Insulin Resistance and Alzheimer´s Disease: Molecular Links & Clinical Implications

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Abstract: Hyperinsulinemia as well as type II diabetes mellitus are among the risk factors for Alzheimer´s disease (AD). However, the molecular and cellular basis that link insulin resistance disorders and diabetes with AD are far from clear. Here, we discuss the potential molecular mechanisms that may explain the participation of these metabolic disorders in the pathogenesis of AD. The human brain uses glucose as a primary fuel; insulin secreted by the pancreas cross the blood-brain barrier (BBB), reaching neurons and glial cells, and exerts a region-specific effect on glucose metabolism. Glucose homeostasis is critical for energy generation, neuronal maintenance, neurogenesis, neurotransmitter regulation, cell survival and synaptic plasticity. It also plays a key role in cognitive function. In an insulin resistance condition, there is a reduced sensitivity to insulin resulting in hyperinsulinemia; this condition persists for several years before becoming full-blown diabetes. Toxic levels of insulin negatively influence neuronal function and survival, and elevation of peripheral insulin concentration acutely increases its cerebrospinal fluid (CSF) concentration. Peripheral hyperinsulinemia correlates with an abnormal removal of the amyloid beta peptide (Aβ) and an increase of tau hyperphosphorylation as a result of augmented cdk5 and GSK3β activities. This leads to cellular cascades that trigger a neurodegenerative phenotype and decline in cognitive function. Chronic peripheral hyperinsulinemia results in a reduction of insulin transport across the BBB and a reduced insulin signaling in brain, altering all of insulin's actions, including its anti-apoptotic effect. However, the increase in brain insulin levels resulting from its peripheral administration at optimal doses has shown a cognition-enhancing effect in patient with AD. Some drugs utilized in type II diabetes mellitus reduce cognitive impairment associated with AD. The link between insulin resistance and neurodegeneration and AD, and the possible therapeutic targets in preventing the insulin-resistance disorders are analyzed.

Keywords: Insulin, Insulin Resistance, Hyperinsulinemia, Diabetes, Cognition, Neurodegeneration, Alzheimer´s Disease.

A) GLUCOSE & INSULIN SIGNALING

1. Insulin and Insulin Receptor (IR)

Insulin is a small polypeptide, with a molecular weight of about 6,000 kDa, synthesized in significant quantities in β-cells of the pancreas. An increased level of glucose in blood stimulates β-cells in pancreas to secret insulin by exocytosis, which diffuses into the islets capillary blood [1]. Insulin provides both short-term and long-term homeostatic signals, since it is secreted acutely in response to an increase in blood glucose and because plasma insulin levels are directly correlated with the degree of long term increase in body adiposity [2,3].

Insulin exerts its effect on glucose uptake in peripheral tissue by binding to the Insulin Receptor (IRs), a cell surface protein, which belongs to the family of tyrosine kinase receptors. Binding of insulin leads to a rapid autophosphorylation on several tyrosine residues, which provide docking sites for adaptor proteins, such as the insulin receptor substrate (IRS) proteins. Docking of adaptor proteins induces the activation of downstream pathways such as the lipid

kinase phosphatidylinositol 3-kinase (PI3K) and the mitogen activation protein kinase (MAPK) cascade [4,5]. PI3K is associated with almost all of the metabolic actions of insulin (refer to Fig. **1**).

The effects of insulin on the central nervous system (CNS) are affected by its availability to this separate physiological compartment. The term BBB in its most restrictive sense refers to the vascular bed of the CNS, which is specially modified to prevent the unrestricted transfer of molecules between the blood and the extracellular fluid of the CNS [6]. The BBB plays a critical role in the transduction of signals between the CNS and peripheral tissues. It does so through several mechanisms, including the direct transport of peptides and regulatory proteins such as insulin [7].

There is solid evidence that insulin can cross the BBB by a saturable transport process mediated by the insulin receptor protein [6, 8–14]. This transporter is not uniformly distributed throughout the BBB [7]. There is also evidence of local insulin synthesis in brain [12, 14–16], although its function has not been elucidated. Therefore, the origin of brain insulin is a subject under investigation. IRs are present in the CNS, and were localized for the first time by ligand autoradiography and confirmed by immunohistochemistry and autoradiography [17–19]. CNS insulin receptors differ from their peripheral counterparts both in structure, function, and molecu-

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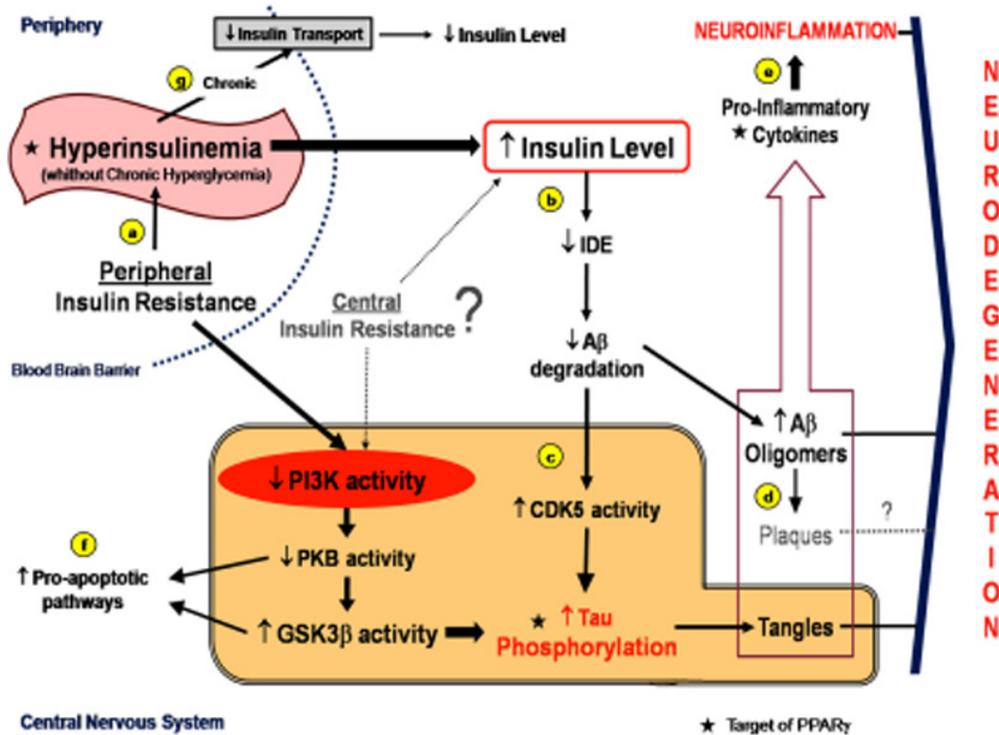
Fig. (1). Schematic representation of molecular pathways linking insulin resistance and neurodegeneration, and therapeutic target of PPARγ ligands.

(a) Peripheral insulin resistance results in periods of hyperinsulinemia to overcome the hyperglycemia. (b) Hyperinsulinemia, with the possible contribution of central insulin resistance, leads to increased insulin levels in the CNS, that kidnaps IDE resulting in a decrease of Aβ degradation, which upregulates cdk5 with consequent tau hyperphosphorylation, in the meantime that increases Aβ oligomers. (c) Peripheral insulin resistance is associated with decreased insulin signaling in neuron, that also can be a result of central insulin resistance, with decreased PI3K and PKB activity, leading an increased GSK3β activity. Cdk5 and GSK3β activities increase tau phosphorylation and tangles formation. (d) Aβ oligomers are more toxic than Senile Plaques, they are one of the responsible elements for activation of the innate immune response in the glia. (e) These oligomers are among factors that lead to Neuroinflammation. (f) Downregulation of PKB activity and a high GSK3β activity are also related with pro-apoptotic pathways that result in neurodegeneration. (g) Thus, chronic hyperinsulinemia (without hyperglicemia) results in down-regulation of insulin transporters in the blood brain barrier and decreases the levels of insulin in the CNS.

lar weight [20]. IRs are widely distributed in the brain with the highest concentration in the olfactory bulb, hypothalamus, cerebral cortex, cerebellum and hippocampus. They are expressed in all regions of brain in both neurons and glial cells. IRs are distributed throughout the neuronal processes and the cell body, but are concentrated in the synaptic endings [4, 21–24].

The signaling and biological effects of the insulin have been widely studied mainly in the "classical" insulin target tissues, e.g. liver, fat and skeletal muscle, with respect to glucose uptake, regulation of cell proliferation, gene expression and the suppression of hepatic glucose production [4]. However, insulin plays many roles within the CNS. It has been shown that some of the CNS effects of insulin are the opposite to those exerted in peripheral tissues. In particular, CNS insulin increases glucose and inhibits feeding, whereas serum insulin decreases glucose and increases feeding [14,25]. Thus, to some extent, insulin acts as its own counterregulatory hormone, with CNS insulin producing features of insulin resistance [6]. Thus, in the CNS, insulin participates in the regulation of feeding behavior and energy homeostasis, neuronal maintenance, neurogenesis, and neurotransmitter regulation. In addition, it has a role on cognitive functions as supported by neuronal activity, and in the control of aging-related processes [26].

2. Glucose, Insulin and Cognition

The brain consumes metabolic energy disproportionate to its size. It uses glucose (primary fuel), largely through oxidative metabolism. Glucose deprivation leads to coma, seizures, and produces potentially permanent brain damage. Glucose is continually supplied from cerebral blood flow and must be transported into the brain through the endothelial cells that form the BBB to reach neurons and glial cells by facilitated diffusion. Carriers of glucose are the glucose transport proteins (GLUT), which allow glucose entry into individual cells (neurons and glial cells) [27]. Animal models show that the cerebral blood flux depends on glucose levels in the blood stream; there is a compensatory mechanism to maintain adequate delivery of glucose fuel to the brain [28,29].

Evidence suggests regionally specific effects of insulin on brain glucose metabolism. Insulin does not seem to influence basal cerebral glucose metabolism or transport of glucose into the brain [30–35]. Insulin affects the use of glucose

in specific regions of the brain, most likely by selective distribution of insulin-sensitive GLUT isoforms, which overlap with the distribution of insulin and IRs in the brain [36–39]. Insulin, through the IRs localized in the hypothalamus, contributes to the regulation of food intake and energy homeostasis and leads to changes in body weight by anorexigenic or orexigenic effects produced by increasing or decreasing levels of insulin in brain respectively. Moreover, insulin in the CNS modifies peripheral glucose metabolism by increasing insulin sensitivity in peripheral tissue [10,40,41].

In brain, insulin is not a major regulator of glucose metabolism. *In vitro* studies showed that insulin regulates glucose uptake of glial cells, but did not influence neuronal glucose uptake [42,43]. Insulin can influence neurons directly by mechanisms unrelated to modulation of glucose uptake. Neurotransmitter release, neuronal-outgrowth, tubulin activity, neuronal survival and synaptic plasticity are all directly modulated by insulin [5, 44–46]

Studies in human and animal models have shown that an increase in brain insulin has a cognition-enhancing effect, independently of changes in peripheral glucose [47–51]. Moreover, there is cumulative evidence to support the effects of insulin and IRs on cognition, mediated by a modulatory role in learning and memory processes [1,4,5,26,52]. Insulin also modulates CNS concentration of neurotransmitters associated with important roles in cognition such as acetylcholine, norepinephine and dopamine [53,54]. It has been shown that, in an early stage of memory formation, an alteration of gene expression of IRs in the rat hippocampus in response to learning experiences occurs [55]. Taken together, all these findings suggest that insulin may influence normal memory function (Figs. **1** and **2**).

The biological basis of learning and memory processes resides in synaptic strength, where insulin signaling plays a modulator role on synaptic long-term potentiation (LTP) and long-term depression (LTD), two opposite forms of activity-dependent synaptic modifications. Insulin signaling modulates synaptic plasticity by: 1) Promoting the recruitment of GABA receptors on post-synaptic membranes; 2) Influencing NMDA receptor conductance (neuronal Ca^{2+} influx); and 3) Regulating AMPA receptor cycling [56–62].

B) INSULIN RESISTANCE

1. Insulin Resistance

Reduced sensitivity to insulin by the main target organs (liver, fat, muscle) is known as Insulin Resistance, where there is an elevated level of insulin in the bloodstream (hyperinsulinemia). This reduced sensitivity also results in impaired response to oral glucose (referred to as Impaired Glucose Tolerance), where it takes longer to restore normal glucose levels after eating. Peripheral Insulin Resistance is known to be the major contributor to the progression to hyperglycemia and Type II Diabetes Mellitus, since the pancreas can not secrete enough insulin to overcome the insulin resistance and prevent these events. Hyperinsulinemia without hyperglycemia is an indication of Insulin Resistance, a pre-diabetic condition. There is evidence of an increased risk of cognitive decline and neurodegeneration in populations with peripheral insulin resistance (hyperinsulinemia without hyperglycemia) [63–66].

2. Hyperinsulinemia/Hyperglicemia in the Brain

Alterations in circulating glucose levels can negatively affect the CNS because neurons have a consistently high glucose demand. Neuronal glucose uptake depends on extracellular glucose concentration, but chronic hyperglycemia results in cellular damage (glucose neurotoxicity). Therefore, a tight metabolic control is very important [67–69]. Studies on hyperglycemic rodents have shown cognitive impairment in addition to functional and structural alterations in the brain [68].

A growing body of evidence suggests that peripheral insulin abnormalities increase the risk for memory loss and neurodegenerative disorders such as AD, but acute and chronic hyperinsulinemia have opposing effects on memory performance (Fig. **2**). A possible explanation for that is discussed below. Chronic hyperinsulinemia has a negative influence on memory, since type II Diabetes Mellitus has been associated with long-term impairment in cognitive function in humans and animal model studies. On the other hand, acute increases of peripheral or brain insulin have an enhanced memory performance effect [47,49, 63, 70–73]. Prediabetic conditions with hyperinsulinemia but not chronic hyperglycemia may persist for many years before progression to type II Diabetes Mellitus. Hyperinsulinemia exposes the cells (including neurons) to unphysiologically high levels of insulin for a long period of time. Studies have shown that high concentrations of insulin affect the function and survival of neurons in culture by sensitizing them to toxin and stress-induced insults [74].
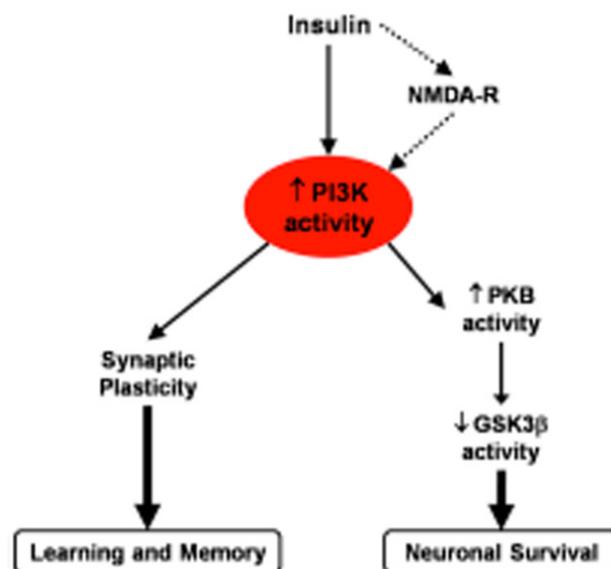
Fig. (2). Schematic representation of the relationships between insulin signaling, learning, memory and neuronal survival.
Acute high levels of insulin in the CNS have been related with the improvement of cognitive function and Neuronal Survival by a pathway that it involves the NMDA-R, PI3K and PKB activity and inactivation of GSK3β.

Moreover, a relationship between glycosylation of tau proteins, its aggregation ability and the neurodegenerative phenotype has been revealed [75]. Peripheral injection of

high doses of insulin in mice caused a rapid and dose-dependent increase in tau phosphorylation in the CNS [76]. *In vitro* studies indicate that insulin modulates the levels of Aβ peptide by promoting the release of intracellular Aβ. Physiological insulin levels promote Aβ clearance by peptide degradation, a mechanism that involves insulin degrading enzyme (IDE) activity (detailed below in section III.1) [77–79]. A low level of insulin in brain reduces Aβ release from intracellular to extracellular compartments and high levels reduce Aβ degradation in the extracellular compartment [80–84].

Impaired verbal memory has been reported in individuals with hyperinsulinemia and not chronic hyperglycemia [85]. Epidemiological studies have indicated that hyperinsulinemia, independent of glucose levels, constitutes one of the risk factors for dementia in the type II diabetes mellitus population as well as in the non-diabetic population, since the raising of peripheral insulin concentration acutely increases its concentration in the CSF and brain [63,65,70,86]. Prolonged peripheral hyperinsulinemia downregulates BBB functions and the IR activity and reduces insulin transport into the brain [86, 87]. Thus, hyperinsulinemia during the development of type II diabetes mellitus is neurotoxic. Development of these complications depend on the duration of a diabetic condition, upregulation of circulating glucose, glycosylated proteins, etc., and the quality of metabolic control [67].

Insulin resistance, hyperinsulinemia and type II Diabetes Mellitus are associated with elevated inflammatory markers and increased risk for AD [63,65,88–90] (Fig. **1**). In adults with type II Diabetes Mellitus and impaired glucose tolerance, an abnormal level of soluble TNF-R1 (death receptor domain) and TNF-R2 (cell survival) has been reported. Increased levels of TNF-R1 and decreased levels of TNF-R2 have also been observed in AD brain [91–93].

3. Impaired Glucose Uptake and Metabolism in the AD Brain

Studies using positron emission tomography (PET) have demonstrated that glucose metabolism is reduced markedly in the cerebral cortex in early stage AD and in the mild cognitive impairment (MCI) that is believed to be a precursor of AD, and that the reduction parallels the worsening of dementia symptoms, establishing that glucose uptake and metabolism are impaired in AD brain [94]. However, the causes of the impairment of glucose uptake/metabolism in AD brain are not well understood. Several findings suggest that this impairment is a cause, rather than a consequence, of neurodegeneration. The impaired cerebral glucose consumption is more severe than the impaired oxygen consumption in AD, suggesting that the former is not the result of the latter.

The brain is highly dependent upon glucose as a source of energy. The impaired glucose uptake/metabolism might lead to deficient synaptic activity and cellular homeostasis, as these are very sensitive to energy deficiency. Impaired glucose uptake/metabolism also causes reduced formation of acetyl-CoA and, consequently, affects the synthesis of acetylcholine [95]. Deficient activity of the cholinergic system is one of the most significant biochemical deficiencies in AD pathology, discovered decades ago [96], and is the basis of

the first generation of AD drugs, the cholinesterase inhibitors donepecil, galantamine and rivastigmine. The reduced cellular availability of acetyl-CoA may also cause decreased formation of both intracellular cholesterol and neurosteroids, which are the main lipid components of cell membranes. Membrane abnormalities have been observed in AD brains.

The Aβ peptide (or Aβ oligomers) has been shown to cause decreased glucose uptake/metabolism [97]. Furthermore, apolipoprotein E4 (apoE4) and diabetes mellitus are known to be major risk factors for AD [98]. Individuals with apoE4 alleles have lower brain glucose metabolism than those carrying apoE2 or apoE3 alleles. Diabetes patients are characterized by deficient glucose uptake/metabolism. Hence, it can be speculated that apoE4 genotype together with diabetes may increase the risk for AD by impairment in brain glucose uptake/metabolism.

C) LINK BETWEEN INSULIN RESISTANCE AND ALZHEIMER DISEASE

1. Relationships between Insulin Resistance and AD Pathogenesis

Abnormalities in insulin metabolism, characteristic of type II diabetes, are among the major factors thought to mechanistically influence the onset of AD. These abnormalities are thought to play a role in AD via their influence on the synthesis and degradation of Aβ and as a consequence of the cascade of neuronal alterations resulting from the effects of danger/alarm signals from oligomeric amyloid species (Fig. **1**) [99]. Additionally, recent studies have indicated that certain signal transduction pathways downstream of the insulin receptor may also promote the generation of Aβ peptides by modulating the cleavage of the parent Aβ precursor protein (AβPP) at the γ-secretase site, a cleavage site necessary for Aβ amyloidogenicity [100].

Although this evidence tentatively suggests that type II Diabetes Mellitus might play an important role in AD through mechanisms that involve Aβ peptide generation, alternative studies suggest that insulin may also provoke amyloid accumulation by limiting Aβ degradation via direct competition for the IDE. Another major substrate of IDE is the Aβ peptide. The IDE degrades monomeric but not the oligomeric Aβ peptide [77,78]. IDE is a zinc-metallo-peptidase that preferentially cleaves proteins with a propensity to form β-pleated sheet-rich amyloid fibrils, such as Aβ peptides. This relationship of IDE with Aβ is supported by recent evidence indicating that IDE activity in the brain is negatively correlated with Aβ content, and that IDE expression is decreased in the AD brain [83]. Insulin regulates the levels of IDE. Hyperinsulinemia reduces Aβ degradation by reduction of IDE levels and binding competition (Fig. **1**) [80–84].

It has been reported that Aβ40 and Aβ42 reduce insulin binding and insulin receptor autophosphorylation. The reduction in this binding seems to be caused by a decrease in the affinity of insulin to the insulin receptor. This suggests that Aβ is a direct competitive inhibitor of insulin binding and action [101], an aspect that demands further investigation.

The strikingly reduced CNS expression of genes encoding insulin, IGF-I, and IGF-II, as well as the insulin and IGF-I receptors in AD led to some authors to suggest that AD may represent a neuro-endocrine disorder that resembles diabetes mellitus [102]. In addition, the same researchers demonstrated that alterations insulin levels and insulin-like growth factor expression and deterioration of insulin function with the course of AD progression, were linked with an acetylcholine decrease in the brain [103]. There is evidence supporting the notion that high plasma insulin levels and peripheral insulin resistance affect Aβ42 levels, inflammation in the CNS and cognitive performance of individuals [104]. From such evidence, a model can be constructed describing how this metabolic profile contributes to the pathogenesis of AD. There are several etiological factors leading to the final common expression in the AD pathology [103].

Insulin plays an important role in memory and brain function in general. Peripheral hyperinsulinemia and insulin resistance induce a number of deleterious effects in the CNS that interfere with these functions, in a manner that is exacerbated by obesity and aging. In particular, effects on Aβ regulation and neuroinflammation are potential culprits in promoting aging-related memory impairment in some cases of AD (see Fig. **1**). This possibility has obvious relevance for adults with type II Diabetes Mellitus. However, it is worth noting that hyperinsulinemia and insulin resistance afflict many non-diabetic adults with conditions such as obesity, impaired glucose tolerance, cardiovascular disease, and hypertension [105–107].

Indeed, in recent years, cumulative evidence has been gained on the involvement of alteration in neuronal lipoproteins activity, as well as a role of cholesterol and other lipids in the pathogenesis of this neurodegenerative disorder. In relation to hypercholesterolemia, several reports have shown that elevated serum cholesterol levels and elevated levels of Aβ are linked with AD risk. Cholesterol influences the activity of the enzymes involved in the metabolism of the amyloid precursor protein and in the production of Aβ, but the mechanism by which cholesterol affects Aβ production and metabolism is not fully understood [108].

2. Metabolomics of Insulin in the Context of Neuronal Survival and AD Onset

To understand the link between insulin, insulin resistance, neuronal survival and AD onset, it is important identify the link between the key molecules involved in the intracellular pathways utilized by insulin to exert its effect. Insulin resistance in the periphery produces acute episodes of hyperinsulinemia without chronic hyperglycemia. High levels of insulin in plasma are correlated with high levels of insulin in brain (Fig. **1**) [86], leading to neurotoxic effects [74].

Studies in transgenic mouse models of AD have shown that diet-induced peripheral insulin resistance promotes amyloidosis suggesting that peripheral insulin resistance can influence Aβ production in the brain [84,109]. These findings in association of a reduced basal signaling of insulin in cortex with an increased degree of AD neuropathology argue that peripheral insulin resistance promotes neuronal insulin

resistance when genetic background predisposes to AD. However, further studies are necessary to clarify this.

Insulin binding to the IRs leads to autophosphorylation of the IRs which initiates several signaling cascades. One of these is the lipid kinase phosphatidylinositol 3-kinase (PI3K) cascade, which is associated with almost all of the metabolic action of insulin. PI3K activation leads to the activation of Protein Kinase B (PKB or Akt) and Glycogen Synthase Kinase 3 β (GSK3β). Activation of PKB inactives GSK3β by phosphorylation [110,111]. The neuroprotective effect of IGF-1 results from activation of PKB [112]. Expression of PKB protects neurons against toxin-induced death and protects PC12 cells against Aβ peptide-induced death [113–115].

Cole and coworkers have reviewed the molecular link between insulin action, diabetes and AD [5]. Insulin resistance in the periphery produces hyperinsulinemia, while in the brain it decreases IDE activity. The effects of IDE include abnormal Aβ removal and plaque formation, increased cdk5 activity, and increased activity of GSK3β (Fig. **1**). Dysregulation of cdk5 is a major molecular event in the pathway to neurodegeneration [116–118]. GSK3β activity has been implicated in the pathology of AD in different ways. AD brain exhibits a dysregulated expression of this kinase as well as changes in its activity [119,120], leading to hyperphosphorylation of tau and tangle formation. Several studies have shown that GSK3β activity is required for induction of neuronal apoptosis (refer to Fig. **1**), while inhibition of GSK3β promotes neuronal survival (illustrated schematically in Fig. **2**) (reviewed by Cole *et al,* 2007 [5]). Insulin signaling induces the phosphorylation and inhibition of GSK3β [110].

All of the negative effects of hyperinsulinemia in the CNS and their associated functions could be exacerbated or similarly produced by a central insulin resistance in conjunction with the peripheral insulin resistance or secondary to it. This is supported by the evidence of central molecular insulin resistance in a mouse model of AD with induced peripheral insulin resistance, where a reduced basal signaling (reduced phosphorylation of IR, PKB and GSK3, as well as decreased PI3K activity) was shown in the cerebral cortex (Fig. **1**) [84,109].

Studies of insulin action in brain are focused on the basic effects of insulin signaling. Insulin acts like a neurotrophic factor, since it promotes neuronal survival [46,121]. Studies *in vitro* of intracellular pathways utilized by insulin for synaptic plasticity have identified a link to neuronal protection against cell death [46,121]. Intracellular pathways utilized by insulin to influence synaptic plasticity and neuronal survival converge on the PI3K pathway (Fig. **2**) (reviewed by van der Heide *et al.* 2006 [1]). The increased catalytic activity of PI3K results in the phosphorylation and activation of anti-apoptotic substrates [1]. PKB is of major importance in mediating the effects of PI3K in neuronal survival, and *in vitro* and *in vivo* studies have shown that activated PKB protects against apoptosis. Dominant negative PKB does not [122–124]. Studies on insulin-facilitated LTP and LTD induction show that this process is completely NMDA receptor-dependent [62]. PI3K activity is required for LTP maintenance in the hippocampal CA1 region [125]. An increased

gene expression of IR in the rat hippocampus in response to learning experiences has been shown [55]. All together, the effect of insulin on neuronal survival and its effects on NMDA-dependent synaptic plasticity, require similar intracellular signaling routes (schematically illustrated in Fig. **2**).

Clinical Studies on Insulin Resistance and AD

In the last five years a growing body of evidence has been developed suggesting that glucose metabolism is associated with the pathogenesis of AD, age-related cognitive decline and neuroinflamation [90,126–128]. Intravenous insulin infusion in healthy, eugylcemic older subjects (a procedure known as hyperinsulinemic-euglycemic clamp) induces a facilitation of memory while it increases cerebrospinal fluid levels of $A\beta_{1-42}$ [126]. Insulin mediated memory facilitation is less marked at supraoptimal levels of insulin and in subjects with AD the hyperinsulinemic facilitation of memory is obtained at higher concentrations of insulin when compared with normal subjects. This phenomenon is more clear in patients who are not carriers of the APOE-ε4 gene [51].

The clinical effects of insulin and/or glucose administration on memory in patients with AD have been extensively reviewed [128,129]. As a result, it has been suggested that moderate administration of both glucose and insulin could improve memory in patients. These observations appear to be in disagreement with Gispen and Biessels [68] who described that both chronic hyperglycemia and chronic hyperinsulinemia are associated with accelerated cognitive decline in the elderly. This apparent discrepancy may be explained in part by the mechanisms underlying spontaneous chronic hyperglycemia and chronic hyperinsulinaemia during insulin resistance. Hoyer [130] has comprehensively addressed this issue in his recent review. He classified AD as an ''insulin resistant brain state''. This interesting hypothesis is supported by a recent clinical trial performed by Reger *et al.* [73] where the administration of intranasal insulin improved cognitive performance in elderly patients. In this pilot study, the authors administered 20 IU BID intranasal insulin to 13 patients and compared them with 12 controls receiving placebo. They found no changes in plasma levels of glucose and insulin with the treatment. However the insulin-treated group retained more verbal information after a delay and showed improved attention and functional status. Insulin treatment also increased the $A\beta_{1-40}/A\beta_{1-42}$ ratio in plasma. Previous studies by the same author [131] demonstrated that this effect was restricted to APOE-ε4 negative subjects.

Modulation of the response to insulin by the APOE genotype has led to speculation regarding whether APOE-ε4 is an independent risk factor for AD, so that people carrying this allele do not have a greater risk of being insulin resistant than the general population. In the other hand, people that are insulin resistant are at risk of developing AD regardless of their APOE-ε4 status [128]. The evidence that favors the role of insulin resistance in AD has opened new perspectives for treatment options. The family of antidiabetic Thiazolidinedione drugs function as agonists of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ and have been shown to improve sensitivity to insulin by decreasing circulating insulin and increasing insulin medi-

ated glucose uptake. In addition to these functions, they also have been demonstrated to diminish the levels of multiple inflammatory mediators (Fig. **1**) (reviewed by Jiang, 2008, Rojo *et al.*, 2008 [90,133]). Studies in cellular models have shown that PPARγ agonists down-regulate Aβ peptide deposition that occurs in AD, although the mechanisms of this phenomenon demand further investigation [133–135]. Troglitazone, a thiazolidinedione, significantly reduced phosphorylation of tau protein at Ser202 and Ser396/404, residues phosphorylated in early and later stages of neurofibrillary tangle accumulation in AD and other neurodegenerative disorders (Fig. **1**, PPARγ ligands) [136].

Interestingly, the PPARγ Pro12Ala polymorphism, which is associated with an increased risk of type II diabetes [137], has been found to influence plasma 24S-hydroxycholesterol/ cholesterol ratio in AD patients. This fact is important considering that elevated cholesterol in blood is a risk factor for AD and 24S-hydroxycholesterol is the major product of brain cholesterol metabolism and is released into the blood stream [108]. At present pioglitazone and rosiglitazone are the two thiazolidinediones that are available for clinical use. Both share the adverse side-effect of generating edema and potentially heart failure [138]. A recent meta-analysis [139] revealed a possible increased risk of myocardial infarction and a borderline increase in the risk of death from cardiovascular causes in patients receiving rosiglitazone. However, the significance of the data has been debated [140]. Thus, rosiglitazone as a possible therapy for AD is an active field of clinical investigation.

Rosiglitazone has been shown to preserve memory function in AD patients when compared with a placebo-assigned group. In a double blind trial, 20 mild AD or MCI patients who received 4 mg of rosiglitazone for 6 months showed improvements in memory and selective attention, associated with less reduction in plasma Aβ-40 and Aβ-42 when compared to 10 control patients [129]. This stabilization of plasma Aβ could be of importance, considering that plasma $A\beta_{1-42}$ decreases with AD progression, as previously described [82]. Another study on 511 AD patients treated with 2 mg, 4 mg or 8 mg of rosiglitazone or placebo found that there is an improvement in cognitive function when evaluated by the Alzheimer's Disease Assesment Scale-cognitive (ADAS-cog) for patients treated with 8 mg of rosiglitazone, and that this result is restricted to those patients who are not carriers of APOE-ε4 [141]. This modulation of the effect of rosiglitazone by the APOE genotype strongly resembles what has been observed for insulin and support the recommendation for APOE genotyping of patients at risk of cognitive decline [142]. Even though this evidence is still preliminary, it supports the role of PPARγ ligands with their dual action over insulin resistance and on neuroinflammation as a novel strategy for the treatment of cognitive decline associated with AD and stress the importance of further studies in this perspective.

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