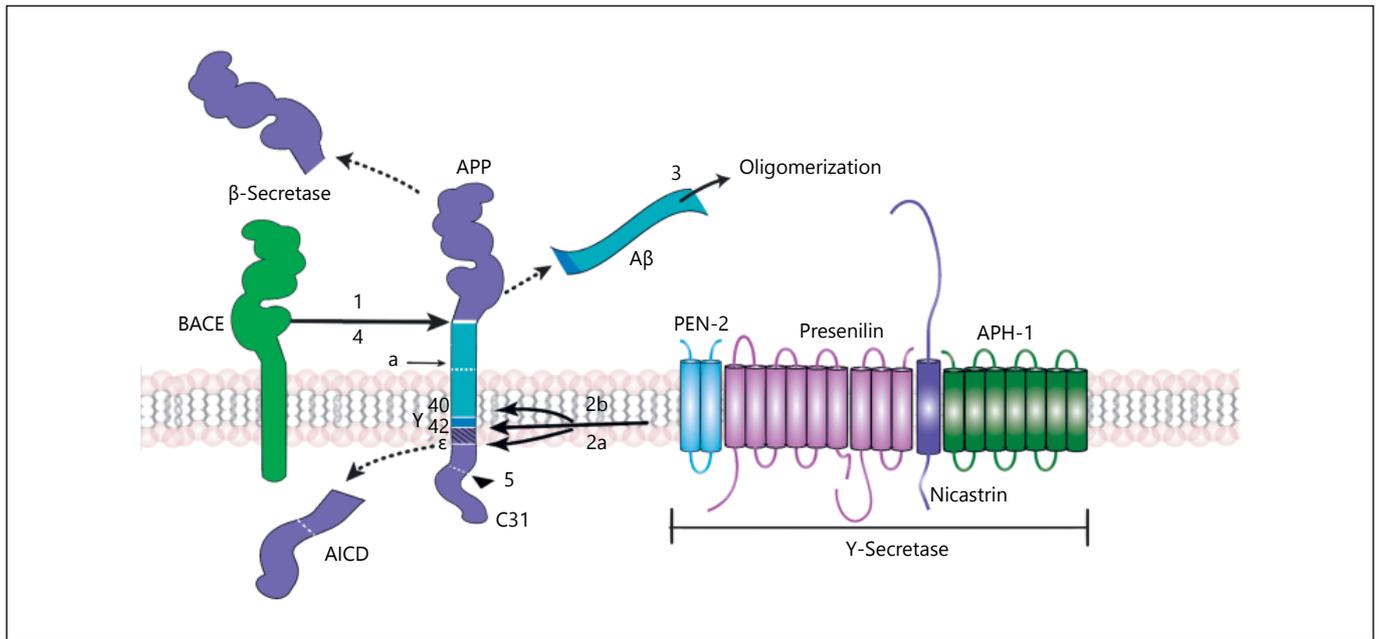
linergic receptor potentiators, NMDA receptor blockers) for AD. Currently there is consensus that the observed relationship between cognitive impairment and decreased cholinergic transmission in the brain plays an important role in AD but by itself does not establish definitive causation of the disease [37, 38].

### The Amyloid Hypothesis

In the amyloid cascade hypothesis of AD, the disease is analyzed as a series of abnormalities in the process and secretion of the amyloid precursor protein (APP), where an inequality between production and clearance of amy-

loid  $\beta$  is the triggering event and the most important factor; responsible for other abnormalities observed in AD [39]. Amyloid  $\beta$  is a peptide with high resistance to proteolytic degradation. It consists of 37–43 amino acids, in which the isoforms 1–40 and 1–42 are the most common [40]. The 1–42 amyloid peptide isoform is the most hydrophobic and is considered to have the greatest toxicity [23]. Because of its physical characteristics, it often acquires the configuration of a  $\beta$ -pleated sheet [41], showing a greater tendency to aggregate and form the core of the amyloid plaque [42–44]. It is the main component of amyloid neuritic plaques [43, 45].

The processing of APP at the plasma membrane constitutes the origin of the amyloid peptide (Fig. 1). APP is



**Fig. 2.** Production of amyloid  $\beta$  peptide from sequential proteolytic breaks of the APP. The figure shows the sequence of phases in the processing of APP in the formation of amyloid  $\beta$  peptide and its oligomerization. Numbers (1, 2a, 2b, 3, 4, and 5) refer to intervention sites where the production of amyloid  $\beta$  peptide could be modulated

with possible therapeutic utility.  $\alpha$ ,  $\alpha$ -secretase cut site;  $\gamma$ ,  $\gamma$ -secretase cut site;  $\epsilon$ , cut site  $\epsilon$  of  $\gamma$ -secretase; BACE,  $\beta$ -secretase;  $A\beta$ , amyloid  $\beta$  peptide; AICD, carboxy-terminal fragment referred to as APP intracellular domain.

a transmembrane glycoprotein of type I and its specific function is unclear; however it is known that its expression is increased during cellular stress phenomena. APP processing is performed by a series of ruptures, which involves an initial breakdown by enzymes with  $\alpha$ -secretase activity, mainly enzymes belonging to the disintegrin and metalloprotease (ADAM) family including: ADAM10, ADAM17, and ADAM19 [45, 46]. ADAM activity can be modulated by different situations such as receptor activation, growth factors, cytokines, and hormones. The excision produced by  $\alpha$ -secretase leads to the formation and release of a peptide of the amino terminal portion called APPs  $\alpha$ , which is soluble under certain conditions [47] and a fragment of the carboxyl terminal portion (C83) [48]. The APPs  $\alpha$  is found in lower quantities in AD patients [49] and has been associated with trophic and neuroprotective functions [50].

In patients with AD, the first APP rupture at an extracellular level, generates a shorter soluble amino terminal portion (APPs  $\beta$ ) and a longer terminal carboxyl fragment (C99) [51, 52]. This split is performed mainly by BACE1 ( $\beta$ -site - APP - cleaving enzyme), an ubiquitous transmembrane protease with  $\beta$ -secretase activity. BACE1 expression can be modulated by frequently seen situations

in neurodegenerative diseases and aging such as OS, ischemia, inflammation, hypoxia, and trauma [51, 53–55].

After  $\gamma$ -secretase, consisting of a heteromeric complex of 4 subunits called: presenilins (PSEN1 and PSEN2), nicastrin (NCSTN), APh-1, from the acronym anterior pharynx defensive phenotype 1 (APh-1a and APh-1b) and PEN - 2 (PS-enhancer-2) [56] produces a cut in the  $\gamma$  site releasing the carboxyl terminal fragment named APP intracellular domain (AICD) and producing the amyloid  $\beta$  peptide, which is secreted, aggregated, and accumulated in extracellular plaques due to its low solubility (Fig. 2) [57]. It has been observed that APh-1 inhibits the production of amyloid  $\beta$  peptide while the PEN-2 favors it [58, 59].

Several studies have shown the *in vitro* and *in vivo* neurotoxicities of various forms of amyloid  $\beta$  [60]; however, the exact mechanisms are quite complex and not fully understood [37, 52]. So far the relation between the specific site of action of amyloid  $\beta$  and its structural diversity is unknown; what is clear is that the amino acid sequence that is contained between positions 25 and 35 of the primary structure has greater neurotoxicity.

Concentrations of amyloid  $\beta$  peptide are determined by the balance between generation and clearance; in AD

patients, a clearance abnormality leads to the accumulation of amyloid  $\beta$  in the brain [61]. The transport of amyloid  $\beta$  through the blood-brain barrier is mediated by receptors, the passage of the peptide from the brain to the blood occurs by interaction with LRP-1 receptors (Lipoprotein receptor-related protein-1) and the action of p-glycoproteins. Increased levels of amyloid  $\beta$  and aging, decrease of the expression of LRP-1 receptors results in an accumulation of amyloid peptide in the central nervous system (CNS).

The transit of amyloid  $\beta$  peptide into the brain through the blood-brain barrier [62] also occurs via receptors, primarily through multi-ligand AGE (advanced glycation end products) receptors or RAGE. RAGE expression is determined by the concentration of its own ligands. In contrast to the expression decrease of LRP-1s due to high levels of amyloid  $\beta$  in the brain, RAGE increases the expression under these conditions.

Since RAGE interaction with amyloid  $\beta$  causes: inflammatory responses at the endothelium level, endothelial cell apoptosis, decreased cerebral blood flow, and suppression of LTP; RAGE may play a role in the development of neurovascular changes observed in AD [62–65].

Proteolytic degradation of amyloid  $\beta$  peptide is carried out mainly by neprilysin (NEP) and the insulin-degrading enzyme (IDE). During aging and in AD patients, the expression of NEP and IDE decreases, causing an increase in the concentration of amyloid  $\beta$  peptide in the brain [61, 66]. In patients with AD, decreased quantity and activity of NEP is seen especially in the cortex and hippocampus, but not in other regions of the brain.

The secretion of IDE in the brain is regulated by the microglia, and similar to NEP its distribution and concentration also presents differences in AD patients. Lower concentrations have been identified in the cortex and hippocampus, where the predominant form has a higher degree of oxidation [9].

The endosomal lysosomal pathway is also an important regulator of the processing of APP [67, 68] and of the tau protein metabolism [69]. Because of the importance of this pathway for cellular maintenance and its role in the immune system, recent studies suggest that dysfunctions in neuronal autophagy causing an increase in the amount and size of endosomes at the cellular level, may be involved in the pathogenesis of AD [70–74], as these changes are seen before the appearance of senile plaques and neurofibrillary tangles in the brain [72, 74]. Other studies report the absence of a di-

rect relationship between quantity and size of lysosomes and AD [75].

The amyloid cascade hypothesis has the highest acceptance rate; however, current studies support the idea that not only amyloid  $\beta$  but other fragments from the processing of APP, such as C83 or AICD, contribute to the pathogenesis of AD resulting in difficulty to attribute the pathological features of the disease exclusively to the amyloid peptide [60].

### The Tau ( $\tau$ ) Protein

The amyloid cascade hypothesis consists of the production and accumulation of amyloid  $\beta$  peptide as the beginning of the disease process; however, it does not completely explain the etiopathogenesis of AD. In this hypothesis, the  $\tau$  protein arises as a secondary pathogenic event that subsequently causes neurodegeneration [76, 77]. In vitro experiments using various cell types, ranging from neuronal cell lines to primary hippocampal and cortical neurons and hippocampal organotypic cultures, have demonstrated that amyloid  $\beta$  induces  $\tau$ -alterations [78–80]. These include mainly an increased phosphorylation and cytoplasmic and dendritic translocation often linked to neurodegeneration [81].

The  $\tau$  protein is a highly soluble protein that relates to the microtubules and its function under normal conditions consists of stabilizing them. These microtubules provide support for structural changes, axonal transport, and neuronal growth [69, 82, 83]. In the CNS, the  $\tau$  protein presents itself in 6 different isoforms that vary in the number of binding sites for microtubules and the amount of exons they possess [82]. In AD, dysfunctions occur in phosphorylation processes of  $\tau$  protein, resulting in a hyperphosphorylation of the molecule.

Hyperphosphorylated protein  $\tau$  presents aberrant aggregation with the cytoskeletal proteins; it shows a lower grade of interaction with microtubules which favors an increase of free tau protein that leads to greater aggregation and fibrillization of itself, with the consequent malfunction of axonal transport [84, 85].

Scientific literature reports changes in  $\tau$  protein and amyloid  $\beta$  oligomers as the most important factors responsible for neuronal dysfunction in the pathogenesis of AD [46, 86]. Likewise neurofibrillary tangles observed initially in the EC and hippocampus subsequently extend to the amygdala and cortical areas (temporal, frontal, and parietal) [57, 85].

## Contribution of OS in the Pathogenesis of AD

It has been long recognized that one of the common characteristics in neurodegenerative diseases is the relationship between OS and neuronal apoptosis [87]. OS is a condition in which the balance between production of active reactive oxygen species (ROS) and the level of antioxidants is significantly disturbed, resulting in cell damage. ROS chemically interact with biological molecules such as nucleic acids, proteins and lipids, and cell organelles [88].

In addition to the established pathology of senile plaques and neurofibrillary tangles, the presence of extensive OS is a characteristic of AD brains. The accumulation of free radical damage, alterations in the activities or expression of antioxidant enzymes such as superoxide dismutase and catalase are also present in AD patients [89]. Although OS is an important factor in AD pathogenesis, the mechanisms by which the redox balance is altered and the sources of free radicals are not exactly known. It has been demonstrated that abnormal accumulation of amyloid  $\beta$  is capable of promoting the formation of ROS through a mechanism that involves the activation of NMDA receptors [90], and that OS may augment amyloid  $\beta$  production and aggregation as well facilitate tau phosphorylation and polymerization, forming a vicious cycle that promotes the initiation and progression of AD [91].

Neuronal mitochondria (essential for cellular metabolism) show metabolic abnormalities in AD models. It has been demonstrated that mitochondria are quite vulnerable to OS, which may directly disrupt its functions (energy production, decrease of antioxidant enzymes, and loss of membrane potential), generating a further increase in ROS levels that finally produce cell death by caspase activation and apoptosis [92–94].

## Calcium Homeostasis in AD

Calcium, an ubiquitous intracellular messenger, regulates multiple physiological functions, generating concentration gradients and binding to several proteins, receptors, and ion channels. The regulation of intracellular calcium homeostasis is a very complex mechanism that is vital for several cellular pathways and is thus involved in cell survival and death. Two organelles play a major role in calcium homeostasis, the endoplasmic reticulum (ER) and mitochondria, whereas ATPase calcium pump and

the sodium-calcium exchanger are the 2 main systems involved in calcium efflux through the plasma membrane [95, 96].  $\text{Ca}^{2+}$  is continuously exchanged between the cytosol and the lumen of the ER. Overload of intracellular calcium due to a blockage or dysfunction of the transport system leads to the cleavage of several proteins and other substrates, OS, perturbs energy production [97], stimulates protein production (amyloid  $\beta$  and  $\tau$  protein) [98], and induces cell death through necrosis and/or apoptosis [99, 100].

In AD, the ability of neurons to regulate the influx, efflux, and subcellular compartmentalization of calcium is compromised [101]. These disruptions involve several mechanisms, such as alterations of calcium buffering capacities, deregulation of calcium channel activities, excitotoxicity or disruption of mitochondrial functions. Alterations resulting from calcium disruption are the result of age-related OS, metabolic impairment in combination with disease-related accumulation of A $\beta$  oligomers and the presence of mutations of genes that encode presenilin [102]. Particularly, A $\beta$  may promote cellular calcium overload by inducing membrane-associated OS and forming pores in the membrane [103–105].

## Conclusion

During the last decades, advances in cellular biology have been essential for understanding the molecular mechanisms underlying AD. Currently it is known that the molecular pathogenesis of AD is complex and involves several theories or hypothesis where many diverse factors interrelate. However, none of these postulates alone is able to clarify the entire aspect regarding the pathology, and further studies are required. Aspects like the initial causes of the disease such as the abnormal formation of amyloid  $\beta$ , and the mechanisms by which it affects neurons and nicotinic acetylcholine receptors and the relation between the disruption of cholinergic pathways and the cognitive deficits of AD are still not fully understood.

New discoveries that contribute to the elucidation of the molecular pathogenesis of AD and its relations are crucial because they can allow the development of new therapeutic strategies for the treatment of a condition where current drug therapy lacks the ability to prevent its occurrence and progression.

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