Cell. Mol. Life Sci. 63 (2006) 1226–1235
1420-682X/06/111226-10
DOI 10.1007/s00018-005-5597-y

Cellular and Molecular Life Sciences

Review

Glycogen synthase kinase 3β and Alzheimer's disease: pathophysiological and therapeutic significance

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Received 19 December 2005; received after revision 24 January 2006; accepted 6 February 2006
Online First 29 March 2006

Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive and behavioral dysfunction and is the leading cause of dementia in the elderly. Several studies have implicated molecular and cellular signaling cascades involving the serine-threonine kinase, glycogen synthase kinase β (GSK-3β) in the pathogenesis of AD. GSK-3β may play an important role in the formation of neurofibrillary tangles and senile plaques, the two classical pathological hallmarks of AD. In this review, we discuss the interaction between GSK-3β and several key molecules involved in AD, including the presenilins, amyloid precursor protein, tau, and β-amyloid. We identify the signal transduction pathways involved in the pathogenesis of AD, including Wnt, Notch, and the PI3 kinase/Akt pathway. These may be potential therapeutic targets in AD.

Keywords. Alzheimer's disease, glycogen synthase kinase 3β, amyloid precursor protein, presenilins, tau, β-amyloid, neuronal apoptosis, lithium.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder associated with cognitive and behavioral dysfunction. AD manifests both as an early-onset familial form (FAD) and a late-onset sporadic form. Mutations in the amyloid precursor protein [APP] gene on chromosome 21, presenilin1 (PS1) gene on chromosome 14 and PS2 gene on chromosome 1 have been linked to FAD and the early onset of Alzheimer's pathology and symptoms [1–3]; mutations in PS1 account for approximately 50% of FAD cases [4]. Sporadic AD occurs later in life, with incidence increasing almost exponentially with age. However, with both forms of AD, the clinical and pathological features observed are identical.

Senile plaques and neurofibrillary tangles (NFTs) are the two major lesions seen in the hippocampus and neocortex of AD patients [5]. Senile plaques, seen outside the neurons, are made up of less-soluble β-amyloid [Aβ] peptides generated from the cleavage of APP [6, 7]. NFTs, mostly seen inside the neurons, are polymers of paired helical filaments (PHFs) generated by the aggregation of a microtubule-associated protein called tau in the hyperphosphorylated state [8]. Aggregated forms of Aβ peptide have been shown to be neurotoxic and might contribute to the cell death and neuronal loss through apoptosis seen in AD [9–11]. Evidence is accumulating that a complex interplay of various molecules and gene products, including APP, Aβ peptide, presenilins, apo E4, tau, Wnt and Notch [12, 13], and products of oxidative stress [14, 15], as well as mediators of apoptosis [16], are involved in the progression to the pathology of AD. More recently the serine-threonine kinase glycogen synthase

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kinase 3β (GSJ-3β) has been demonstrated to be involved, at some level, in many of these interactions, making this molecule a promising therapeutic target.

GSK-3β is a serine-threonine kinase first identified as an enzyme that phosphorylates glycogen synthase in the glycogen synthesis pathway. It plays an important role in many processes such as cellular signaling pathways, metabolic control, embryogenesis, cell death and oncogenesis [17]. GSK-3β has been implicated in a wide range of disorders including neurodegenerative disorders, bipolar mood disorders, stroke, and diabetes [17]. Unlike most protein kinases, GSK-3β is normally constitutively active in all cells, and is primarily regulated through inhibition [18]. GSK-3β activity is approximately 200-fold higher upon phosphorylation at its Tyr216 residue [19]. GSK-3β is inhibited via phosphorylation at specific serine residues (serine 9 for GSK-3β and serine 21 for GSK-3α), which allows the N terminus of the GSK molecule to bind within its own active-site-binding pocket [20]. GSK-3β is also regulated by the Wnt signaling pathway. Wnt binding to frizzled receptor recruits disheveled protein, which in turn inhibits GSK-3β and stabilizes β-catenin. Disheveled protein might inhibit GSK-3β through activation of protein kinase C (PKC) [21] or through formation of a complex with GSK-3β-binding proteins [GBPs] [22]. GSK-3β activity is enhanced in the absence of Wnt through a complex formation with adenomatosis polyposis coli (APC) protein, Axin and β-catenin [23]. In the absence of Wnt, there is no inhibition of GSK activity, and β-catenin is phosphorylated [24]. This leads to the degradation of β-catenin through the ubiquitin-proteasome mechanism. While much evidence exists for the potential of GSK-3β inhibition to prove beneficial in treatment of AD, concerns regarding conflicting evidence for the *in vivo* relationship of GSK activity to AD, possible unintended effects of GSK-3β inhibition, and potential deleterious effects of molecules such as lithium [25] are well-founded and emphasize the need for further examination of this issue. In this review, we will discuss the recent evidence pointing to the role of GSK-3β in AD, and the potential role of inhibitors of GSK-3β in the treatment of AD.

Interaction of GSK-3β, APP, and the Aβ peptide

APP is a transmembrane phosphoprotein present in a variety of tissues but predominating in the brain [26]. The progressive cleavage of APP is regulated by several enzymes called secretases [27]. The initial APP processing involves the cleavage of APP by α-secretase. This does not give rise to amyloidogenic fragments and hence is referred to as the non-amyloidogenic pathway. [27]. Physiologic cleavage of APP (within the Aβ domain of the protein) [28] by α-secretase results in the production of a soluble APP (soluble or secreted αAPP) and a C-terminal fragment (CTF) [29]. There is some evidence that soluble APP is actually neurotrophic [30] as well as possibly neuroprotective [31]. A second pathway of APP proteolysis referred to as the amyloidogenic pathway involves the sequential action of β-secretase followed by γ-secretase. In humans, two β-secretases have been identified, referred to as BACE-1 and BACE-2, however, only BACE-1 is significantly expressed in the brain. The product of β-secretase cleavage of APP becomes a substrate for the site-specific proteolysis by γ-secretase, generating two predominant Aβ peptides either 40 or 42 amino acids in length and a short intracellular fragment, AICD (APP intracellular domain) that may function as a transcriptional activator [27, 32].

In some cases of FAD, mutations in the APP gene cause a gain of function, resulting in a shift of the cleavage of APP with an increase in the activity of β-secretase (BACE-1) [33, 34] and resultant increase in Aβ42. Aβ42 is less soluble and more neurotoxic than Aβ40, and readily aggregates into plaques [35]. GSK-3β appears to be involved in several steps of this process, including the effects of the various CTFs, the neurodegeneration induced by Aβ42, and the cleavage of APP.

APP products such as CTFs and AICD have been shown to be associated with increased GSK-3β activity. Studies with neuronal cells expressing the CTFs showed an increase in the active form of GSK-3β and a subsequent increase in tau phosphorylation and apoptosis [36]. Another group has shown evidence that the presence of these tau molecules may be necessary for Aβ neurotoxicity to manifest [37]. Further *in vitro* studies have shown that AICD enters the nucleus and activates gene transcription, including an increase in GSK-3β mRNA and protein product [38]. AICD transgenic mice show activation of GSK-3β and phosphorylation of CRMP2 protein, a GSK-3β substrate [39].

Evidence for the potential of GSK inhibition in altering APP processing comes from data in the PDAPP transgenic mice that overexpress the FAD mutant human APP gene. These mice, when treated with the GSK-3β inhibitors lithium or valproic acid, demonstrated a reduction in the increase of Aβ and plaques in the hippocampal region of the brain [40] This effect was mediated through the inhibition by lithium of GSK-3β, as the presence of GSK-3β dominant-negative constructs or antisense molecules mimicked the effects of lithium. However, another group has recently shown that lithium decreased Aβ production by interfering with the action of γ-secretase mediated through selective inhibition of GSK-3α rather than GSK-3β [41]. In fact, selective GSK-3β inhibition in this study actually showed a small increase in Aβ production. In addition, another recent study shows evidence that lithium increases the β-cleavage of APP, resulting in increased substrate for γ-secretase and therefore increased Aβ pro-

duction, and that this effect is independent of GSK-3β inhibition. Therefore, some controversy exists regarding the effects of lithium in altering the processing of APP.

Intraneuronal Aβ accumulation might promote the neuronal apoptosis and other neurodegenerative changes seen in AD through signaling cascades involving GSK-3β. Effects of Aβ on neurons have been studied using *in vitro* models such as overexpression of Aβ25–35 and *in vivo* in mouse models [42]. An increase in neuronal cell death was observed when hippocampal neurons were treated with both Aβ and tau protein kinase I (TPKI)/GSK-3β sense oligonucleotide. Neuronal cell death was reduced by addition of TPKI/GSK-3β antisense oligonucleotides to the Aβ-treated culture, suggesting that TPKI/GSK-3β is one of the key elements in Aβ-induced neurotoxicity and cell death [43]. Interestingly, another study by the same group showed that application of Aβ25–35 to rat hippocampal neurons resulted in accumulation of soluble APP (sAPP), CTFs, and other cleavage products of APP in the cytoplasm of neurons. Treatment of neurons with GSK-3β antisense oligonucleotides prevented the accumulation of sAPP and rescued the neurons from apoptotic death. Increased GSK-3β activity is hypothesized to affect axonal transport through tau phosphorylation and in turn alter APP metabolism and processing [44]. The same group has also shown that the Aβ25–35-induced GSK-3β activation in hippocampal neurons might be accomplished through the inactivation of phosphatidyl inositol 3 kinase (PI3K) [45]. Increased GSK-3β activity inactivates mitochondrial pyruvate dehydrogenase (PDH)]. This enzyme is involved in the conversion of pyruvate to acetyl-CoA in cholinergic neurons, and its inactivation results in mitochondrial dysfunction, decreased acetylcholine production, and contributes to neuronal death; this suggests that Aβ, through activation of GSK-3β, may play a role in the loss of cholinergic signaling seen in AD [46].

Interaction of GSK-3β and presenilins

Presenilins are transmembrane proteins confined to intracellular membranous compartments which are vital to cellular-fate-signaling pathways during development [47, 48]. Mutations in the PS1 and PS2 genes induce the pathological changes seen in FAD. There are several theories regarding the mechanism by which PS mutations lead to these changes, including alteration of APP cleavage, loss of GSK inhibition, and facilitation of tau phosphorylation (Fig. 1).

Presenilins play a significant role in APP processing. PS1 is necessary for the γ-secretase step of APP processing. By altering the proteolytic cleavage of APP, presenilin mutations increase production of the neurotoxic Aβ42 peptide, the major component of senile neural plaques. In cell lines, transgenic mice, and even in human brain tissue [49], expression of presenilin mutations results in increased levels of Aβ42. Studies in transgenic mice have shown that co-expression of the FAD-linked PS1 and APP mutations is associated with earlier development of amyloid deposits [50]. Knockout of the PS1 gene leads to a significant decrease in Aβ42 production [51]. Several groups have shown that knockout of both PS1 and PS2 results in virtually no Aβ production [52, 53]. More recently, the specific role of PS1 has been further delineated. PS1, in association with membrane proteins named nicastrin (Nct), APH-1, and PEN-2, is now known to form a complex that performs the function of γ-secretase [32, 47, 53–55].

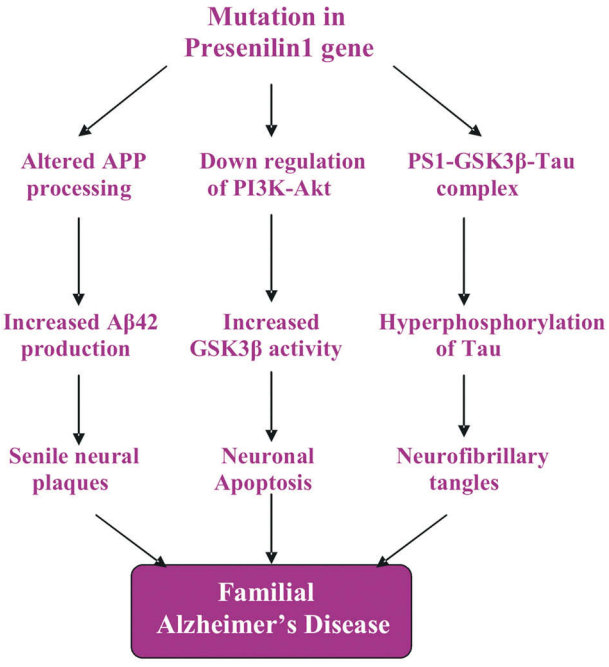

Figure 1. Role of GSK-3β in the pathogenesis of familial Alzheimer's disease. PS1, presenilin 1; APP, amyloid precursor protein; GSK-3β, glycogen synthase kinase 3β; PI3K, phosphatidyl inositol 3 kinase; Akt, protein kinase; Aβ42, β-amyloid with 42 amino acids.

PS1 mutations expressed in primary cultured hippocampal neurons led to apoptosis, a downregulation of the pro-survival Akt/PKB pathway, and subsequent increase in GSK-3β activity [56]. Other studies have confirmed the role of PS1 in promotion of cell survival by demonstrating that PS1 activates the P13K/Akt pathway, promotes the inactivation of GSK-3, and prevents apoptosis of confluent cells, and that these effects are unaffected by inhibitors of γ-secretase activity [57]. Another group has shown evidence that GSK-3β binds a particular residue of PS1, and that mutations of the PS1 gene result in an increase in this binding [58]. This group also showed that tau binds the same residue of the PS1 protein, implying that PS mutations might also facilitate tau phosphorylation by physical proximity of tau and GSK-3β. There has been some work examining the possibility that PS1 mu-

tations might compromise neuronal function. Studies of mice with either mutant PS1 or absent PS1 had an increase in the relative activity of GSK-3β as well as a reduction in kinesin-1-driven organelle motility [59]. PS1 can also be phosphorylated by GSK-3β [60] and GSK-3β in turn can regulate PS1 C-terminal levels [61].

Interaction of GSK-3β and tau

Tau is a microtubule-associated protein which plays an important role in microtubule assembly and stability [62]. GSK-3β regulates tau by phosphorylation as well as by regulating splice variance. Hyperphosphorylated tau has less affinity toward the microtubules, and readily aggregates into PHFs and NFTs, disrupting microtubule stability. This in turn might contribute to alteration in the synaptic plasticity and neurodegenerative changes seen in AD [63]. Some studies have shown that hyperphosphorylated tau itself is neurotoxic and can promote the neuronal apoptosis and cell death seen in AD [64]. GSK-3β and CDK5 have been identified as the two kinases associated with phosphorylation of tau [65]. Protein kinases isolated from the microtubule fractions of rat and bovine brain extracts associated with phosphorylation of tau were named TPKI and TPKII [66, 67]. GSK-3β has now been shown to be identical to TPKI [68] and the catalytic subunit of TPKII is identical to CDK5 [69].

TPKI/GSK-3β phosphorylates the Ser199, Thr231, Ser396, and Ser413 residues on tau [70] and TPKII phosphorylates the Ser202, Thr205, Ser235 and Ser404 on tau [71]. Twenty phosphorylation sites were identified for PHF-tau from Alzheimer's brain, of which the eight TPKI and TPKII sites were the major ones. Antibodies raised against these eight tau phosphorylation sites strongly stained the hippocampus of AD brains, including antibodies against the phospho-Ser413 residue of tau, which is phosphorylated only by GSK-3β. These antibodies stained the major lesions (NFT and neural plaques) associated with AD [71], suggesting the importance of GSK-3β in NFT formation. GSK-3β can phosphorylate tau at both primed and unprimed sites at Ser-Thr-Pro motifs. Examples of unprimed substrates are Axin and the APC gene product. In other cases, the substrate must be first phosphorylated at a site that is four amino acids C-terminal to the target site, which primes the target for GSK3β. The phosphorylation of tau at primed sites has been shown to play a more significant role in regulating the interaction of tau with microtubules than phosphorylation at unprimed epitopes [72].

GSK-3β is also capable of altering tau protein morphology by mechanisms other than hyperphosphorylation. Tau exists in two isoforms due to alternate splicing of exon 10, a 4R isoform (a 4-repeat region that has a microtubule-binding domain) with increased microtubule-binding capacity, and a 3R isoform with a reduced microtubule-binding capacity. The 3R isoform is formed in the presence of the splicing factor SC35. Inhibition of GSK-3β activity results in the co-localization of GSK-3β with SC35 in the nucleus, and in the absence of SC35 there is increased transcription of exon 10 and therefore the formation of the 4R isoform. Cultured cortical neurons treated with the GSK-3β inhibitors lithium or AR-18 showed an increase in the tau 4R isoforms [73]. Increased GSK-3β activity is associated with the 3R isoform, and the associated decrease in microtubule stability may contribute to the lesions seen in AD. Given the clear associations of GSK-3β with tau, cellular insults leading to GSK-3β overactivity might result in hyperphosphorylated and altered states of tau that bind poorly to microtubules, altering microtubule stability and neuronal integrity, and leading to the changes seen in AD.

Transgenic mice overexpressing GSK-3β via tetracycline(Tet)-regulated systems in the forebrain were used to study the deregulation of GSK-3β in AD. GSK-3β expression was increased in the hippocampus and cortex of Tet/GSK-3β transgenic mice compared with wild-type mice. Immunohistochemistry showed increased GSK-3β in layer I and II pyramidal neurons of the frontal cortex, CA2 neurons and dentate gyrus of the hippocampus. An increase in tau phosphorylation was observed in the hippocampus using PHF-tau antibody [74]. Thus, GSK-3β-mediated phosphorylation of tau plays an important role in the pathophysiology of AD. However, as will be discussed in further detail below, while GSK-3β overexpression is deleterious, GSK-3β is of vital importance to brain function and development [75], and deletion of GSK-3β leads to embryonic lethality [76].

Recent studies have shown that polymorphisms in certain apolipoprotein (apo) E isoforms, particularly apo E4, are a major risk factor for AD. Though there are several theories implicating apo E4 isoforms in AD, of particular interest is the interaction of apo E4 isoforms with tau kinases and phosphatases. Human SHSY-5Y neuroblastoma cells treated with apo E4 initially showed an increase in GSK-3β activity leading to apoptosis and tau hyperphosphorylation, and later a reduction in GSK-3β activity to compensate the effects of apo E4 and Aβ 1–42 on GSK-3β. Polymorphisms in the apo E4 gene could lead to deregulation of GSK-3β activity and might contribute to tau hyperphosphorylation and subsequently the pathological lesions seen in AD [77].

Further evidence for the role of the interaction of apo E4 and GSK-3β in tau phosphorylation comes from knockout studies involving reeler and yotari mice. Apo E and Reelin are ligands of the apo E receptor. They bind to the apo E receptor and recruit disabled protein, an intracellular adapter protein. This results in inhibition of GSK-3β activity. Mice lacking the apo E receptor ligand Reelin or lacking both Reelin and apo E show an increase in GSK-

3β activity and subsequent increase in site-specific tau phosphorylation. Mice lacking disabled protein showed the same results. The lack of binding of apo E and Reelin proteins to apo E receptor reduces recruitment of disabled protein, resulting in an increase in GSK-3β activity and subsequent tau phosphorylation [78].

Relationship of GSK-3β to neuronal cell death and survival pathways

Neuronal loss is associated with AD. However, what mechanisms underlie this neuronal loss and if neuronal loss is directly responsible for the progressive dementia of AD is not clear. There is substantial evidence for neuronal loss in AD relative to the level of cognitive impairment. Evidence that synaptic loss rather than neuronal loss is responsible for dementia has been presented. The attrition hypothesis states that activation of the effector caspase-6 in AD due to one or a variety of insults is responsible for the breakdown of the cytoskeletal structure of neurites and damages proper trafficking of proteins and organelles, thus resulting in the observed clinical and pathological features of AD [79]. GSK-3β is a molecule with an extremely diverse number of actions in intracellular signaling pathways, in addition to the roles already discussed above; this evidence has generated strong interest in other possible etiologies in the pathogenesis of AD apart from the traditional amyloidogenic hypothesis. GSK-3β has been shown to regulate cell survival by facilitating various pro-apoptotic pathways [17]. It is known to interact with the cell cycle via several pathways, including the Wnt and Hh pathways [80]. Given these data, there is a legitimate concern that inhibition of GSK-3β, if not completely specific, could have significant detrimental effects on the cell [18].

Several studies have shown the importance of hypoxic injury, oxidative stress, and reactive oxygen species (ROS) in the pathogenesis of neurodegenerative disorders. The interaction between GSK-3β activity and oxidative stress has been well described [81]. Transient hypoxia induces abnormalities seen in AD such as increases in phospho-tau expression, increased cleavage of APP into Aβ40 and Aβ42, and increased GSK-3β activity [82]. Oxidative stress and ROS might promote neurodegeneration and cell death through DNA fragmentation, lipid peroxidation, and mitochondrial pro-apoptotic pathways involving caspases and GSK-3β [83]. Efforts to understand the molecular mechanisms underlying the resistance of the murine hippocampal cell line HT22 to glutamate- and hydrogen-peroxide-induced oxidative stress have shown that reduced GSK-3β activity might be essential to the survival of those cells. Also, treatment of HT22 cells with the GSK-3β inhibitor lithium resulted in increased resistance to glutamate- and hydrogen-peroxide-induced oxidative stress [84]. Interestingly, there is some evidence that the inhibition of GSK-3β specifically protects cells from intrinsic oxidative stress [85].

Recently, great interest has been shown in the role of GSK-3β and its substrates in neuronal apoptosis and cell death. GSK-3β plays an important role in the PI3K and protein kinase Akt-mediated cell survival pathways. PI3K and its substrate Akt are essential for growth-factor-mediated neuronal cell survival. PC12 cells treated with nerve growth factor (NGF) showed reduced GSK-3β activity, and pretreatment with PI3K inhibitors resulted in the reversal of GSK-3β inhibition. Overexpression of GSK-3 in PC12 and rat fibroblast cells induced apoptosis which was reversed by inhibition of GSK-3β, suggesting that GSK-3β inhibition is central to the PI3K-Akt-mediated cell survival pathway. [86].

One of the mechanisms by which GSK-3β might potentiate apoptosis and cell death is by regulation of transcription factors. There are many transcription factors inhibited by GSK-3β, including heat shock factor-1 (HSF-1), cyclic-AMP-response element-binding protein (CREB) and nuclear factor kappa B (NFκB); these may play an important role in the apoptosis and cell death seen in AD [17]. NFκB is a transcription factor which has been shown to be a key mediator of responses to tumor necrosis factor alpha (TNF-α) [87, 88]. NFκB suppresses the signal for cell death [89]. GSK-3β stabilizes the p105 precursor of the NFκB p50 subunit in resting conditions, thus promoting cell survival, and it primes p105 for degradation after treatment with TNF-α [90]. Other groups have shown that inhibition of GSK-3β leads to the suppression of NFκB activity [91], and that this decrease in NFκB activity leads to an increase in caspase activity and an increased sensitivity of cells to TNF-α [76]. The ability of GSK-3β to increase the susceptibility of neurons to apoptosis assumes significance in the context that neuronal apoptosis plays a role in the pathogenesis of AD [16, 92–94].

Recently, much interest has been shown in the role of GSK-3β, Wnt signaling and Notch signaling in the pathogenesis of AD. Wnt in vertebrates is homologous to wingless in *Drosophila*. Wnt proteins are extracellular glycoproteins involved in cell fate decisions and pattern formation in *Drosophila* [95]. There is some evidence that these pathways are involved in the pathogenesis of AD [12, 13], and that this might involve the action of GSK-3β [13]. The Notch pathway is also involved in *Drosophila* cell fate determination. Presenilins, as a part of γ-secretase, are thought to be involved in the release of the Notch intracellular domain (NICD) and activation of the Notch signaling pathway. This raises the possibility of interaction between the Notch signaling pathway and APP cleavage at the level of presenilins and γ-secretase. The Notch and Wnt signaling pathways may also interact at the level of disheveled protein [13]. Altered neurogenesis in the

adult hippocampus has been shown to be associated with PS1 A246E mutation [96]. The interplay between Wnt signaling, Notch signaling, presenilins, APP processing and GSK-3β overactivity may contribute to the pathogenesis of AD.

GSK-3β inhibitors and therapeutic significance

While there has been an abundance of evidence which links GSK-3β activity *in vitro* to the pathogenesis of AD, the evidence regarding the *in vivo* situation remains equivocal. Although several groups have demonstrated increased levels of GSK-3β in AD brain [97, 98], there has been no evidence that the activity of GSK-3β is increased in this setting. One group has found repeatedly that while there is an approximate 50% increase in the amount of GSK enzyme in the postsynaptosomal supernatant of AD brain, there is no demonstrable increase in GSK activity compared with that of control brain tissue [97]. There is a theory that, in the AD brain, an overall reduction in phosphatase activity might make even a normal activity level of GSK-3β sufficient to hyperphosphorylate tau; however, this has not been substantiated experimentally. Other groups have shown that there is actually a decrease in GSK-3β activity in AD brain, while total protein expression remains unchanged [99]. More data from another study show that there is a strong expression of the inactive form of GSK-3β in AD plaques as well as a subpopulation of NFTs [100]. One study has shown some evidence that the active form of GSK-3β co-localizes with areas of granuvacuolar degeneration in AD plaques, but found only a very loose association with the NFTs [101]. Thus, there is still significant controversy regarding the actual *in vivo* role played by GSK-3β in AD. For this reason, as well as the possible detrimental effects of GSK inhibition, further investigation is warranted before GSK-3β inhibition is incorporated into the treatment of AD.

GSK-3β is of vital importance to brain function and development [75], and deletion of GSK-3β leads to embryonic lethality [76]. There is also evidence that GSK inhibition can be detrimental. Recently, inhibition of GSK-3β by excessive PI3K-Akt pathway activity has been shown to be associated with neurodegeneration in mouse models of Niemann-Pick type C disease [102]. Therefore, there is substantiation of concerns regarding GSK inhibition, such as that by lithium, as a treatment for AD.

Lithium has been in use for years in the treatment of mood disorders, prior to the knowledge of its exact signaling mechanism. Lithium has been shown to affect such varied processes as embryonic development, cell metabolism, neuronal communication, and cell proliferation. In the neuron, lithium can affect neurotransmitter release, neuron signal transduction, and expression of neurofilament proteins [41]. Efforts to understand the cellular targets of lithium have led to the interesting discovery that lithium specifically inhibits inositol monophosphatase [103], phosphomonoesterases [104], and GSK-3β [105, 106]. This inhibition of GSK-3β has been demonstrated to be through the competition of lithium with magnesium [107]. Other studies suggest that lithium might act through both direct competitive inhibition of GSK-3β and indirect inhibition through the activation of the PI3K-Akt pathway resulting in the inhibition of GSK-3β. Human neuroblastoma SH-SY5Y cells treated with lithium showed increased levels of phosphorylation at the Ser9 position of GSK-3β, suggesting an inhibitory state, without affecting the total GSK-3β level [108]. Lithium does have cellular effects outside those related to inhibition of GSK-3β, which are very important to examine and consider when discussing its use as a therapeutic agent.

There is some evidence that lithium has the potential to limit the pathological changes seen in AD associated with Aβ. Treatment of HEK293 cells, transfected by a stable Swedish mutant of APP, with lithium resulted in decreased production of total Aβ and Aβ1–42 without evidence of toxicity. Similar results were obtained by treatment of PDAPP mice with lithium chloride; the hippocampus of these mice showed a reduction in both Aβ isoforms [109]. Lithium also confers protection against Aβ-induced cell death in cultured cortical neurons and prevents Aβ-induced hyperphosphorylation of tau [110]. Further, transgenic mice overexpressing the human mutant tau protein, treated with lithium chloride showed a significant reduction in hyperphosphorylated tau and tangle formation which correlated with reduced GSK-3 activity [111]. Another, very recent, study has shown that lithium can decrease tau lesions by promoting ubiquitination of tau [112].

However, despite these promising results in animal models and cell line research, a recent case control study has shown an increased risk of the diagnosis of dementia in patients treated with lithium [113]. Although this may represent some effect of the underlying disorder for which lithium was prescribed, it does show that further research is needed before lithium can be recommended as prevention for AD. As mentioned earlier, one group has demonstrated that treatment with lithium can actually increase Aβ production [25].

There are several other GSK-3β small-molecule inhibitors, such as maleimide derivatives (SB-415286 and SB-216763), thiadiazolidinones and AR-A014418, which have been demonstrated to be neuroprotective [114, 115]. These may have more potential than lithium; however, this has to be confirmed through more investigations in transgenic animal models of AD. Recently, an increase in the total GSK-3β protein was demonstrated in white blood cells of patients with AD, and patients with mild cognitive impairment, compared with healthy elderly control subjects [116]. Thus, GSK-3β could potentially become a marker for disease in easily accessible tissue.

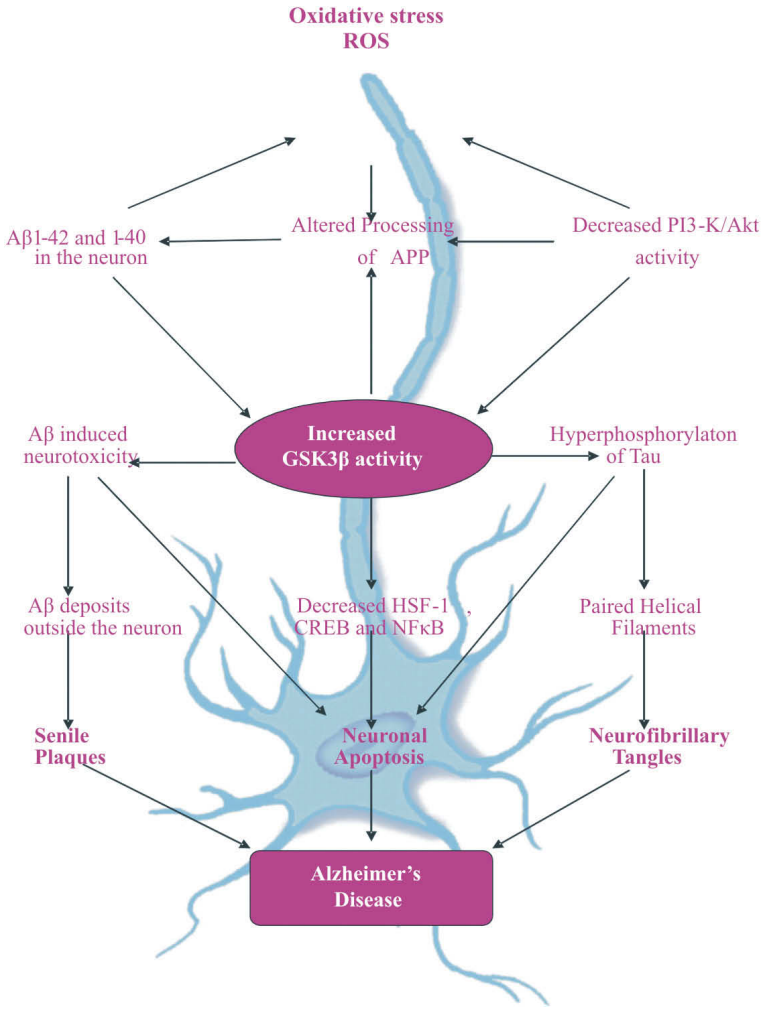

Figure 2. Role of GSK-3β in the pathogenesis of AD. ROS, reactive oxygen species; APP, amyloid precursor protein; PI3-K, phosphotidyl inositol 3 kinase.

The work done thus far in this field demonstrates the multifaceted involvement of GSK-3β in the pathophysiology of AD (Fig. 2). Given the complexity of the pathological changes and processes involved in AD, including insoluble aggregate formation, apoptosis, and oxidative stress, the ideal therapy will have to target a number of these processes. GSK-3β is an appealing therapeutic target for AD, and future work with GSK-3β inhibitors is promising.

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