Insulin Resistance as a Common Link Between Current Alzheimer’s Disease Hypotheses

Alves, Suélen Santos; Silva-Junior, Rui Milton Patrício da; Servilha-Menezes, Gabriel; Homolak, Jan; Šalković-Petrišić, Melita; Garcia-Cairasco, Norberto

The scientific path towards Alzheimer's disease understanding: insulin resistance as a common link between current hypotheses

Suélen Santos Alves[1]; Rui Milton Patrício da Silva Junior[2,4]; Gabriel Servilha-Menezes[4]; Jan Homolak[3]; Melita Šalković-Petrišić[3] and Norberto Garcia-Cairasco[1,4]

[1]Department of Neurosciences and Behavioral Sciences, Ribeirão Preto Medical School - University of São Paulo (FMRP-USP).

[2]Department of Internal Medicine, Ribeirão Preto Medical School – University of São Paulo (FMRP-USP).

[3]Department of Pharmacology & Croatian Institute for Brain Research. University of Zagreb School of Medicine, Zagreb, Croatia.

[4]Department of Physiology, Ribeirão Preto Medical School – University of São Paulo (FMRP-USP).

Abstract

Almost 115 years ago, Alois Alzheimer, a German psychiatrist, described Alzheimer's disease (AD) for the first time, in Tübingen, Germany. Since then, many hypotheses have been proposed. However, AD remains an enigmatic disease and a severe health public problem. The current medical approaches for AD are limited to symptomatic interventions and the complexity of this disease has led to a failure rate of approximately 99.6% in AD clinical trials. In fact, no new drug has been approved for AD treatment since 2003. These failures indicate that, because we still do not fully understand the pathophysiology of AD, we are failing in mimicking this disease in experimental models, or, at least, its sporadic form. Although most studies have focused on the amyloid cascade hypothesis of AD, the literature has made clear that AD is rather a multifactorial disorder. Therefore, the persistence in a single theory has resulted in lost opportunities, since numerous alternative hypotheses have been proposed all over the years and

did not receive equal attention, for example to those based upon the presence/detection of the triad: amyloid-β peptide, hyperphosphorylated Tau protein and neurodegeneration. In this review, we aim to present the striking points of the long scientific path followed since the description of the first AD case and the main AD hypotheses discussed over the last decades. We also highlight a rather new one, the "type 3 diabetes" hypothesis, which has presented consistent findings and proposed insulin resistance as a common link between many other hypotheses.

Running title: Alzheimer's disease and brain insulin resistance

Correspondence: Norberto Garcia-Cairasco, PhD

Department of Physiology, Ribeirão Preto Medical School – University of São Paulo. Av. Dos Bandeirantes 3900 – 14049-900 – Ribeirão Preto – SP, Brazil.

E-mail address: ngcairas@usp.

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INTRODUCTION

Since the first description of Alzheimer's disease (AD) in 1906 [1], researchers have coursed a long scientific path seeking for a better understanding of this neurological disorder. Many hypotheses have been proposed over the last decades [2,3],however, AD has remained an enigmatic and complex disease with etiopathogenetic mechanisms yet to be elucidated. Currently, besides neurodegeneration, AD is mainly characterized by the accumulation of the amyloid-β peptide (Aβ), which tends to aggregate and form Aβ plaques, and by presence of tangles, caused by accumulation of hyperphosphorylated forms of Tau protein [4].

Worldwide, there are approximately 50 million people living with AD or other dementias [5]. AD is a progressive neurodegenerative condition and the most frequent type of dementia, corresponding to 60-80% of the cases [6]. In the United States, it is estimated that one in 10 people age 65 and older has AD, a total number of 5.8 million Americans [6,7]. Furthermore, epidemiological data suggests an increasing trend in prevalence with estimations being 40 million patients suffering from AD in 2016 [8] and 131 million in year 2050 [9]. Due to its complexity, AD is usually divided into familial AD (fAD) and sporadic AD (sAD). fAD accounts for approximately 1-5% of all cases and it is usually caused by autosomal mutations in the amyloid-β precursor protein (AβPP), presenilin 1 (PS1), and/or presenilin 2 (PS2) . Conversely, sAD, responsible for a majority of the cases (approximately 95-99%), does not present a well-defined etiology. It is believed that an interplay of genetic, environmental, behavioral and metabolic factors might be responsible for the development of the sporadic form of this disorder [10].

Nowadays, more than one century after the discovery of this disorder [11], AD is still a chronic condition with no cure or effective interventions to delay its progression [12]. The current medical approaches for AD are limited to symptomatic interventions and the complexity of the disease has led us to constant failures in clinical trials [13]. The pharmacology of AD is

currently limited to cholinesterase inhibitors (rivastigmine, galantamine and donepezil) and memantine, which is an N-Methyl-D-aspartate (NMDA) receptor antagonist. These treatments are not able to prevent or reverse the progression of AD and are often accompanied by many adverse effects. In fact, no new drug has been approved for AD treatment since 2003 [14].

Some authors believe that AD drugs have failed mainly because of an inadequate target, since the majority of studies have focused on the drugs targeting amyloid [15,16], however other factors such as the stage of the disease at the moment of therapy initiation and the heterogeneity of factors implicated in AD pathophysiology should also be considered [17,18]. Consequently, the multifactorial hypothesis of AD has been proposed by some to ponder divergent thinking and investigate multiple and diverse etiological factors that might be converging in a common brain pathology [19]. In this review, we aim to present the striking of the long scientific path since the first description of an AD case and the main AD hypotheses proposed over the last decades. Additionally, the "type 3 diabetes" hypothesis is discussed as accumulated evidence points towards insulin as an important factor implicated in etiopathogenesis of AD, and dysfunctional insulin signaling in the brain provides a common link between other proposed hypotheses.

THE PIONEERING DESCRIPTION OF ALZHEIMER'S DISEASE

Alois Alzheimer, a German psychiatrist, described an AD case for the first time on November 4th of 1906, at the 37th annual conference of German psychiatrists, in Tübingen, Germany [20]. In his lecture, Alzheimer reported a case study describing a "peculiar severe disease process of the cerebral cortex". Although Alzheimer's presentation did not arouse the interest of the numerous scientists present in the audience, the case was published one year after the conference [1]. Alzheimer's talk was based on the case of a 51-year-old woman (August D.) from Frankfurt who had presented with psychiatric symptoms of progressive cognitive impairment, aggression, and hallucinations, with subsequent autopsy revealing atherosclerotic

changes of the larger brain vessels, and specific neurofibrillary changes unknown at the time [21].

Briefly, on November 26th, 1901, a man took his wife to the Community Psychiatric Hospital at Frankfurt am Main after observing substantive changes in her personality and behavior. The first behavioral changes presented by August D. were characterized by bouts of excessive jealousy toward her husband which evolved into significant memory impairment, delusions and psychosocial incompetence [1,21]. At that time, Alzheimer was an assistant physician, and with the consent of Emil Sioli, the Director of the Frankfurt Hospital, he decided to examine and interview August D. However, in 1903, Alzheimer moved to Heidelberg to work with Emil Kraepelin, one of the main psychiatrists at that time, and shortly afterwards, they both moved to Munich, where Alzheimer supervised the completion of a new University Hospital for Psychiatry and assumed the control of a modern histopathological laboratory, where he continued his histopathological research with important scientists from all over the world, including Friedrich H. Lewy, the famous neurologist who discovered the Lewy bodies [22].

August D.'s case worsened, and she died on April 8th 1906. Following her death, Emil Sioli informed Alzheimer, who investigated the histological sections of August D.'s brain. Alzheimer analyzed the histological sections stained with Bielschowsky's silver stain and described the main hallmarks of AD for the first time: cell death, Aβ plaques and neurofibrillary tangles [23]. Alzheimer and Gaetano Perusini, an Italian physician specialized in dementia, kept working on other cases, and in 1909, Perusini published three more cases similar to that of Auguste D [24].

In 1910, Kraepelin included Auguste D.'s case in the 8th edition of his textbook *Psychiatrie* and proposed the term Alzheimer's disease for the first time [20]. In 1911, Alzheimer published again [25]. In this paper, he reported the case of the male patient Johann

F. who died in Munich in 1910 after being hospitalized for three years and examined by Alzheimer and Kraepelin. Johann F's case had already been mentioned by Kraepelin in his textbook, even before death, which suggest that Johann F was probably the first patient to be clinically diagnosed with AD [22]. His case presented many similarities to August D.'s case, however, Alzheimer did not identify neurofibrillary tangles in his brain slices, only Aβ plaques [23].

In 1998, Möller and Graeber re-examined the original histological brain slides of August D. and Johann F. with more advanced techniques [26]. They concluded that the differences observed in these two cases could be attributed to different stages of the same disease. Therefore, Alzheimer had not only reported the first case of AD, but had also described one important stage in the physiopathology of this disorder [22]. These findings laid the foundations for the most traditional and accepted theory of AD, the amyloid cascade hypothesis [27]. Nevertheless, the original debate on whether the amyloid was the cause or the consequence of the disease actually dates back to the time of Alois Alzheimer [28].

THE AMYLOID CASCADE HYPOTHESIS OF ALZHEIMER'S DISEASE

The Aβ, a peptide derived from a larger protein known as AβPP, was isolated in 1984, by Glenner and Wong at the University of California, in San Diego [29]. The mechanism responsible for the cleavage of AβPP and the production of Aβ is now well known [30]. AβPP is first cleaved off by the enzyme β-secretase (BACE 1), giving rise to two fragments: sAPP-β (N-terminal fragment), being released in the extracellular space and C-terminal fragment β (CTFβ,CT99 or CT89), which remain bound to the cell membrane. Then, the γ-secretase complex (Nicastrin, Anterior Pharynx defective 1, Presenilin enhancer 2, Presenilin 1 and or Presenilin 2) cleaves the remaining membrane-bound portion of the protein releasing the extracellular fragment Aβ (Fig. 1). Due to heterogeneous γ-secretase cleavage, γ-secretase can

cut AβPP at different sites, producing a 37 to 49 amino acid residue peptide. Therefore, Aβ can vary in length [31]. On the other hand, in the non-amyloidogenic pathway, AβPP is firstly cleaved by α-secretase within Aβ sequence, producing soluble α-APP fragments (sAPPα) and C-terminal fragment α (CTFα, CT83), and, posteriorly, CTFα is cut by γ-secretase, releasing non-toxic fragments [32].

In 1991, a group led by John Hardy demonstrated that mutations in the gene AβPP could cause a development of AD [33]. Subsequently, in 1996, mutations in PS1 and PS2, two genes that code for proteins from the γ-secretase complex, were found to be implicated in fAD [34]. These autosomal dominant mutations result in increased production and longer variants of Aβ associated with aggregation and formation of oligomers. Further agglomeration promotes the formation of insoluble fibrils, which tend to deposit in plaques. Although both types of aggregates are involved in the pathogenesis of AD [35], soluble oligomers are considered to be more toxic [36,37].

The gene encoding AβPP was found to be located on the chromosome 21 [38,39], individuals with trisomy 21 known as Down's syndrome, seem to be at an increased risk of developing AD, due to an extra copy of the gene and consequent overexpression of AβPP [40]. These factors laid the groundworks for amyloid cascade hypothesis, proposed by Hardy and Higgins in 1992 [27]. According to this theory, an acute noxious stimulus, such as head trauma, triggers the pathophysiological cascade that induces disturbances in AβPP metabolism by altering production, clearance and deposition of Aβ. The Aβ protein, in turn, leads to intracellular calcium (Ca^{2+}) dysregulation, inducing neurofibrillary tangle formation and cell death [41]. Consequently, Aβ has been implied as a triggering factor in both forms of the disease: fAD and sAD.

On the other hand, it has already been demonstrated that Aβ plaques deposition can be present in elderly individuals without cognitive impairment [42–50] and it is still a matter of

debate whether or not this reflects a predisposition or a preclinical state of AD [51]. Additionally, elevated Aβ and the presence of plaques in individuals with Down syndrome do not always lead to the development of dementia [52]. Furthermore, the severity of dementia in humans is not proportional to quantity of Aβ plaques, but it is in positive correlation with the formation of neurofibrillary tangles in the neocortex, which can occur even when no plaques are present [53,54]. In fact, it has already been demonstrated that the removal of Aβ plaques from the brain does not prevent AD progression and the propagation of Tau pathology [55].

Another information casting doubt on the amyloid hypothesis is the fact that, although several pre-clinical studies using transgenic mice overexpressing human mutant AβPP/Aβ have been successful, the failure rate in AD clinical trials is approximately 99.6% [56,57]. These failures indicate that, because we still do not fully understand the pathophysiology of AD, we are failing in mimicking this disease in experimental models, or, at least, its sporadic form. Besides the fact that genetic mutations have not been sufficient to mimic sAD [58], the non–deterministic genes related to the development of the sporadic form of AD are related to lipid and glucose metabolism and not to Aβ production [59].

In this context, despite promising results in experimental studies with animal models, many anti-amyloid drugs have failed over the years [12,60]. In the enzyme inhibitors group, γ-secretase inhibitors, such as Semagacestat from Eli Lilly, failed mainly because of the numerous other cellular substrates of γ-secretase, which ended up worsening the cognitive impairment and increasing skin cancers and infections cases [61,62]. BACE inhibitors, such as Verubecestat (MK-8931), have also been developed and, although they seemed to be safer than γ-secretase inhibitors, these drugs were not able to promote any improvement in cognitive function [63,64].

Active and passive immunization against Aβ have also been tested [12,65]. In humans, active immunization against aggregated human $A\beta_{1-42}$ (AN1792, Elan Pharmaceuticals) that demonstrated desirable effects on plaque burden and cognitive performance in transgenic AD

mice [66], resulted in removal of amyloid plaques in a few patients, but provoked aseptic meningoencephalitis in others [67–69]. Due to this adverse effect, the study was interrupted with drug dosing terminated in January 2002. Nevertheless, thorough clinical follow-up and monitoring of the non-affected patients continued under blinded conditions enabling retrospective analyses [67]. In one such retrospective analysis of the cohort, Nicoll and colleagues (2019) executed a 15-year post-mortem neuropathological follow-up of individuals who participated in the first trial of Aβ immunotherapy . The authors concluded that, although a clear evidence of plaque removal was observed, most patients progressed to severe cases of dementia, possibly due to propagation of Tau as extensive distribution of tangles (Braak V/VI) was found in a substantial number of patients [55].

Other Aβ-targeting antibodies (Solanezumab, Crenezumab, Gantenerumab, Bapineuzumab) were also tested, but although some positive effects have been observed, the results were not always replicable [70–74]. Defenders of the amyloid cascade hypothesis believe that the failures in these trials occurred because of difficulties in establishing adequate protocols. Problems of inappropriate dosing and administration of the drug in the late irreversible stages of the disease could explain the failure rate of clinical trials [75]. Indeed, the stage of the disease in which the drugs have been administered may have a huge impact in AD progress, since alterations in Aβ production, clearance and aggregation might start decades before the appearance of the first cognitive symptoms [76].

On the other hand, critics of this hypothesis argue that, besides the fact that AD is a heterogeneous disorder, the relationship between Aβ and AD is at least indirect. In this sense, Aβ might represent an end-stage of the condition rather than a cause. For them, persisting in this theory may result in loss of opportunity to consider other options, since numerous alternative hypotheses have been proposed all over the years and did not receive equal attention [15,18].

Nowadays, the two anti-Aβ antibodies Aducanumad and BAN2401 have shown benefits, but are still on trial [77]. Aducanumab was discontinued after a phase III futility analysis. However, after Biogen's request, the U.S. Food and Drug Administration (FDA) approved a re-dosing study [78,79]. Aducanumab has given not just support for the amyloid cascade hypothesis, but also hope to society, because, if approved, this drug will be the first medication with the ability both to remove the amyloid and slow down the cognitive decline.

ALZHEIMER'S DISEASE AS A MULTIFACTORIAL DISORDER: PROPOSAL OF OTHER HYPOTHESES

Despite all the attention the amyloid hypothesis received in the recent years, other important theories have been proposed (Table 1; Fig. 2). One example is the **cholinergic hypothesis** proposed by Davies and Maloney in 1976 [80] that provided a critical insight in the role of cholinergic transmission in the context of AD etiopathogenesis, and paved the way for development of AD drugs that are in use today [81]. The involvement of the cholinergic system in dementia was first implied by the studies which demonstrated that anticholinergic drugs could exert amnestic effect [82–84] and further corroborated with findings suggesting its reversal upon treatment with a cholinergic agonist [85]. Furthermore, the activity of the enzyme choline acetyltransferase (ChAT), responsible for the synthesis of acetylcholine (ACh), was found to be significantly decreased in postmortem samples from the amygdala, cortex and hippocampus of AD patients [80,86–88].

Shortly after, Whitehouse and colleagues observed a substantial loss of neurons in the nucleus basalis of Meynert (NbM), the source of cortical cholinergic innervation in the brain [89]. Ever since, the neurodegeneration of cholinergic projections from the NbM to the neocortex and the hippocampus has been considered as one of the main events in the pathophysiology of AD [90]. In addition, the proportion of cholinergic neurodegeneration was

found to have a positive correlation with the severity of dementia in AD [91,92]. Based on these findings, lesions in cholinergic projections from the basal forebrain to the cortex and hippocampus have been employed for induction of animal models of AD [93].

The cholinergic hypothesis was a stepping stone in the process of development of most drugs approved to treat AD - the acetylcholinesterase inhibitors (tacrine, rivastigmine, galantamine, donepezil) [94]. These drugs ameliorate cognitive symptoms, but, unfortunately, they are not able to decrease the risk, slow up the onset, or stop the progression of AD. Moreover, individual responses to these drugs may vary. Tacrine, the first drug approved by the FDA and introduced in the US marketing in 1993 [95], had quite poor adherence and presented many adverse effects, including hepatotoxicity, which lead to its discontinuation in 2013 [96]. Donepezil, the second drug approved by the FDA, was marketed in 1996 for the treatment of mild and moderate AD. However, in 2010, a higher dose was approved to treat more severe cases. Donepezil is accompanied by side effects, such as nausea, diarrhea, dizziness and insomnia, and cardiac adverse effects have been reported in some rare cases [97,98].

Galantamine [99] and rivastigmine [100] were both approved in 2000 for the treatment of AD. However, rivastigmine has also been used to treat Parkinson's dementia [101]. Recently, Ray and colleagues demonstrated that rivastigmine is able to direct AβPP processing away from the amyloidogenic pathway, by promoting α-secretase activity and, therefore, it might be explored as a disease-modifying treatment [102].

The cholinergic hypothesis has given support to the amyloid cascade theory [103] since the discovery that stimulation of cholinergic receptors regulates AβPP metabolism [104] and that Aβ toxicity can promote cholinergic impairment [105]. Other studies have demonstrated that nicotinic cholinergic receptor stimulation can modulate phosphorylated Tau aggregation [103] also corroborating the Tau hypothesis of AD [106].

The **Tau hypothesis** claims that Tau hyperphosphorylation precedes neurodegeneration and, in association with convergent signaling mechanisms, results in AD pathophysiology [107,108]. Moreover, there is evidence that alterations in Tau phosphorylation occur before Aβ accumulation [109]. The microtubule-associated protein Tau is the main component of neurofibrillary tangles [107]. Tau interacts with tubulin to promote microtubule polymerization and stabilization. However, Tau functions are regulated by its phosphorylation state. In a hyperphosphorylated state, the interaction of Tau and microtubules is hindered, resulting in destabilization and loss of cytoskeletal structure [110].

The human Tau gene is localized on chromosome 17. There are six Tau isoforms expressed in the adult brain, as a result of alternative mRNA splicing [11]. In AD, all six protein isoforms may be abnormally hyperphosphorylated, resulting in the formation of neurofibrillary tangles and destabilization of the microtubule network [111]. Therefore, in AD-impaired neurons, degenerating neuronal microtubules might be gradually replaced by tangles [112].

The fact that the severity of AD cases correlates well with Tau pathology in the brain has contributed to the confirmation of Tau hypothesis [113]. Tau pathology is usually classified according to Braak and Braak [114], affecting primarily the transentorhinal region in stages I and II, the limbic system in stages III and IV, and neocortical fields, mainly temporal and parietal areas, in more advanced stages (stages V and IV) [106]. In this sense, Tau has been investigated as a potential target in AD treatment. However, similar to the anti-amyloid therapies, strategies focused on Tau have also failed in clinical trials. The Tau aggregation blocker TRx0237 [115] and the Tau-targeted passive vaccine IVIG [116], for example, have both failed in phase III trials. Other trials are in progress, yet, since we do not completely understand the pathogenesis of AD, Tau-based therapeutic approaches still remain challenging [117].

Besides microtubule dysfunction, abnormal Tau phosphorylation promotes defective axonal transport of mitochondria and other organelles [118]. In fact, mitochondrial dysfunction has been frequently reported in AD [119], and, therefore, it has given support to another theory, the so-called **mitochondrial cascade hypothesis** [120–122].

In 1989, Parker suggested that mitochondrial DNA inheritance could influence AD risk [120,123]. Other authors claimed that somatic mitochondrial DNA mutations were able to influence the aging process [124–127] and, more specifically, AD development [128–131]. Then, in 2004, Swerdlow and Khan proposed that, since the individual's baseline mitochondrial function is defined by genetic inheritance, interactions between genetic and environmental factors would define the rhythm at which mitochondrial dysfunction accumulates and, therefore, would determine the AD onset [122]. Subsequently, other studies demonstrated that a maternal family history of AD increases the risk of developing the disease, when compared to a paternal family history of this disorder, which indicates that the maternally inherited mitochondria might play an important role in mitochondrial dysfunction and mediate the risk for the development of AD [132,133].

The mitochondrial cascade hypothesis may be linked to other theories [3] especially the amyloid cascade theory as mitochondrial dysfunction affects both AβPP expression and metabolism [134–137]. Indeed, besides the fact that functional mitochondria are essential for Aβ neurotoxicity [134], AβPP and Aβ co-localize with the mitochondrial network [135]. Therefore, it has been suggested that mitochondria mediate Aβ toxicity [119]. Furthermore, AβPP overexpression and Aβ exposure alter mitochondrial function in transgenic mice, cultured cells and autopsy brains [119,136–139].

Besides Aβ accumulation [139], there is evidence that mitochondrial dysfunction promotes Tau hyperphosphorylation [138] inflammation [140] and oxidative stress [141]. Moreover, mitochondrial changes, such as decreased rate of metabolism [140], decreased

mitochondrial concentration in the cerebrospinal fluid [141], and mitochondrial morphological alterations [142,143] have also been described in AD [144]. For this reason, mitochondrial enzymes and energy metabolism have been investigated as potential targets of drugs for the treatment of AD [145].

Mitochondrial dysfunction has also been implicated in the pathogenesis of AD through the generation of reactive oxygen species (ROS) [146]. ROS are oxygen-containing chemicals with reactive properties that play a fundamental role in maintenance of cellular homeostasis. ROS are constantly being produced as by-products of non-enzymatic reactions in the respiratory chains, or enzymatically by macrophages upon recognition of pathogen-associated molecular patterns. Physiologically, enzymes and other compounds usually control and maintain ROS at low levels in a defined homeostatic range, as they cannot be totally eliminated because of their function as specific second messengers in signaling cascades related to cell proliferation and differentiation. However, accumulation of high levels of ROS, usually due to overproduction or inadequate clearance, disrupts cellular homeostasis by a pathophysiological process known as oxidative stress. Since oxidative stress can damage cells, proteins, lipids, Ca^{2+} homeostasis, and DNA, it is considered harmful to the human body and a strong contributor to the process of aging [147].

Central nervous system is particularly susceptible to free radical damage due to its large oxygen demand and high mitochondrial respiration rate. Besides that, the central nervous system is characterized by a high lipid content and low capacity of enzymatic and non-enzymatic antioxidant systems, which may promote cumulative oxidative damage over time and contribute to AD pathogenesis [148].

In AD, different biomolecules from the neuronal membrane, such as lipids, fatty acids, and proteins can undergo oxidation [149]. There is evidence showing that high ROS levels may be present in earlier stages of AD, even before the appearance of Aβ accumulation or clinical

symptoms [150]. And, besides the high amount of evidence showing that Aβ induces oxidative stress in AD [151], regions with elevated levels of Aβ, such as cortex and hippocampus, present higher levels of oxidation products when compared to regions with low Aβ levels, such as the cerebellum [150–152]. Moreover, oxidative stress seems to be related to modifications of protein Tau conformation, which contributes to the formation of neurofibrillary tangles [153,154]. Alterations in Tau conformation, in turn, can potentiate oxidation of DNA and RNA [147,155] , evolving into a pathological cycle. Biometals also play an important role in neurodegeneration [156]. In this sense, increased concentrations of redox-active transition metals such as iron and copper, and the redox-inactive metal, zinc have been observed in Aβ plaques and surrounding tissues [152,157].

Corroborating the **oxidative stress hypothesis**, studies have demonstrated that AD patients present depletion of plasma antioxidants when compared to controls and that a good antioxidant status may be able to protect against cognitive impairment [158]. Moreover, since oxidative stress seems to be only one of many features of AD, neuroprotective potential of antioxidant compounds has been studied as a potential treatment option, but usually in combination with other therapies [159]. In this context, the phytocannabinoid cannabidiol (CBD), a constituent of *Cannabis sativa*, has been considered as a potential compound for AD treatment [160,161]. CBD is especially attractive as, besides its antioxidative and antioxidant properties, it also presents anti-inflammatory features [162] and neuroprotective effects on memory [163–167]. In addition, CBD appears to alleviate the hyperphosphorylation of Tau protein by attenuating glycogen synthase kinase 3 beta (GSK-3β) activity [168]. Furthermore, CBD has been shown to promote hippocampal neurogenesis [169] and prevent cortical and hippocampal neurodegeneration [170].

Another metabolic condition that has been implicated in AD along with mitochondrial dysfunction and oxidative stress is neuroinflammation [171–173]. This pathophysiological

process, characterized mainly by the accumulation of glial cells and upregulation of pro-inflammatory cytokines in the central nervous system, has been investigated as the crucial event in AD pathogenesis for more than two decades [174–177]. The peripheral immune system is linked to the brain through different mechanisms, including direct passage of cytokines from the blood through leaky regions in the blood-brain barrier (BBB), carrier-mediated transport of cytokines into the brain, and stimulation of cytokine synthesis by microglia activation after detection of a peripheral immune response via vagal afferents [178]. The immune system became particularly relevant to AD research once genome-wide association studies (GWAS) discovered that numerous immune genes are risk factors for sAD [179,180].

According to the **neuroinflammation hypothesis** of AD, an initial inflammatory stimulus, which could be a trauma, a pathogenic infection or even Aβ toxicity, triggers microglial activation. Microglia, in turn, secretes numerous pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNFα), and releases ROS, attracting more microglia and astrocytes towards the lesion area [181]. Physiologically, this process is critical for reparation of the damaged area. However, in pathological aging, and, more specifically, in AD, this stimulus is persistent and results in excessive activation of microglia, which initiates an auto-destructive process, culminating in neurodegeneration and AD pathogenesis [176,182].

Microglial activation is usually beneficial, as microglia participates in Aβ clearance and degradation [183], but its persistent activation may result in neurotoxic effects [184]. In fact, there is evidence that hyper-reactive microglia is present even in early stages of sAD [185]. In this sense, studies have demonstrated that constant microglial activation stimulated by Aβ, increases Aβ production and diminishes its clearance [186,187]. However, the inflammatory process induced by other agents is also able to increase Aβ production, via β-secretase cleavage [184]. Furthermore, hyperphosphorylated Tau leads to the activation of microglial cells, and

synthesis and production of pro-inflammatory cytokines [188]. Pathologically changed astrocytes have also been described in AD [189] and, although astrogliosis has been observed in regions without Aβ pathology, in AD brain tissue, astrogliosis is correlated with the degree of cognitive impairment [190].

In summary, the response to inflammatory stress induces hyperphosphorylation of Tau and increases Aβ synthesis. In addition, both Aβ accumulation and Tau hyperphosphorylation dysregulate the immune system and activate a constant and persistent inflammatory process, leading to a deleterious microglial and astrocytic reactivity, and, consequently, trigger a vicious circle of neurotoxic pro-inflammatory response [191].

Studies have consistently reported elevated levels of pro-inflammatory cytokines in serum and brain tissue of AD patients relative to controls, especially in severe AD [192–194]. More recently, it has been proposed that ambient air pollution might be able to trigger microglial activation and, consequently, provoke a constant inflammatory process accompanied by a permanent elevation of pro-inflammatory cytokines and ROS that could lead to AD and other neurodegenerative diseases [195,196]. Indeed, alterations in microglial morphology, increased proinflammatory cytokines and elevated oxidative stress have been observed in brains of humans and animals exposed to high levels of ambient urban air pollution [197–203].

Although the effects of the exposure to anti-inflammatory drugs in AD, specially nonsteroidal anti-inflammatory drugs (NSAIDs), are still controversial, some studies have observed benefits in the use of this type of medication before the onset of AD [204]. The early-stage responsiveness of AD to NSAIDs might be explained by the fact that, in advanced stages of the disease, the overactive microglia would go through a process of senescence and become non-functional, reaching a dystrophic status. At this stage, the senescent microglia would not be able to accomplish its physiological roles such as neuroprotection and clearance, but would maintain its ability to produce pro-inflammatory cytokines, thus accelerating the

disease progress [205,206]. Furthermore, it has been proposed that the activation of the innate immune system might act as a disease-promoting factor in which the senescent microglia is the initial trigger of AD pathogenesis [207]. In this case, AD should be considered an immune senescent disease rather than a neuroinflammatory disorder, as stated by the **innate immunity hypothesis** [208–210].

Another pathophysiological event that has been proposed as both the cause and the consequence of metabolic, oxidative, and proteotoxic stress in AD is dysregulation of Ca^{2+} homeostasis [211]. In this sense, **the calcium hypothesis**, postulated more than 30 years ago [212–214], proposes that sustained alterations in Ca^{2+} signaling in neurons might be a key event of AD pathogenesis [215]. This hypothesis has been supported by the findings suggesting that gene mutations that increase the risk for developing AD are usually related to dysfunctional Ca^{2+} signaling [211].

The Ca^{2+} ion works as an intracellular messenger in many signal-transducing pathways and as a regulator in diverse physiological functions. Because of the importance of Ca2+ homeostasis, a number of cellular regulatory mechanisms, such as ion channels, buffers and ATP-dependent ion pumps, are working to keep the level of Ca^{2+} at low nanomolar concentrations under resting conditions [216]. Homeostasis is particularly important as action potential-regulated influx and efflux of calcium is indispensable for proper neuronal signaling. Consequently, regulation of a complex network of calcium channels and transporters, as well as conserved activity of endoplasmic reticulum (ER) and mitochondria, two main organelles responsible for intracellular buffering, is a prerequisite for maintenance of structure and function of the central nervous system. Failures of this system results in the inability to maintain calcium homeostasis and leads to neurodegeneration [217]. Although astrocytes, the main homeostatic regulatory cells in the central nervous system, cannot generate action

potentials, they sense fluctuations in intracellular concentration of ions, especially Ca^{2+}, in order to respond to neuronal activity [218].

Corroborating the calcium hypothesis, several studies have shown a bidirectional relationship between Ca^{2+} and the Aβ peptide in pathogenesis of AD [219]. In this context, it has already been demonstrated that Aβ aggregates disrupt Ca^{2+} signaling in numerous ways and that Ca^{2+} dysregulation may also alter AβPP metabolism [220–225]. This link has been confirmed by the findings of the longitudinal aging study suggesting that individuals who use calcium channel blockers (CCBs) in the antihypertensive treatment present a significantly slower rate of progression to dementia. This effect could be related to a significant CCBs-induced decrease in Aβ1-42 levels found in neuroglioma cultures overexpressing APP [226].

Tau pathology might also be linked to disruption in Ca^{2+} signaling once microtubule dysfunction promoted by hyperphosphorylation of Tau impairs dynamics and axonal transport of organelles and vesicles, including mitochondria and ER [118]. When these components are affected, they end up directly influencing calcium signaling pathway, especially in neurons where the communication networks between ER, mitochondria and plasma membrane are fundamental for the regulation of temporal and spatial aspects of Ca^{2+} signaling [227].

Recently, Jadiya and colleagues (2019) demonstrated that impaired mitochondrial calcium efflux stimulates disease progression in AD models, by accelerating memory alterations, Aβ pathology, Tau hyperphosphorylation and development of histopathological changes [228]. In fact, some authors believe that, since mitochondrial Ca^{2+} overload may appear before the typical pathological features of AD, it should be considered a priority among therapeutic targets for AD [229]. Finally, all proposed hypotheses should not be considered individually, but as pieces of the pathophysiological puzzle contributing to understanding of the etiopathogenesis of AD. Some hypotheses, such as the mitochondrial hypothesis of the disease, and oxidative stress hypothesis as well as the calcium hypotheses are more closely

related as mitochondrial dysfunction is often considered as a key contributor to cellular ROS burden, and bidirectional interaction between the ER calcium and mitochondria make it difficult distinguish their cause-effect relationship. Nevertheless, a number of less obvious interconnections exist between all factors proposed as the main drivers of the disease, and current understanding of molecular mechanisms suggests all have the potential to trigger pathogenic cascade of AD. Recently, accumulated evidence on the importance of metabolic factors in the context of AD provided additional information that, when considered in the context of other hypotheses, might enable deeper understanding of the pathogenesis of the disease and reveal some links that might have been overlooked.
Due to numerous metabolic alterations described in AD, it was proposed that this disorder contains a significant metabolic component [230]. One of the main features of AD hypothesized as a metabolic disorder is the consistent findings suggestive of impaired insulin signaling in AD brains. In fact, the term "type 3 diabetes" has been proposed in order to englobe the cellular and molecular mechanisms by which insulin plays an important role in the pathology of AD. Interestingly, alterations in the regulation of the insulin signaling pathway, just like Aβ peptide accumulation, seem to be related to many aspects of AD discussed in this review.

ALZHEIMER'S DISEASE HYPOTHESIS OF A "TYPE 3" DIABETES

Although cognitive dysfunction in *Diabetes mellitus* (DM) has been frequently reported over the last decades [231–238], the first study showing worse performance in attention and memory tests in diabetic patients was made in Boston by Miles and Root, almost a century ago [239]. At that time, these findings were not well understood, however, in 1983, Bucht and colleagues found important results suggestive of decreased insulin sensitivity in AD patients [240]. These data implied for the first time that the hormone insulin could somehow be involved in the etiopathogenesis of AD. In 1998, Frölich and colleagues described alterations in the neuronal insulin signal transduction pathway in AD brains [241], which culminated in the

proposal that AD is a brain type of non-insulin dependent DM, made by Hoyer [242] in the same volume. After extensive work, in 2005, a group led by Suzanne de La Monte at the Brown Medical School proposed the term “type 3 diabetes” to refer to AD as a neuroendocrine disorder, similar, but also distinct, from DM types 1 (T1DM) and 2 (T2DM) [243].

More recently, studies have demonstrated that DM is a risk factor for developing dementia [244–247]. According to Chatterjee and colleagues, this risk is approximately 60% greater for diabetic patients compared with those without diabetes [248]. The most prominent factors that seem to be shared by T2DM and AD as common risks could be found often combined, from aging and age-related alterations like metabolic, hormonal and vascular pathology to environmental factors. Additionally, although the link between the two diseases is still not fully understood, associations have been reported also at the genetic level [249]. Caberlotto and colleagues have recently analyzed transcriptomic data of post-mortem AD and T2DM human brains and identified a central role for the autophagy pathway in both diseases. In addition, the authors used genetically modified animal AD models to confirm the role of autophagy-related genes in AD pathogenesis. These results suggest that autophagy dysregulation might be a common pathophysiological mechanism underlying AD and T2DM [250].

Considering the metabolic factors, particularly glucose metabolism in the brain, decreased glucose utilization and altered energy metabolism have been reported since early stages of AD [248], especially in regions associated with the process of learning and memory [251,252].

Evidence has gathered supporting the link between AD and T2DM based on the presence of AD biomarkers in the brain tissue of diabetic patients without clinical signs of dementia [253–256] and alterations in insulin signaling pathways found in the brain of both AD patients post mortem and AD animal models [241,243,257–259]. The insulin signal

transduction pathway is particularly important in the brain because of its functions related to neuronal survival, central regulation of body metabolism and modulation of memory and other cognitive and emotional processes [260].

The insulin signal transduction pathway in the AD brain

The presence of insulin in the central nervous system, as well as its origin and functions have been widely debated over the decades [261–265] mainly because glucose uptake by neurons is not insulin dependent. After extensive research in this field, it has become evident that both insulin and insulin receptors (IR) are distributed in a region-specific manner in the brain, with highest density in the hippocampus, cerebral cortex, olfactory bulb and hypothalamus [266–270]. Moreover, it is now known that glucose uptake in the brain can be influenced by insulin in conditions of high energy demand induced by increased neuronal activity. Increased glucose uptake upon insulin binding is mediated by the stimulation of the translocation of glucose transporters type 3 (GLUT3) and type 4 (GLUT4) to the plasma membrane in the conditions of increased energy demand, such as hippocampal-dependent tasks [271,272]. Studies have also demonstrated that systemic insulin may be actively transported through the BBB to the central nervous system [266,270,273,274], but a small portion of insulin can also be locally produced by neurons [275–279]. There is a higher density of IR in neurons as compared to glia, but astrocytes express both IR isoforms (IR-A and IR-B; IR-A, in contrast to IR-B, shows no negative cooperativity, indicating different functional regulation upon insulin binding), while neurons express exclusively the IR-A isoform [280].

IRs are composed of dimers of alpha (extracellular) and beta (intracellular) subunits joined by disulfide bonds. Insulin, or insulin-like growth factors (IGF), bind to the alpha subunits of IR inducing autophosphorylation of its beta subunit on tyrosine residues, thereby promoting the transduction of many signaling pathways [281], especially related to cell proliferation and metabolism. Then, the signal is transduced through the phosphorylation of

insulin receptor substrates (IRS), which are usually composed of six members (IRS1-6), also on tyrosine residues [282]. The IRS-1 is one of the most well described in humans and it is involved in modulation of essential functions in the cerebral cortex [260]. Phosphorylation of the IRS promotes conformational changes that enable the binding between IRS and another enzyme, phosphoinositide 3-kinases (PI3K). PI3K activation, in turn, phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) at the cell membrane and results in the formation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Then, PIP3 enables protein kinase (AKT/PKB) signaling pathway [283], which regulates the activation of many intracellular proteins in pathways related to cell proliferation and survival, such as the mammalian target of rapamycin (mTOR), forkhead box (FOX) proteins and Glycogen synthase kinase-3 (GSK3), besides the facilitation of the translocation of GLUT4 to the cell membrane and glucose uptake into the cell by the metabolic pathway [284].

GSK-3 activity is extremely relevant to AD pathogenesis and has emerged as an important target for AD drug development [285–287]. There are two isoforms of GSK-3 in mammals, the isoforms α and β, encoded by two different genes [288]. While GSK-3α regulates Aβ production [289], GSK-3β modulates phosphorylation of Tau [290]. In fact, GSK-3β is the main kinase of Tau protein, and it is able to phosphorylate at least 12 Ser/Thr of its pro-sites [291–293]. Moreover, GSK3-β activity may also be involved in Aβ production through the modulation of AβPP cleavage, as PS1 has been identified as a GSK-3β substrate and GSK3-β over-activation or overexpression stimulates the cleavage of AβPP by BACE1 [288]. The activities of GSK-3 α/β are inhibited through phosphorylation of GSK-3 by AKT at serines 21 and 9, respectively [294].

GSK-3 is expressed in all tissues, but it is particularly abundant in the brain, especially in the hippocampus [295]. It can inhibit insulin signaling through serine phosphorylation of the IRS 1 and 2 [296–298]. It is also the main suppressor of Wnt signaling pathway, one of the

most important developmental pathways that regulates fundamental aspects of cell fate determination, such as cell migration and neural patterning [299], which means that GSK-3 is able to influence cell differentiation and reproduction [295,300–302]. GSK-3 is also involved in regulation of learning and memory functions, and processes of neurodegeneration, neurogenesis, inflammation and synaptic plasticity, therefore alterations in GSK-3 activity found in AD could provide a molecular background for some of the neuropathological hallmarks of the disease [295,303]. Since GSK-3 phosphorylation at serine inhibits its activity, it would be expected to observe decreased phosphorylated GSK-3 α/β levels in AD brains. Curiously, AD studies have been contradictory and, while some authors identified increased levels of GSK-3 α/β in its active form [304], others observed increased GSK-3 phosphorylation [305,306]. Elevated expression and over-activity of GSK-3 has also been reported in T2DM [307] providing further support to dysfunctional IR signaling cascade as an underlying pathology linking AD and T2DM. Furthermore, increased expression and activation of GSK-3 have also been observed in other diseases, such as bipolar disorder [308–310], Parkinson's disease [311–313], and Huntington's disease [314,315].

Therefore, inhibition of GSK-3 has been investigated as a candidate pharmacological target for the treatment of many diseases, especially AD [287]. One of the most well studied drugs in this field is lithium, a non-selective GSK-3 inhibitor. However, the obtained results are contradictory and inconclusive [316–318]. Similar results were found with a small molecule non-ATP-competitive and irreversible GSK-3 inhibitor tideglusib (NP12) [319,320]. More recently, a meta-analysis performed by Matsunagaa, Fujishirob and Takechia suggested that GSK-3 inhibitors might not be effective in AD treatment. However, the protocol established in the analyzed studies might have not been adequate, and non-selective inhibition of GSK-3 in a number of different cell types with consequent modulation of important signaling pathways

might account for both ineffectiveness and side-effects of such a treatment. Hence, further studies are needed to obtain final conclusions about GSK-3 inhibitors [287].

Besides changes in GSK-3 activity, other upstream alterations in the insulin signaling cascade have also been reported in AD pathogenesis. For example, decreased levels of insulin, IGF-1, and their receptors have already been identified in AD brains [241,243,321]. Lower levels of IRS-1 [322] and increased IRS-1 serine phosphorylation, which disable normal transmission of signal through the IR-IRS signaling pathway and may result in insulin resistance [323,324], have been described in AD [325–327], even a decade before the clinical onset of AD [328]. Serine IRS-1 phosphorylation might be associated with Tau dysfunction in AD. In Tau knockout mice, serine phosphorylation of IRS-1 is increased, and insulin-induced hippocampal tyrosine phosphorylation of IRS-1 is decreased [329]. Decreased levels of PI3K and reduced phosphorylation of Akt have also been reported in AD [273,322]. Dysfunctional PI3K/Akt pathway has important downstream signaling consequences in AD, since it has been recognized as a molecular regulator of GSK-3, mTOR, glucose transporter trafficking, and autophagy, all recognized to be altered in the process of neurodegeneration.

IR signaling pathway also leads to the activation of the mitogen-activated protein kinase (MAPK) pathway, which regulates cell differentiation, proliferation, survival, death and metabolic activity. The expression of MAPK is increased in AD brain tissue and it is found to also be involved in the process of Aβ plaques formation, Tau hyperphosphorylation, neuroinflammation, oxidative stress and synaptic plasticity. Furthermore, MAPK seems to be involved in the regulation of cognitive function [258,330] . Consequently, MAPK has also been proposed as a possible therapeutic target in AD [331] and some candidate molecules have been tested in this context. For example, brain-permeable orally bioavailable small molecule isoform-selective inhibitor of p38α MAPK MW181 was reported to improve working memory,

reduce Tau phosphorylation and inflammation in Tau transgenic mouse model of tauopathy [332].

Since many studies have demonstrated that impairment in both peripheral and central metabolism is related to cognitive decline and dementia [333–335] insulin levels and sensitivity became therapeutic targets in AD treatment [336]. In line with that, both insulin secretagogues like glucagon-like peptide 1 (GLP-1) receptor agonists insulin sensitizers, such as thioglitazones and biguanides, have been shown to improve cognitive function in both AD patients and animal models [337–339]. Metformin, a biguanide that decreases gluconeogenesis in the liver and ameliorates insulin resistance, has been associated with a reduced risk of developing AD in older people with DM [340]. Although some results have been contradictory, studies have shown that this drug is able to interfere with the formation of Aβ plaques and neurofibrillary tangles and improve insulin signaling in the brain [258,341–343].

Many antidiabetic drugs have been investigated in AD treatment. These drugs may present numerous positive effects, such as improvement of insulin resistance and cell metabolism, which might result in amelioration of cognitive impairment [330]. Recently, in a literature review, Meng and colleagues summarized the available clinical and experimental studies reporting the effects and the potential mechanism of action for 14 antidiabetic drugs that have been considered for AD treatment [344]. Among them, insulin administration has led to, besides other benefits, significant improvement in cognitive function in both humans and animal models.

Effects of insulin administration on memory and Alzheimer's disease

Numerous studies have investigated the effects of insulin administration in the central nervous system in order to better understand the role of this hormone in cognition, and, more specifically, in AD [345]. Curiously, while peripheral insulin administration promotes memory deficits in rodents [346], probably through the induction of hypoglycemia [347], intranasal

insulin administration has shown positive effects in cognitive function in both clinical and experimental studies [306,348–350].

When insulin is applied to the nasal mucosa, its transport to the brain is facilitated by the axon bundles of the receptor cells in the roof of the nasal cavity that are involved in the process of olfaction. In this context olfactory bulb and hippocampus stand out as the most important brain structures involved in the process. Trigeminal pathways and rostral migratory flow also appear to be involved in this transport [351–354]. This type of administration does not alter peripheral insulin or glucose levels [355] and it usually takes 1 hour for insulin to bind to IRs in the hippocampus and frontal cortex in animals [356] and humans [351,357]. The importance of findings related to this treatment modality are further corroborated by the fact that the National Institutes of Health (NIH) appointed intranasal insulin administration as one of the most promising therapeutic strategies for the treatment and prevention of AD until the year 2025 [358].

Studies have demonstrated that even a single dose of intranasal insulin is sufficient to improve cognitive function in cognitively normal individuals [359–362]. In AD patients, most studies, in general, have reported positive results on cognition, [352,357,361,363–366]. Regarding the duration of treatment, literature data are inconsistent and indicate that the response might also depend on the type of insulin used for the treatment (e.g. short-acting versus long-acting) [365].

Overall, insulin treatment seems to be beneficial for the quality of life of AD patients, since studies have reported the treatment to be associated with improved functional status and daily activity [367]. Recently, the first pilot study of a single dose rapid-acting intranasal insulin in Down syndrome patients was performed by Rosenbloom and colleagues in order to verify the safety and viability of this potential treatment as this population is at high risk to develop

AD. Although the treatment was well tolerated by the subjects, the study was not powered to identify effects on cognitive function [368].

Besides the consistent reports of positive effects of insulin on memory [306,363–365,369–371], many other benefits have been observed in AD studies. Vandal and colleagues demonstrated that a single injection of insulin is able to decrease Aβ accumulation in a mouse model of AD [372]. In a clinical study, chronic treatment with intranasal insulin was able to modulate Aβ levels in early AD [366]. It has also been shown that chronic treatment with intranasal insulin decreases Tau hyperphosphorylation and improves the regulation of the insulin signaling pathway in animals [306]. Regarding neuroinflammation, one week of daily intranasal insulin treatment decreases microglial activation and increases synaptic proteins levels [373].

Although intranasal insulin administration seems to be a promising strategy in AD treatment, few studies have reported controversial results [361]. A minority of studies have observed no effect [374,375] or a reverse effect of insulin administration on memory [376]. However, some clinical studies have suggested that response to intranasal insulin may depend on other factors [367]. Four month-treatment with a short-acting insulin (20 IU or 40 IU) administered by a nasal delivery device to participants with mild cognitive impairment or AD, stratified as APOE4-carriers or -non-carriers, indicated a dose-, APOE4 status- and sex-dependent response in cognitive performance, compared to placebo [377]. The low dose group demonstrated overall treatment effect which was not seen in the high dose group where in APOE4-non-carriers cognitive improvement was observed in men and not in women while their APOE4-carrier counterparts remained cognitively stable. The results of studies with intranasal insulin treatment in cognitively impaired patients indicate that some people are better responders than the others to the treatment which targets insulin resistance in the brain, supporting thus the idea of the existence of different endophenotypes of AD and underlying the

necessity of identifying the brain insulin resistance-endophenotype features for a more successful therapy.

Some animal studies also reported conflicting results. In one study, glial overactivation has been observed following intranasal insulin treatment in F344 rats [376]. In one other study insulin treatment induced little effect on the regulation of proteins from the insulin signaling pathway [374]. Interestingly, in both studies, the treatment was tested in healthy animals, suggesting that, among other factors, the treatment effect might depend on the presence of underlying pathophysiological changes.

The link between diabetes and insulin signaling to other AD hypotheses

Evidence has gathered indicating dysfunctional insulin signaling in the brain of AD patients and animal AD models, which supports the hypothesis of AD as a "type 3 diabetes", however, alterations in the insulin signaling cascade seem to be shared by other hypotheses of AD as well**.** Regarding **the amyloid cascade hypothesis**, a bidirectional link between insulin and AβPP metabolism has already been identified [378]. The secretion of sAPPα, the product of normal APP processing in the non-amyloidogenic pathway related to neuronal health and brain development, is increased after insulin treatment, which indicates that insulin signaling may have functions related to the expression and activation of α-secretases that favor anti-amyloidogenic processing of AβPP and prevent Aβ accumulation [379,380]. Moreover, both insulin and IGFs present neuroprotective effects against Aβ toxicity [381–384]. These neuroprotective effects occur through the modulation of Akt and extracellular signal-regulated kinases (ERK) phosphorylation [385].

Furthermore, the insulin degrading enzyme (IDE) is one of the main factors responsible for Aβ degradation [386]. However, given its higher affinity, IDE binds preferentially to insulin, when compared to other substrates, including Aβ. Thus, insulin or pathological conditions that affect its levels, such as DM, can indirectly modulate circulating

Aβ levels. In this sense, conditions that decrease insulin sensitivity and increase insulin levels may result in greater accumulation of Aβ and, consequently, gradual deposition in senile plaques [387–389]. It has already been demonstrated that IDE's activity in the brain decreases during the process of aging and is significantly reduced in early stages of AD [390,391]. Conversely, insulin can also increase IDE protein levels via PI3K pathway and, therefore, deficient insulin signaling is correlated with decreased IDE in AD brains [392].

On the other hand, the Aβ peptide can form oligomers that bind to IRs, acting under certain conditions as insulin antagonists and interfering with the regulation of the insulin signaling cascade through the reduction of Akt activation and the increase of GSK-3 α/β activity [393]. Aβ can also induce serine phosphorylation of IRS-1, which inhibits insulin signaling and initiates a positive feedback loop, leading to an increase in AβPP processing and Aβ production [380]. There is also evidence that Aβ is able to promote the loss and redistribution of neuronal surface IRs, which might be related to the first clinical symptoms of AD [394,395].

Tau protein is related to insulin signaling in more complex ways. Marciniak and colleagues revealed this complexity when they identified brain insulin resistance in Tau knockout mice [329]. It is well known that insulin and IGF-1 modulate both Tau phosphorylation and Aβ production through the inhibition of GSK-3 by the PI3K-Akt signaling pathway [289,396]. However, there is also evidence that peripheral hyperinsulinemia, one of the main features T2DM, is able to alter brain insulin signaling and promote Tau hyperphosphorylation [397]. In this context, insulin resistance in T2DM is associated with elevated cerebrospinal fluid levels of Tau [398]. These findings are extremely relevant for understanding the underlying mechanism leading to an increased risk of diabetic patients to develop AD.

On the other hand, it has already been shown that intranasal insulin administration reduces Tau hyperphosphorylation in the brain of T2DM rat models induced by a high protein,

high glucose, and high fat diet followed by intraperitoneal injection of streptozotocin (STZ) [399]. STZ is a glucosamine-nitrosourea substance with alkylating properties that destroys pancreatic β-cells and leads to decreased insulin secretion and hyperglycemia, and, consequently, induces diabetes in experimental animals. Curiously, central administration of this compound produces memory deficits, impaired insulin signaling, neuroinflammation, neurodegeneration, and other molecular and pathological features that mimic those in patients with sporadic AD, and has, therefore, been considered a model of type 3 diabetes [400–403]. Chronic treatment with intranasal insulin decreases Tau hyperphosphorylation, improves cognitive function, ameliorates microglial activation and increases neurogenesis in this model [306].

Intracerebroventricular injection of STZ also generates oxidative stress and mitochondrial dysfunction, which may contribute to cognitive impairment [404,405]. Several mechanisms have been proposed to explain STZ-induced oxidative stress, and dysregulation of insulin/IGF signaling, an important regulator of redox homeostasis, provides one possible explanation [406]. Insulin resistance in T2DM is accompanied by hyperglycemia which generates the accumulation of advanced glycation end (AGE) products that promote ROS generation, and increased levels of AGE as well as overexpression of its receptor (RAGE) have also been observed in AD brains [407]. Besides oxidative stress, an imbalance between pro-oxidants and antioxidants and lipid peroxidation leading to cell damage have been identified in both AD and DM [408]. Moreover, insulin antagonizes the deleterious effects of oxidative stress in the brain. It presents neuroprotective effects against oxidative stress by restoring antioxidants and energy metabolism and modifying anti-apoptotic-associated protein synthesis through the stimulation of the PI3K/Akt pathway and inhibition of GSK-3β [409,410]. Moreover, it has already been shown that insulin sensitizers are able to protect against mitochondrial dysfunction caused by APOE4, a genetic risk factor for AD [411].

With regards to **the cholinergic hypothesis**, Hoyer was the first researcher to associate the cholinergic system to the brain insulin signal transduction system in AD [412] based on the information that glucose and energy metabolism are fundamental to the formation of the neurotransmitter acetylcholine [406]. However, many other authors have contributed to the understanding that the memory-enhancing effects of glucose are mediated by the cholinergic system [347]. Studies with hypoglycemia due to hyperinsulinemia have demonstrated that systemic insulin administration produces memory impairments in rodents [346,347]. In addition, the authors observed that these effects were mediated by cholinergic changes, which suggested that insulin had an important role in the modulation of cholinergic influences on memory [347]. Subsequently, Rivera and colleagues demonstrated that alterations in insulin and IGF-I signaling promote brain deficiencies in acetylcholine biosynthesis [413].

Chronic **inflammation** and high levels of inflammatory markers are other two main features of DM and AD. The connection between DM and AD inflammation is corroborated by the fact that adipose-derived inflammatory mediators, usually found in T2DM, can cross the blood–brain barrier and act together with the cytokines produced by microglia, increasing brain inflammation [414]. Moreover, GSK-3 may be a key mediator between impaired insulin signaling and neuroinflammation. Increased levels of TNFα, secreted mainly by microglial cells in response to central nervous system injuries, have been identified in both DM and AD, and it has been shown that GSK-3 is able to increase its production [415]. Increased levels of TNFα have also been observed in the cerebrospinal fluid of healthy individuals after peripheral administration of a single dose of insulin [416].

In addition, it has been shown that TNFα, as well as other inflammatory cytokines and stress-sensitive kinases, can promote insulin resistance [417–419] by stimulating the serine phosphorylation of IRS via the activation of c-Jun N-terminal kinase (JNK) [257,414] and that the intracerebroventricular administration of an anti-TNF agent is able to ameliorate insulin

signaling in rats [420]. On the other hand, the anti-inflammatory cytokine IL-4 increases insulin sensitivity [418,421]. Najem and colleagues proposed that neuroinflammation, insulin resistance and Aβ accumulation may act together to drive the pathogenesis of AD [418]. Their proposal was based on findings that insulin signaling modulates Aβ-induced inflammatory response [420] and soluble oligomers of Aβ promote IRS-1 inhibition via TNFα activation [325]. Therefore, they suggested that AD research should focus on understanding the possible link between these three events [418].

Regarding **the calcium hypothesis**, calcium homeostasis also presents a bidirectional link with insulin signaling. Calcium flux is involved in modulation of insulin release from the pancreatic islets cells [422]. On the other hand, insulin can control calcium distribution [423]. In addition, it has already been demonstrated that PI3K-Akt signaling pathway plays important roles in the voltage-dependent calcium channel trafficking to the plasma membrane, which suggests that insulin participates in the regulation of calcium entry in excitable cells [424].

More recently, it has been shown that acute insulin treatment is able to decrease calcium transients, which may affect intracellular calcium channel functions. These results suggest that insulin-mediated changes in calcium homeostasis may contribute to the positive effects of insulin in the brain [425]. On the other hand, in the central nervous system, increased levels of intracellular calcium are related to dysfunctional glucose metabolism [426,427]. Moreover, according to De Felice, aberrant calcium influx may be related to insulin resistance in AD since neuronal response to insulin can be inhibited by the calcium chelator BAPTA-AM [428].

A possible link between AD and T2DM could also be discussed at the level of cerebrovascular pathology found in diabetic and many AD patients indicating an additive effect on dementia [429,430]. Bearing in mind the heterogeneous etiopathogenesis of vascular cognitive impairments [431] it could not be excluded that the underlying mechanisms, besides factors like hyperglycaemia, maybe also linked to insulin regulation of vascular function [336].

At normal concentrations insulin acts as vasodilator (binding to its receptors on endothelial cells stimulates release of nitric oxide via the PI3K pathway) while at high concentrations it acts as a vasoconstrictor (stimulation of endothelin-1 production via the MAPK pathway) [432]. In a T2DM condition of chronic hyperinsulinemia due to insulin resistance, the vasoconstrictory role of insulin prevails resulting in reduced cerebral perfusion which may be detected years before the cognitive impairment [336].

CONCLUSIONS

Alzheimer's disease is a severe health public problem, with no cure or interventions to delay its progression. Although numerous researchers have focused on the understanding of this disease over the last decades, AD is still a not well understood disorder, with a complex pathogenesis. A lack of perception of AD as a heterogeneous pathological condition with a multifactorial etiology might be contributing to the constant failures in AD clinical trials. After gathering all the main features and hypotheses proposed in the context of the development of AD, we can infer that a single theory that could explain all its enigmas has not yet been proposed. We believe that AD is rather a multifactorial condition that can be influenced by numerous factors and different processes and that an adequate approach of AD should englobe the multiple aspects of this disorder.

Although most studies have focused on the amyloid cascade theory of AD, the metabolic hypothesis of AD, suggesting that AD is a metabolic disorder gained a lot of attention and provided consistent basic and clinical evidence in recent years. Based on this, some authors even proposed that AD should be considered a "type 3 diabetes" to further emphasize the importance of metabolic changes in the context of etiopathogenesis of the disease. The most interesting feature of this hypothesis is the fact that it provides an integrative framework indispensable for understanding individual pathomechanisms proposed by other hypotheses and often considered individually. By adding an additional contextual layer, and providing missing

links, this integrative hypothesis of AD taking into account dysfunctional insulin signaling cascade as a missing link between many of the other proposed hypotheses, may help us deepen our understanding of the AD pathophysiology, gain different perspectives, and design better prevention and treatment strategies.

CONFLIT OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

SSA conceived the original idea, participated in literature review and in writing the first draft of the manuscript. RMPSJ, GSM, JH, MSP, and NGC participated in literature review and revised the article. GSM prepared the figures for the manuscript. All authors have read and approved the final manuscript.

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FIGURES

Figure 1

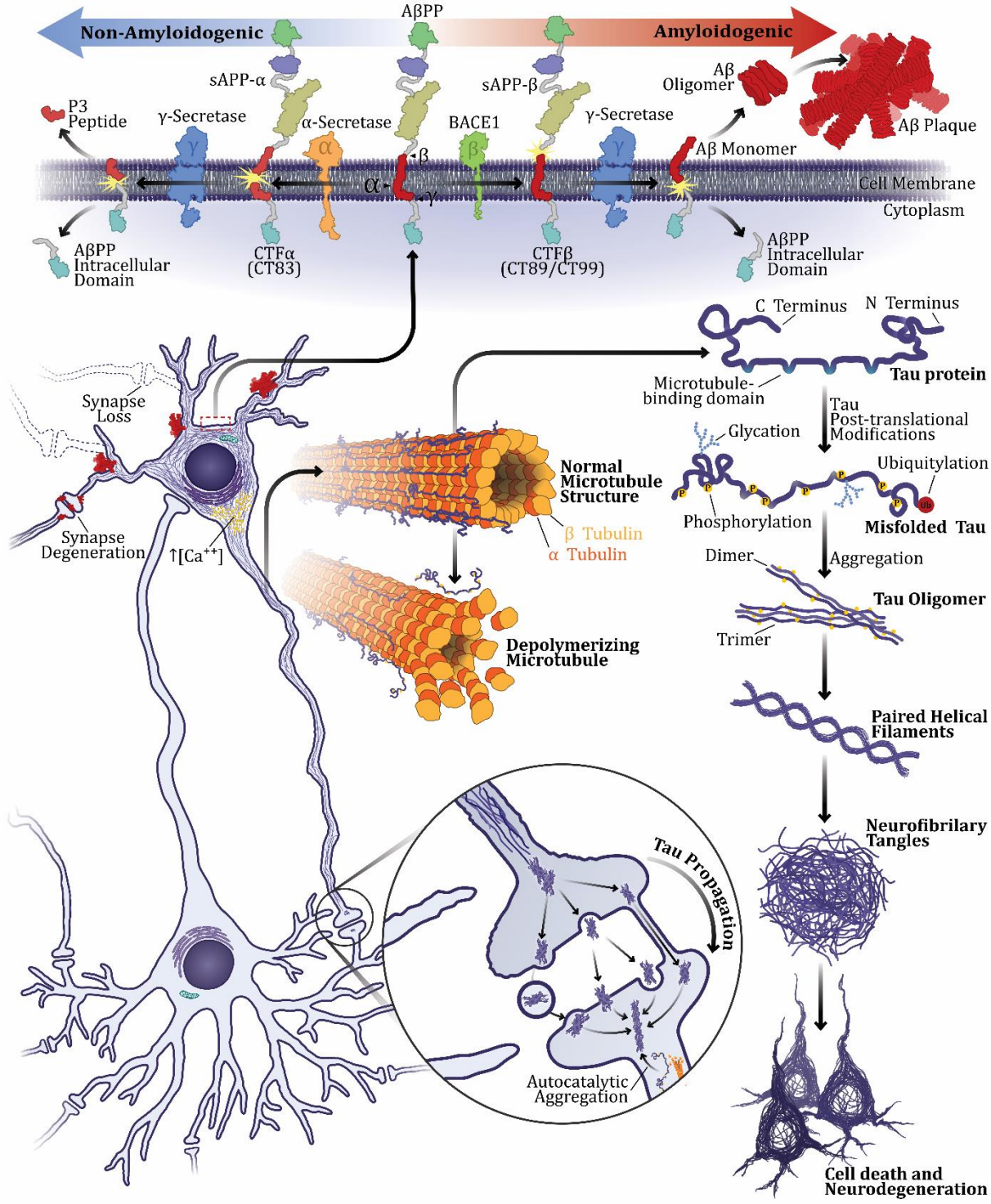

Figure 2

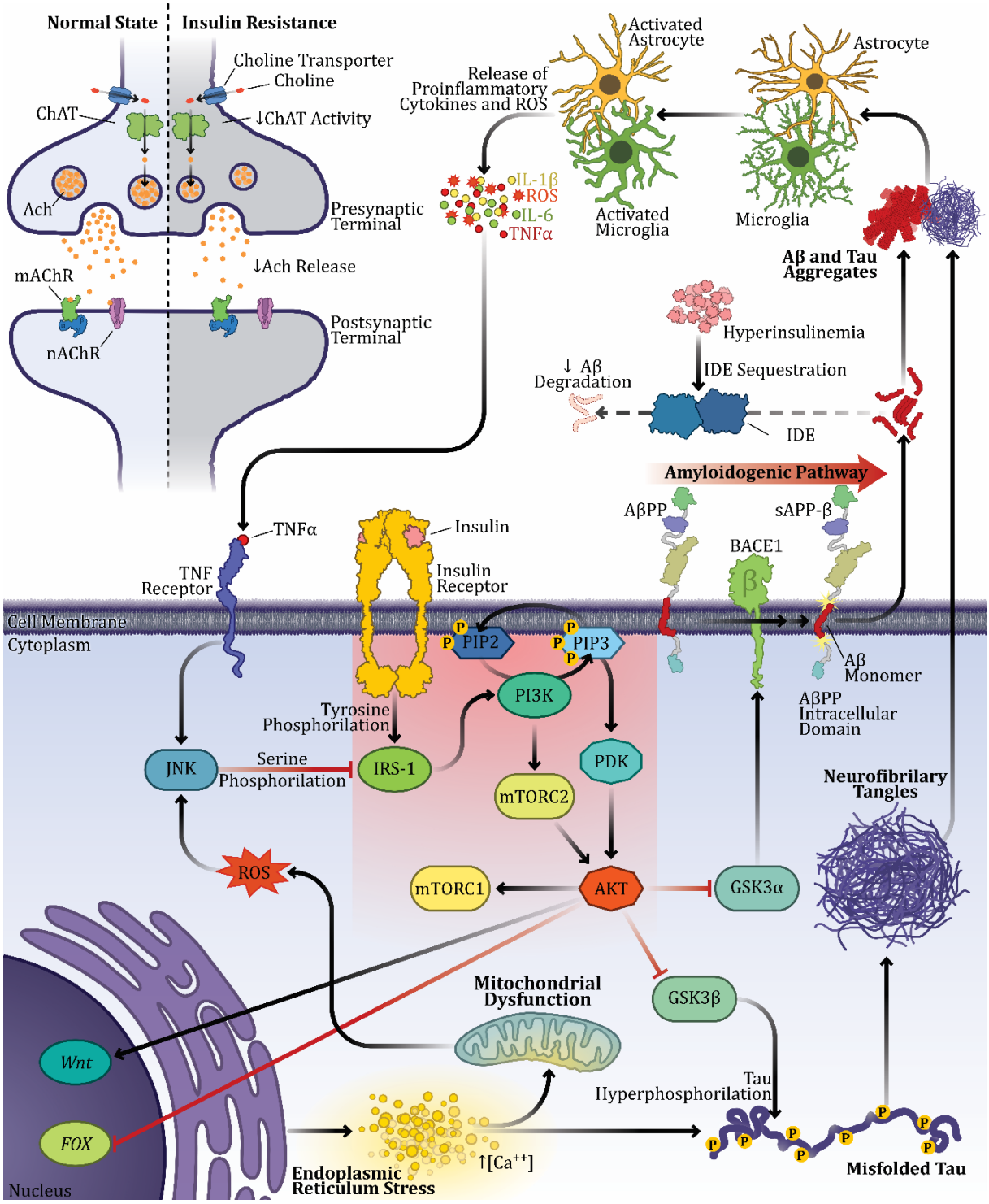
Normal State
Insulin Resistance
Choline Transporter
Choline
ChAT
↓ChAT Activity
Ach
Presynaptic Terminal
↓Ach Release
mAChR
nAChR
Postsynaptic Terminal
Activated Astrocyte
Astrocyte
Release of Proinflammatory Cytokines and ROS
IL-1β
ROS
IL-6
TNFα
Activated Microglia
Microglia
Aβ and Tau Aggregates
Hyperinsulinemia
IDE Sequestration
↓ Aβ Degradation
IDE
Amyloidogenic Pathway
AβPP
sAPP-β
BACE1
β
Aβ Monomer
AβPP Intracellular Domain
TNFα
TNF Receptor
Insulin
Insulin Receptor
Cell Membrane
Cytoplasm
PIP2
PIP3
PI3K
Tyrosine Phosphorilation
JNK
Serine Phosphorilation
IRS-1
PDK
mTORC2
ROS
mTORC1
AKT
GSK3α
Neurofibrilary Tangles
Mitochondrial Dysfunction
GSK3β
Wnt
FOX
Tau Hyperphosphorilation
↑[Ca++]
Endoplasmic Reticulum Stress
Misfolded Tau
Nucleus

FIGURE LEGENDS

Fig. 1. The traditional triad in Alzheimer's disease pathogenesis: amyloid-β peptide, hyperphosphorylated Tau protein and neurodegeneration. Aβ plaques, neurofibrillary tangles, and cell death had been seen the main neuropathological hallmarks and major factors in AD pathogenesis for more than a century. According to the amyloid cascade hypothesis, the most traditional hypothesis of AD, disturbances in AβPP metabolism are the triggering event in AD. In the amyloidogenic pathway, AβPP is first cleaved off by the enzyme β-secretase (BACE 1), giving rise to two fragments: sAPP-β (N-terminal fragment) and CT99 or CT89. Then, the γ-secretase complex cleaves the remaining membrane-bound portion of the protein releasing the extracellular fragment Aβ. On the other hand, in the non-amyloidogenic pathway, AβPP is firstly cleaved by α-secretase within Aβ sequence, producing soluble α-APP fragments (sAPPα) and C-terminal fragment α (CTFα, C83), posteriorly, CTFα is cut by γ-secretase, releasing non-toxic fragments (P3 peptide and AβPP intracellular domain). Alterations in AβPP processing usually result in increased Aβ production in the amyloidogenic pathway. Excessive Aβ production, aggregation and deposition into plaques, in turn, lead to intracellular Ca2+ dysregulation and induce Tau protein hyperphosphorylation and cell death. Tau is highly abundant in neurons and interacts with tubulin to promote microtubule polymerization and stabilization. However, when hyperphosphorylated, the ability of Tau to interact with microtubules is impaired. Hyperphosphorylated Tau undergoes conformational changes and self-aggregates into oligomers. Elongated oligomers usually form paired helical filaments, which culminate in neurofibrillary tangles formation, inducing neurodegeneration. Moreover, hyperphosphorylated Tau can sequester normal tau into filamentous tau aggregates.

Fig.2. Alzheimer's disease as a multifactorial disorder: multiple factors converging into a single disease. Interactions between different events proposed in AD pathogenesis over the last decades: Aβ overproduction, tau hyperphosphorylation, neuroinflammation, alterations in the

cholinergic system, mitochondrial dysfunction, oxidative stress, calcium imbalance, and insulin resistance as the main link connecting different factors. The PI3K-Akt signaling pathway is particularly important in AD pathogenesis due to its numerous interactions with AD events, but mainly because of its modulation of Aβ production and Tau protein hyperphosphorylation through GSK-3 activity. Insulin binds to the alpha subunits of the insulin receptor (IR) by inducing autophosphorylation of its beta subunit on tyrosine residues. Then, the signal is transduced through the phosphorylation of insulin receptor substrates (IRS), also on tyrosine residues. Phosphorylation of the IRS promotes conformational changes that enable the binding between IRS and another enzyme known as phosphoinositide 3-kinases (PI3K). PI3K activation, in turn, phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) in the cell membrane and results in phosphatidylinositol (3,4,5)-trisphosphate (PIP3) formation. Then, PIP3 enables protein kinase (AKT/PKB) signaling pathway, which regulates the activation of many intracellular proteins in pathways related to cell proliferation and survival, such as the mammalian target of rapamycin (mTOR), forkhead box (FOX) proteins and Glycogen synthase kinase-3 (GSK3). There are two isoforms of GSK-3 in mammals, the isoforms α and β. While GSK-3α regulates Aβ production, GSK-3β modulates Tau phosphorylation. Besides GSK-3 activity, alterations of many other proteins in the insulin signaling cascade have also been reported in AD.

TABLE

Table 1. Main hypotheses on the pathogenesis of sporadic Alzheimer's disease and associated therapies

Hypothesis	Main concept	Related therapies	References
Amyloid cascade hypothesis	The Aβ peptide is the triggering factor of AD. Acute effects, such as head trauma, promote disturbances in AβPP metabolism, altering Aβ production, clearance and deposition. The Aβ protein, in turn, promotes intracellular calcium dysregulation, inducing neurofibrillary tangle formation and cell death.	Vaccines, antibodies and molecules targeting monomeric, oligomeric and fibrillar Aβ species, soluble and insoluble Aβ, Aβ protofibrils, Aβ oligomer receptor, Aβ synthesis, Aβ aggregation, Aβ-glycosaminoglycan binding, and pyroglutamate-Aβ (ABvac40, Adubanumab, Bapineuzumab, Solanezumab BAN2401, acitretin,	[27,433]

		atabacestat, semagacestat, elenbecestat bexarotene, Alzhemed, PQ912, etc.)	
Cholinergic hypothesis	AD is a brain cholinergic system failure and the cognitive symptoms observed in this disorder are caused mainly by degeneration of cholinergic neurons in the basal forebrain and by cholinergic synaptic loss in the cerebral cortex.	Cholinesterase inhibitors (tacrine, donezepil, rivastigmine, galantamine).	[80,96]
Tau hypothesis	Tau hyperphosphorylation precedes neurodegeneration	Modulators of tau posttranslational modifications, Tau aggregation	[107,108,117,434]

	and Aβ accumulation, and in association with convergent signaling mechanisms, result in AD pathogenesis.	inhibitors, tau disaggregating agents, stabilizing microtubules (ACI35, AADvac-1, RG6100, ABBv-8E12, lithium, tideglusib, saracatinib, salsalate, ASN120290, epothilone D, methylene blue, nilotinib, TRx0237, etc.).	
Mitochondrial cascade hypothesis	The individual's baseline mitochondrial function is defined by genetic inheritance, and interactions between genetic and environmental factors define the	Antioxidants, bioenergetic medicine, vitamins, cofactors, electron acceptors, redox molecule precursors, intermediate compounds of the Krebs cycle and	[122,435,436]

	rhythm at which mitochondrial dysfunction accumulates and, therefore, determine the AD onset.	gluconeogenesis, intermediate compounds of mitochondrial metabolic pathways, peroxisome proliferator-activated receptor gamma Phenylpropanoids, antihistaminic drug, actions on the lifestyle (vitamin E and C, coenzyme Q10, selenium, mitoquinone mesylate, melatonin, α-lipoic acid, catalase, resveratrol, curcumin rapamycin, Dimebon, nicotinamide	

		adenine dinucleotide, physical exercise, calories restriction, , etc.).	
Oxidative stress hypothesis	The CNS presents a high lipid content and decreased amount of antioxidant enzymes relative to other systems, which may promote cumulative oxidative damage over time and result in AD pathogenesis. Different biomolecules from the neuronal membrane, such as lipids, fatty acids, and proteins undergo oxidation,	Antioxidant, vitamins, supplementary diets, polyphenolic compounds, Flavonoids, medicinal plants (rosmarinic acid, quercetin, epicatechin, cannabidiol, melatonin, vitamins A, C, and E, β-carotene, B-complex vitamins, proanthocyanidin, *Centella asiatica*, *Aloe arborescens*, *Capparis spinosa L.*, Alpinia	[148,149,162,437]

	even in earlier stages of AD.	galanga L., *Abelmoschus esculentus*, *Curcuma longa*, etc.).	
Neuroinflammation hypothesis	A persistent inflammatory stimulus, (trauma, pathogenic infection, Aβ toxicity) triggers microglia activation. Microglia, in turn, secrete numerous pro-inflammatory cytokines and release ROS, attracting more microglia and astrocytes migrating towards the lesion area. Microglia become	Non-steroidal anti-inflammatory drugs, antioxidants, probiotics, steroid and phenolic phytochemicals, Terpenoid-Derived phytochemicals, alkaloidal phytochemicals, Tumor necrosis factor-alpha inhibitor, 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG-CoA) reductase inhibitor, p38 mitogen-activated	[176,177,438–440]

	overactive, which initiates an auto-destructive process, culminating in neurodegeneration and AD pathogenesis.	serine/threonine protein kinase p38 MAPK (p38 MAPKα) selective inhibitor, receptor for advanced glycation endproducts (RAGE) inhibitor, peroxisome-proliferator activated receptor γ (PPARγ) agonists (ibuprofen, tarenflurbil, salsalate, celecoxib, resveratrol, etanercept, simvastatin, neflamapimod, azeliragon,	

		Diosgenin, Prosapogenin III, quercetin, Ginkgolide, berberine, etc.).	
Innate immunity hypothesis	The activation of the innate immune system is the disease-promoting factor and the activation of a senescent and non-functional microglia is the initial trigger of AD pathogenesis.	Pharmacological and genetic therapies targeting impaired microglial clearance, nonsteroidal anti-inflammatory drugs, actions in lifestyle (galantamine, thiazolidinedione, interleukin 33, etc.).	[208–210,441]
Calcium hypothesis	Sustained alterations in Ca2+ signaling in neurons is a key event of AD pathogenesis.	Modulation of Ca2+ related proteins and pathways, therapeutic strategies to balance calcium	[212,442–446]

		homeostasis, and actions in lifestyle. (nilvadipine, memantine, amlodipine, MEM-1003, EVT-101, physical exercise, etc.).	
Type 3 diabetes hypothesis	AD is a neuroendocrine disorder, similar, but also distinct, from DM types 1 and 2. Insulin deficiency and alterations in the insulin signaling pathway play major roles in AD pathogenesis.	Agents that treat hyperglycemia and ameliorates insulin sensitivity and the regulation of insulin signaling pathway, antidiabetic drugs, amylin analog drugs, insulin, glucagon-like peptide-1 receptor agonists, thiazolidinediones, sulfonylurea, inhibitors of dipeptidyl	[243,344,447]

		peptidase 4, biguanides and gliflozin class drugs, glucosidase inhibitors, and meglitinides (liraglutide, pioglitazone, sitagliptin, pramlintide, glimepiride, metformin, canagliflozin, acarbose, repaglinide, etc.).	