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Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: Relevance to Alzheimer's disease

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Abstract. Interest in characterizing the role of impaired insulin actions in Alzheimer's disease (AD) and vascular dementia is growing exponentially. This review details what is currently known about insulin, insulin-like growth factor type I (IGF-I) and IGF-II proteins and their corresponding receptors in the brain, and delineates the major controversies pertaining to alterations in the expression and function of these molecules in AD. The various experimental animal models generated by over-expression, mutation, or depletion of genes that are critical to the insulin or IGF signaling cascades are summarized, noting the degrees to which they reproduce the histopathological, biochemical, molecular, or behavioral abnormalities associated with AD. Although no single model was determined to be truly representative of AD, depletion of the neuronal insulin receptor and intracerebroventricular injection of Streptozotocin reproduce a number of important aspects of AD-type neurodegeneration, and therefore provide supportive evidence that AD may be caused in part by neuronal insulin resistance, i.e. brain diabetes. The extant literature did not resolve whether the CNS insulin resistance in AD represents a local disease process, or complication/extension of peripheral insulin resistance, i.e. chronic hyperglycemia, hyperinsulinemia, and Type 2 diabetes mellitus. The available epidemiological data are largely inconclusive with regard to the contribution of Type 2 diabetes mellitus to cognitive impairment and AD-type neurodegeneration. A major conclusion drawn from this review is that there is a genuine need for thorough and comprehensive study of the neuropathological changes associated with diabetes mellitus, in the presence or absence of superimposed AD or vascular dementia. Strategies for intervention may depend entirely upon whether the CNS disease processes are mediated by peripheral, central, or both types of insulin resistance.

Keywords: Diabetes mellitus, insulin resistance, Alzheimer's disease, animal models, insulin like growth factor, signal transduction, review

1. Insulin and insulin-like growth factor regulate brain development and function

Insulin and insulin-like growth factor type 1 (IGF-I) are important modulators of growth and metabolic function in the central nervous system (CNS), and correspondingly, their receptors are abundantly expressed in the brain. Insulin and IGF-I are neurotropic since they can support neuronal growth, survival, and differentiation in the absence of other growth factors, and they promote neurite outgrowth, migration, protein synthesis, neuronal cytoskeletal protein expression, and nascent synapse formation [1–5]. In addition, IGF-I regulates oligodendrocyte survival, development, and myelination [6], while insulin regulates food intake, glucose homeostasis, growth, and metabolic activity [7].

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Previous studies demonstrated insulin immunoreactivity in CNS neurons distributed in the hippocampus, thalamus, hypothalamus, and amygdale of experimental animal [7] and human [8] brains by immunohistochemical staining, and insulin peptide in human brain homogenates by radioimmunoassay [8]. Data regarding the source of brain insulin is limited and somewhat inconclusive as to whether the immunoreactivity reflects transport and uptake from the blood, endogenous production, or both [7]. Although experimental evidence indicates that insulin can be transported through the blood-brain barrier into cerebrospinal fluid via receptor-mediated uptake [9], its subsequent localization in CNS neurons and glial cells was not demonstrated in those same studies. However, recent evidence suggests that the brain may utilize insulin from both locally produced and peripheral (pancreatic) sources for different functional requirements including cognition [10]. IGF-I and IGF-II are expressed in various regions of fetal [6,11] and adult brains [12]. IGF-I is developmentally regulated such that peak levels coincide with neuronal proliferation and neurite outgrowth [6, 13,14], whereas IGF-II is mainly expressed in cells of mesenchymal and neural crest origin [6].

Insulin and IGF-I receptors are expressed in neurons throughout the CNS, but they are most abundantly distributed in the olfactory bulbs, cerebral cortex, cerebellar cortex, hippocampus, thalamus, hypothalamus, brainstem nuclei, spinal cord, and retina [12,15,16]. IGF-I and IGF-II receptors are widely distributed in both fetal [6,11] and adult [12] brains, and previous studies showed that expression of these molecules is not modulated during development [6,13]. The co-expression of intracellular molecules that are critical for transmitting insulin and IGF-I stimulated signals [12, 15] indicates that both immature and mature CNS neurons are equipped to respond to insulin and IGF-I stimulation [12].

2. Insulin receptor substrate molecules

Insulin and IGF-1 mediate their effects on cell growth, survival, homeostasis, glucose transport, and energy metabolism by signaling downstream through insulin receptor substrate (IRS) molecules. IRS subtypes 1–4 have similar organizational structures in that each has a highly conserved N-terminus and a less conserved C-terminus that contains multiple tyrosine phosphorylation sites responsible for transmitting insulin and IGF-1 stimulated signals downstream to mediate a diverse array of cellular functions. The N-terminus contains three important functional domains including, one pleckstrin homology (PH) region, and two regions homologous to a phosphotyrosine binding (PTB) domain [17,18]. The PH domain mediates IRS interactions with Janus tyrosine kinase Tyk-2, and may also be important for linking IRS to signal transduction pathways that involve interactions with G proteins and phospholipids [18]. The PTB domain interacts with the β-subunit of the insulin and IGF-1 receptors. The C-terminal regions of IRS molecules function by interacting with src homology 2 (SH2) domain-containing proteins. The specificity of signals transmitted through IRS molecules is mediated by differential interactions between the PTB domain and the insulin or IGF-1 receptor, and variability in the structure of the C-terminal region which enables selective interactions between IRS molecules and SH2 domain-containing proteins that mediate particular cellular responses [18]. In addition, selective insulin and IGF-1 signaling responses are mediated by tissue-specific expression of the different IRS subtypes.

3. Insulin and IGF-I signaling mechanisms

The stimulatory effects of insulin and IGF-I are mediated through complex intracellular signaling pathways, beginning with ligand binding to the corresponding receptors and activation of the intrinsic receptor tyrosine kinases [19]. Insulin and IGF-I receptor tyrosine kinases phosphorylate a number of cytosolic molecules, including their major substrates, IRS proteins [20,21]. IRS signaling mechanisms are activated by tyrosine phosphorylation of specific motifs located in the C-terminal regions of the molecules. Tyrosine phosphorylated (TP) IRS proteins [17,18] transmit intracellular signals that mediate growth, metabolic functions, and viability by interacting with downstream molecules that contain SH2 domains [17,18], including the growth-factor receptor-bound protein 2 (Grb2), SHPTP-2 protein tyrosine phosphatase, and the p85 regulatory subunit of phosphatidylinositol-3 kinase (PI3 kinase) [22]. The binding of TP-IRS to Grb2 results in sequential activation of $p21^{ras}$, mitogen-activated protein kinase kinase (MAPKK), and Erk MAPK [18]. Erk MAPK activation directly contributes to insulin- and IGF-I stimulated mitogenesis, neuritic sprouting, and gene expression [23–25].

Binding of TP-IRS to p85 stimulates glucose transport [26] and inhibits apoptosis by activating

Akt/Protein kinase B [27–31] and inhibiting glycogen synthase kinase-3β (GSK-3β) [32]. Akt kinase inhibits apoptosis by phosphorylating GSK-3β [32,33] and BAD [34], rendering them inactive. Low levels of Akt kinase and high levels of GSK-3β activity or activated BAD are associated with increased neuronal death [31,35,36]. BAD inactivates anti-apoptotic Bcl-family proteins, rendering the mitochondrial membrane more susceptible to pro-apoptotic molecules that promote membrane permeability, cytochrome c release, and caspase activation [37]. Perturbations in mitochondrial membrane permeability increase cellular free radicals that cause mitochondrial DNA damage, impair mitochondrial function, and activate pro-apoptosis cascades [38,39]. Although insulin and IGF-I have virtually identical signaling cascades, their functions are overlapping but not entirely duplicative, and the corresponding receptors are expressed in different cell populations in the developing, mature, and aging CNS.

4. Experimental animal models of insulin receptor, IGF-I receptor, and insulin receptor substrate gene over-expression or depletion

The roles of insulin and IGF-I signaling in relation to CNS growth, development, and function were divulged in part by the analysis of transgenic and knockout mouse models. Targeted gene mutation studies demonstrated that IGF-I and IGF-II both stimulate prenatal brain growth, whereas only IGF-I stimulates postnatal brain growth [40]. During fetal development, insulin stimulated signaling via its own receptor appears to be uncoupled and instead, the insulin receptor is activated by IGF-II [40]. Therefore, using the targeted mutation approach, IGF-II was demonstrated to be a bi-functional ligand capable of activating both insulin and IGF-I signaling mechanisms in the immature brain, although IGF-II is not as effective as insulin for mediating growth, energy metabolism, glucose homeostasis, survival, and cognition [40].

Transgenic mice that over-express IGF-I have significantly larger brains due to increased populations of neurons and oligodendrocytes, as well as increased myelin content [2,6,11,41]. In contrast, genetic depletion of IGF-I or the IGF-I receptor, or over-expression of IGF 1 binding proteins (IGFBPs) that inhibit the actions of IGF-I, severely retards and impairs brain growth and development. These abnormalities are associated with reduced populations of neurons, deficiencies in myelination [2,4,6,42,43], and increased neuronal apoptosis [44] in the CNS. Homozygous knockout of the insulin receptor gene is lethal due to the severe diabetic ketoacidosis that develops during the early postnatal period; however, hemizygous knockout of the insulin receptor produces diabetes in 10% of the affected adults [45–48]. CNS depletion of the gene that encodes the neuronal insulin receptor results in increased food intake, obesity, and insulin resistance [45]. In that context, the CNS neuronal insulin resistance is associated with reduced activation of Akt, increased activation of GSK-3β, and hyper-phosphorylation of *tau* [49], similar to findings in AD and other neurodegenerative diseases.

Genetic depletion of the IRS-1 gene results in retarded somatic growth due to IGF-1 resistance. In addition, IRS-1 knockout mice exhibit substantial reductions in the masses of skeletal muscle, heart, and liver, and relatively small reductions in brain weight [50]. The relative sparing of brain was attributed to intact IGF-I stimulated brain growth [51], indicating that other IRS molecules can transmit IGF-I signals in the CNS. IRS-2, which mediates peripheral insulin actions and beta cell function in the pancreatic islets, is essential for glucose homeostasis. Depletion of the IRS-2 gene causes diabetes due to reduced beta cell mass. In addition, genetic depletion of IRS-2 impairs neuronal proliferation during development, and promotes accumulation of phosphorylated *tau* containing neurofibrillary tangles in the hippocampi of affected old mice [52, 53]. Therefore, this model links insulin resistant diabetes mellitus to AD-type neurodegenerative lesions in the brain. Genetic depletion of IRS-3 or IRS-4 does not produce an obvious phenotype [45].

5. Diabetes mellitus nomenclature

Diabetes mellitus is a metabolic disorder associated with chronic hyperglycemia. The various subtypes of diabetes mellitus differ with respect to etiology, pathogenesis, and insulin availability, but share the same consequences of chronic hyperglycemia and impaired insulin actions. Type 1 diabetes mellitus is caused by destruction (usually autoimmune) of pancreatic islet beta cells and attendant insulin deficiency. Type 2 diabetes, the most common form, is caused by insulin resistance in peripheral tissues, and is most frequently associated with aging, a family history of diabetes, obesity, and failure to exercise. Individuals with Type 2 diabetes have hyperglycemia and hyperinsulinemia. The pathogenesis of insulin resistance in Type 2 diabetes is not

completely understood. However, evidence suggests that the insulin resistance is partly mediated by reduced levels of insulin receptor expression (down regulation), insulin receptor tyrosine kinase activity, IRS-1 expression, and/or PI3 kinase activation in skeletal muscle and adipocytes [52]. Gestational diabetes develops during pregnancy, and usually is associated with insulin deficiency and hyperglycemia. Although gestational diabetes resolves postpartum, it places the affected individuals at risk for later developing Type 2 diabetes. Other less common causes of diabetes mellitus include: genetic defects in beta cell function or insulin action, diseases of the exocrine pancreas, e.g. pancreatitis or cystic fibrosis, endocrinopathies, drug or chemical toxicity, infection, and genetic syndromes, e.g. Down syndrome.

6. Possible relationship between diabetes mellitus and clinically detectable AD

Diabetes mellitus, regardless of subtype or etiology, is associated with a number of pathophysiological disorders including vascular disease, renal disease, peripheral neuropathy, and retinopathy, in part due to injury and functional impairment in the microvasculature supplying the corresponding organs and structures. The consequences of diabetes mellitus with respect to CNS function and disease have not yet been determined due to the lack of systematic and detailed clinical-pathological correlative study. Although in previous literature reviews, some authors attempted to draw an association between diabetes mellitus and dementia, the supporting epidemiological data were weak, since the studies that examined peripheral gluco-regulation in AD produced largely inconsistent results. While small percentages of patients with AD were found to have modest or moderate impairments in insulin sensitivity, the degrees of those abnormalities paled in comparison with the defects typically observed in Type 2 diabetes mellitus. In a retrospective study, a diagnosis of diabetes mellitus was recorded in 63 of 839 (7.5%) hospital records in which a clinical diagnosis of dementia had been rendered. Subsequent investigations demonstrated reduced blood glucose levels and increased insulin levels in patients with late onset AD relative to aged controls or patients with vascular dementia. Although the authors concluded that the findings did not support an association between diabetes and AD [54, 55], the same data were re-interpreted as reflecting an increased prevalence of insulin resistance in AD. The latter conclusion contradicts the finding that glucose administration could both increase plasma insulin levels and improve cognition in AD. Working under the assumption that increased insulin rather than glucose was responsible for the improvements in memory, further studies were used to demonstrate that the administration of either insulin or somatostatin significantly improved memory performance in early AD. In contrast, increases in plasma glucose that were not accompanied by increases in insulin levels were ineffective for improving memory/cognition [56,57].

The Rotterdam Study was one of the first epidemiological surveys to provide convincing evidence for a relationship between diabetes mellitus and dementia by demonstrating a significantly higher prevalence of dementia in patients with insulin-dependent (Type 1) diabetes mellitus relative to non-diabetic aged controls. The cause of dementia in diabetics was said to be mainly vascular in origin, but the prevalence of AD was also significantly higher than in the control group [58]. Further analysis of the data showed that diabetes mellitus doubled the risk for AD, particularly in individuals who required insulin [59]. One caveat regarding the interpretation of these results is that the Rotterdam Study was entirely based on clinical findings, and was particularly flawed because follow-up reports demonstrating accuracy of diagnosis were not published. The importance of conducting a thorough longitudinal analysis of the cases is underscored by the failure to detect a significant correlation between AD and diabetes mellitus in a recent retrospective postmortem study [60].

The possible association between diabetes mellitus/insulin resistance and degree of hippocampal and amygdalar atrophy was investigated *in vivo* by magnetic resonance imaging. Vascular morbidity was taken into account based on the presence and severity of carotid atherosclerosis, white matter lesions, and cerebral infarcts. The study showed that: 1) individuals with diabetes mellitus had greater degrees of hippocampal and amygdalar atrophy compared with subjects who did not have diabetes mellitus; and 2) severity of insulin resistance correlated with degree of amygdalar atrophy [61]. Unfortunately, the conclusion that diabetes mellitus is a causal factor in AD could not be substantiated because cognitive function was not assessed, and cerebral microangiopathy, the likely vascular lesion produced by diabetes mellitus, was not detectable with the methods employed. Moreover, the study did not exclude the co-occurrence of vascular dementia.

The inability to convincingly and consistently demonstrate a correlation between diabetes mellitus

and AD, or find evidence that diabetes mellitus causes AD pathology, led to the alternative hypothesis that diabetes may serve as a co-factor in the pathogenesis of dementia and possibly AD. In this regard, epidemiological studies showed that hyperinsulinemia in patients with an ApoE4-negative genotype was correlated with AD-type dementia, whereas in the absence of diabetes, an ApoE4+ genotype was also correlated with AD [62–64], suggesting that ApoE4 genotype and diabetes mellitus contribute independently to the pathogenesis of AD. Quite different results were obtained in another study in which a greater than two-fold higher risk for developing AD was detected in subjects who had diabetes mellitus and an ApoE4 allele compared with individuals who had an ApoE4 allele but who did not have diabetes. Correspondingly, postmortem studies showed that individuals with diabetes mellitus and an ApoE4 genotype had significantly more abundant Abeta deposits and neurofibrillary tangles compared with diabetics who did not have an ApoE4 allele [65]. Together, these results suggest that diabetes mellitus may represent a risk factor for AD by acting synergistically with ApoE4.

Problems documenting the relationship between diabetes mellitus or insulin resistance and AD stemmed in part from the poor reliability of clinically distinguishing AD from vascular dementia. Diabetes mellitus causes microvascular disease resulting in sclerosis and luminal narrowing of arterioles and capillaries. Therefore, diabetes-associated microangiopathy could cause chronic cerebral ischemia due to hypoperfusion, leading to permanent tissue injury and dementia [66]. Similarly, multi-focal micro-ischemic injury and infarcts caused by hypertensive cerebrovascular disease or vascular occlusions could also contribute to the clinical deterioration and the pathological changes in AD [67]. Another missing component of the equation is a thorough characterization of the CNS pathology produced by long-standing diabetes mellitus, in the presence or absence of AD. Until such information becomes available, the correlations among diabetes mellitus, CNS pathology, and dementia will remain confusing.

7. Evidence for impaired insulin responsiveness in AD

Some of the earliest work on senile dementia, which probably corresponded to AD, vascular dementia, or a combination of both, documented the development of altered brain metabolism soon after the onset of clinical symptoms. The metabolic abnormalities consisted of impaired glucose utilization and energy metabolism, with features that resemble Type 2 diabetes mellitus [68]. In addition, several studies demonstrated that cerebral metabolism declined prior to the deterioration in cognitive function, suggesting that energy failure was one of the earliest reversible hallmarks of AD. These observations led to the hypothesis that AD-associated abnormalities in energy metabolism were caused by insulin resistance or reduced insulin action in the brain, i.e. brain diabetes [69–72].

7.1. Glucose regulatory defect in AD

Glucose, transported from peripheral blood, is the major fuel for oxidative metabolism and function in the CNS. Although previous studies suggested a role for astrocyte-derived lactate as a major energy source for neurons [73], subsequent research casts doubt on the relative importance of this concept [74,75]. Glycemic index studies demonstrated that AD patients had significantly higher levels of plasma glucose [76], and that non-diabetics with either vascular dementia or late-onset AD, had significantly elevated fasting plasma insulin and glucose levels relative to controls [77]. Although this profile of hyperglycemia plus hyperinsulinemia resembles Type 2 diabetes mellitus, investigators reported that glucose administration improved memory due to facilitation of acetylcholine synthesis and release in the brain [78]. However, further studies demonstrated that patients with very early AD, but not those in the late stages of disease, exhibited significant improvements in memory following a rapid therapeutic increase in plasma glucose to 225 mg/dl, with an accompanying increase in plasma insulin [79,80]. Together, these observations suggest that in early AD, the prominent gluco-regulatory abnormalities in the brain may be responsive to peripheral glucose administration at levels that drive insulin release into the plasma.

7.2. Evidence for insulin resistance in AD

Initial evidence that abnormalities in insulin action or insulin receptor expression/function were features of AD stemmed from the findings that individuals in the early stages of AD had 45% lower levels of cerebral glucose utilization, and 17–18% reductions in cerebral blood flow and cerebral metabolic rate of oxygen relative to controls. However, in the late stages of AD, the major metabolic/physiological abnormality was markedly reduced (55–65%) cerebral blood

flow [81]. Subsequent research confirmed the finding that brain glucose metabolism was reduced in AD, and also showed that cerebrospinal fluid (CSF) amino acid and ammonia levels were markedly increased in patients with early-onset AD [82]. The authors interpreted the increased CNS protein catabolism as reflecting a compensatory response to the 44% reduction in glucose metabolism [82].

7.3. Controversies and inconsistencies

Initial studies designed to examine CSF and plasma levels of insulin in AD demonstrated increased CSF insulin levels after an overnight fast, and increased plasma insulin levels after oral glucose administration [83]. However, subsequent studies failed to find a correlation between increased CSF insulin levels and AD [84], or detect significant alterations in plasma insulin levels in AD following intravenous administration of glucose [85]. Moreover, other investigators detected lower CSF insulin, higher plasma insulin, and reduced CSF-to-plasma insulin ratios in AD relative to normal controls, with larger differences detected in the late stages of AD [86]. In other studies, hyperinsulinemia was detected in AD, but the abnormality was attributed to their significantly larger mean body masses [87,88] and significantly greater food intake [88], suggesting that hyperinsulinemia in AD could be explained on the same basis that it occurs in Type 2 diabetes mellitus.

8. Potential mediators of impaired insulin responsiveness in AD

8.1. Growth factor deficiency as a mechanism of neurodegeneration

Impaired insulin responsiveness or insulin resistance in AD could be caused by reduced local CNS levels of insulin. The possibility that AD-type neurodegeneration represents a neuroendocrine disorder with major abnormalities centered in the hippocampus and hypothalamus was investigated nearly two decades ago. In 1986, it was suggested that some of the AD-associated clinical and pathological abnormalities were due to global defects in CNS and endocrine somatostatin, somatostatin-regulated growth hormone, thyroid-stimulating-hormone, somatomedin and insulin, with associated impairments in glucose metabolism [89]. Immunohistochemical staining studies demonstrated increased IGF-I immunoreactivity in astrocytes of AD brains [90], and higher intensities of insulin and c-peptide immunoreactivity in pyramidal neurons of AD relative to control brains, despite aging-associated reductions in insulin and c-peptide concentrations. However, radioimmunoassay studies demonstrated similar mean levels of somatostatin and IGF-I in AD and control CSF samples, and biochemical studies detected similar levels of insulin and c-peptide in AD and normal aged brains [91,92]. Additional evidence favoring the concept that AD represents a neuroendocrine disorder was provided by the finding that plasma IGF-I levels were significantly reduced in familial AD associated with the Swedish amyloid precursor protein mutation [93]. The lack of consistency in results obtained from different studies made it difficult to champion the concept that AD may represent a neuroendocrine disease.

8.2. Altered receptor binding in AD

Another potential cause of impaired insulin or IGF-1 responsiveness is reduced binding of the ligand to its receptor due to lowered affinity, insufficient availability of ligand (see above), or decreased receptor expression. Initial studies utilized radioimmunoassays to assess the role of impaired growth factor receptor expression and function in relation to AD. 125I-IGF-I binding studies using postmortem brain tissue demonstrated increased IGF-I binding in the cerebral cortex of AD relative to control subjects, suggesting that IGF-I receptors were up-regulated due to reduced local levels of IGF-I [94, 95]. However, in subsequent studies, similar IGF-I binding kinetics and receptor abundances were measured in AD and control brains [96,97], although increased IGF-I binding was observed in neuritic plaques which were more abundant in AD [96]. Commercial availability of insulin and IGF-I receptor antibodies enabled receptor expression to be characterized in histological sections. In one study, a selective reduction in insulin receptor expression was detected in the substantia nigra of Parkinson's disease cases relative to other diseases and normal aging [98]. Immunohistochemical staining studies demonstrated aging-associated reductions in insulin receptor expression in the brain, but in AD, insulin receptor expression was increased while IGF-I receptors were unchanged relative to control brains [8,99]. Despite the increased insulin receptor expression, tyrosine kinase activity was reduced in AD [8,99]. One mechanism proposed for the increased insulin receptor expression and reduced tyrosine kinase activity was Abeta-induced insulin resistance with compensatory up-regulation of the receptor [100].

8.3. Impaired signaling through insulin stimulated pathways in AD brains

Much of the discussion concerning the role of impaired insulin signaling in AD has been focused on insulin resistance, meaning that CNS cells respond poorly or not at all to physiological levels of insulin. With the lack of consistent results regarding possible alterations in growth factor and growth factor receptor expression in AD, attention was refocused on detecting abnormalities in the functional activation of insulin and IGF-I signaling mechanisms. Studies in experimental animals demonstrated aging-associated reductions in insulin stimulated tyrosine phosphorylation of both the insulin receptor and Shc, and reduced associations of Shc with Grb2 in the forebrain cortex and cerebellum. In addition, progressive aging was found to be associated with reduced expression of SHP protein tyrosine phosphatase-2 (SHP2), which negatively regulates insulin signaling [101]. Presumably, this observation reflects a compensatory/homeostatic response to the aging associated reduction in tyrosine kinase activity. Studies of human postmortem brains demonstrated reduced levels of tyrosine kinase activity in late onset AD relative to aged controls [8,99]. Potential consequences of impaired insulin signaling in AD include, reduced glucose utilization, increased GSK-3β activation, deficits in energy production, increased oxidative stress, reduced neuronal survival, and advanced glycation of proteins [102].

9. Insulin and IGF-I signaling and mal-signaling in the brain: Contributions to AD-pathology

9.1. Role in tau phosphorylation and hyper-phosphorylation

Insulin and IGF-1 promote neuronal survival, stimulate energy metabolism, provide neuroprotection, and support neuronal cytoskeletal function via phosphorylation of its subunit proteins. Phosphorylation of *tau* is a normal physiological process required for cytoskeleton assembly and stabilization. *In vitro* experiments demonstrated that *tau* phosphorylation is normally regulated by insulin and IGF-I [103]. The major kinases responsible for physiological phosphorylation of *tau* include: Erk MAPK and cyclin dependent kinase 5 (Cdk-5), both of which are activated by insulin and IGF-1 [104–106]. However, impaired insulin or IGF-1 signaling can result in the hyper-phosphorylation of *tau* due to reduced activation of PI3 kinase and Akt and attendant increased levels of GSK-3β activity [49, 53]. In addition, GSK-3β can be activated through inhibition of insulin/IGF-1 signaling through the Wnt pathway [107], which is not dependent on PI3 kinase or Akt phosphorylation. Impairments in Wnt signaling mechanisms have been linked to several of the key molecular abnormalities in AD [108–112].

GSK-3β is a multifunctional serine/threonine kinase that regulates many intracellular signaling pathways, including receptor tyrosine kinases, G-protein-coupled receptors, and responses to Wnt. GSK-3β is functionally important for regulating glycogen metabolism, cell cycle kinetics, proliferation, survival, and cell migration. These effects are mediated by growth factor stimulated phosphorylation and attendant inhibition of GSK-3β activity [107]. Intact insulin signaling is important for promoting neuronal survival and energy metabolism, whereas impaired insulin signaling in CNS neurons results in increased GSK-3β activity, which leads to *tau* hyper-phosphorylation. In addition, GSK-3β can be activated by hypoxic, ischemic, or metabolic injury, irrespective of growth factor stimulation [113,114]. Hyper-phosphorylated *tau* fails to be transported into axons and instead accumulates and aggregates in neuronal perikarya, and thereby promotes further oxidative stress [115] which can cause cell death mediated by apoptosis, mitochondrial dysfunction, or necrosis. The neuronal cytoskeletal lesions that correlate with dementia in AD contain hyper-phosphorylated *tau*.

Although the mechanisms of increased GSK-3β activation in AD can be readily explained on the basis of impaired insulin/IGF-1 signaling, the increased levels of Erk MAPK [116], Akt [117,118], and Cdk-5 [119] detected in AD brains cannot be attributed to these abnormalities. However, as noted above, GSK-3β can also be activated by oxidative stress. Review of the literature revealed that in addition to growth factor stimulation, Erk MAPK [120–122], Akt [121, 123,124], and Cdk-5 [113,114] activities also can be increased in response to oxidative stress. Therefore, the apparently paradoxical increases in the activities of these kinases in AD, most likely reflect either chronic or perimortem oxidative injury. In essence, impaired insulin/IGF-1 signaling mechanisms could lead to neuronal oxidative stress with attendant activation of the kinases that principally contribute to pathogenic *tau* hyper-phosphorylation.

9.2. *Role in amyloid precursor protein and amyloid beta processing and pathology*

Insulin affects the metabolism of amyloid beta (Abeta) peptide, the main constituent of the amyloid deposits that accumulate in brains with aging or AD, by accelerating betaAPP/Abeta trafficking to the plasma membrane from the trans-Golgi network, where it is generated. In addition, insulin increases extracellular levels of Abeta by promoting its secretion and inhibiting its degradation by insulin-degrading enzyme. These effects of insulin on betaAPP metabolism are mediated by downstream signaling through Erk MAPK [125,126]. Therefore, impaired insulin signaling can disrupt the normal physiological processing of betaAPP. At the same time, Abeta can adversely affect insulin signaling by competing with and inhibiting insulin binding or reducing the affinity of insulin binding to its own receptor [100,127]. This suggests that Abeta accumulations can promote *tau* hyperphosphorylation and the formation of the AD dementia associated paired helical filament-containing neuronal cytoskeletal lesions (neurofibrillary tangles, neuritic plaques, and neuropil threads) through functional impairment of the insulin signaling cascade, leading to increased levels of GSK-3β activity.

In vitro experiments demonstrated that Abeta can be neurotoxic [128], and that Abeta-induced toxic death of cultured hippocampal neurons can be prevented by pre-treatment with IGF-1 $>>$ IGF-II [129,130]. In this context, the neuro-protective actions of IGF-I are produced by increased signaling through PI3 kinase-Akt and suppression of GSK-3β activity [131]. However, the neuroprotective actions of IGF-1 are not specific to Abeta-induced neuronal injury and death, since similar effects occur with ethanol-induced neurotoxicity, which impairs insulin signaling and mitochondrial function [132,133]. Neuronal rescue from these adverse effects of ethanol is also mediated by IGF-1 activation of PI3 kinase-Akt and inhibition of GSK-3β [134]. Thus, the neuroprotective actions and potential therapeutic benefits of IGF-I in relation to both Abeta-induced neurotoxicity and impaired insulin signaling have been well documented.

9.3. *Potential role of insulin degrading enzyme in the amyloid precursor protein/amyloid beta* (*Abeta*) *peptide processing*

The study of amyloid-beta (Abeta) peptides and their potential role in neurodegeneration has overwhelmingly dominated research on the pathogenesis of AD. This tidal wave of investigation was unleashed by the characterization and cloning of amyoid precursor protein (APP) in 1984 by Glenner and Wong [135]. However, since the vast majority of AD cases do not exhibit strong genetic inheritance patterns and are not associated with mutations in the APP gene, research efforts have been re-directed toward understand the mechanisms of aberrant cleavage and processing of APP that could result in the accumulation of neurotoxic forms of Abeta. In addition, experimental models have been extensively utilized to identify potential mechanisms by which Abeta exerts its neurotoxic effects [129,136]. One popular theory is that proteolytic enzymes known as secretases malfunction or exhibit altered expression patterns, resulting in aberrant APP cleavage and local accumulations of neurotoxic Abeta peptides [128]. More recently, investigators demonstrated that Abeta(1-40) and Abeta(1-42), the main physiological C-terminal cleavage products of APP, reduced insulin binding and insulin receptor auto-phosphorylation due to reduced affinity of insulin binding to its own receptor [100]. Although corresponding human brain data are lacking, these findings have been used to support the argument that Abeta accumulation impairs insulin signaling in AD. The mechanism requires validation, and additional information is needed to determine if Abeta has agonist or antagonist properties in relation to the insulin receptor and insulin-degrading enzyme.

Insulin degrading enzyme (IDE) catalyzes insulin degradation and thereby negatively regulates insulin signaling. Recent studies demonstrated that IDE can also degrade soluble Abeta [137], and therefore may be important for regulating extracellular levels of soluble Abeta [138–141]. In this regard, in situ tissue based studies demonstrated increased IDE immunoreactivity around senile plaques [142], and reduced IDE expression in AD hippocampi [143]. Moreover, transgenic mice that over-express mutant IDE have hyperinsulinemia, glucose intolerance, and increased levels of Abeta in the brain. These observations provided additional evidence supporting a role for impaired insulin signaling in the pathogenesis of Abeta accumulations in the brain. Objectively speaking, if accumulated Abeta interfered with IDE function, one would predict the outcome to be increased rather than decreased insulin actions in the CNS. On the other hand, if Abeta accumulation reduced the affinity of insulin for its receptor, that phenomenon could contribute to insulin resistance in AD brains.

In a rat model of Type 2 diabetes mellitus, a small chromosomal region containing a mutant IDE allele was shown to be associated with hyperinsulinemia and glucose intolerance. In addition, the partial loss of IDE function was associated with increased neuronal Abeta secretion, although Abeta did not accumulate in the CNS. The authors concluded that the otherwise normal brain can compensate for defects in Abeta secretion [144]. However, in mice that had homozygous deletions of the IDE gene, Abeta degradation in both the brain and cultured cortical neurons was reduced by at least 50%, endogenous Abeta levels were increased in the brain, and the animals exhibited hyperinsulinemia and glucose intolerance. In addition, the mice had elevated levels of the intracellular signaling domain of APP, which is ordinarily degraded by IDE [145]. In contrast, overexpression of IDE and APP together in CNS neurons, resulted in significantly reduced levels of Abeta, reduced formation of Abeta-positive plaques, and rescue of premature lethality in the APP transgenic mice [146].

The finding that Abeta degradation was mediated by IDE prompted human genetic studies to determine if familial AD was linked to mutations or polymorphisms in the IDE gene, which is located on Chromosome 10. Initial studies using parametric and nonparametric analyses demonstrated a significant linkage between familial late-onset AD and markers that map near the IDE gene (D10S1671, D10S583, D10S1710, and D10S566) [147]. In addition, the authors claimed to have found evidence for an allele-specific association between the putative disease locus and marker D10S583, which maps within 195 kB of the IDE gene [147]. However, those findings could not be confirmed by an independent gene linkage analysis [148], and in another study, although a linkage of late onset AD with D10S583 was demonstrated, the linkage was not related to the IDE gene [149]. Finally, single nucleotide polymorphisms and mutations in the IDE gene were not found to be linked to late onset AD [150].

10. Chemical depletion of insulin-responsive cells in the CNS: Relevance to AD

Reflection on some of the earlier findings in AD, including the impaired glucose utilization, mitochondrial dysfunction, reduced ATP production, and energy shortage, prompted consideration of the hypothesis that these abnormalities were mediated by desensitization of the neuronal insulin receptor [151–154]. The stated metabolic abnormalities, as well as several of the classical histopathological lesions of AD, could be attributed in part to reduced insulin levels and reduced insulin receptor function in AD. Hoyer was among the first to suggest that reduced levels of brain insulin may precipitate a cascade resulting in disturbances in cellular glucose, acetylcholine, cholesterol, and ATP levels, impaired membrane function, accumulation of amyloidogenic derivatives, and hyper-phosphorylation of *tau*, i.e. that AD may represent a brain form of Type 2 diabetes mellitus [155,156].

Streptozotocin (2-Deoxy-2 [methyl-nitrosoamino) carbonyl] amino D-glucopyranose), is a glucose analog that is metabolized to a cytotoxic compound. Streptozotocin (STZ) is particularly cytotoxic to beta cells in the pancreatic islets, and consequently it is used to generate experimental models of diabetes mellitus. Intracerebroventricular injection of STZ chronically reduces glucose and glycogen metabolism by 10–30% in the cerebral cortex and hippocampus [157]. These effects are associated with significantly reduced brain glucose utilization and oxidative metabolism [158], inhibition of insulin receptor function despite increased binding [159], and impaired learning [160]. The STZ injected rats exhibit long-term and progressive deficits in learning, memory, cognitive behavior, cerebral energy balance [161]. Therefore, this model provides a close match with the biochemical and physiological abnormalities observed in AD.

11. Potential therapeutic role for insulin sensitizers in the treatment of AD

A major risk factor for developing Type 2 diabetes in obese subjects is the accumulation of lipids in non-adipose tissues, including skeletal muscle. Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors that regulate lipid metabolism. Ligand activation of PPARs can enhance insulin signaling and reduce tissue accumulations of lipids. PPAR-α is a member of the steroid hormone superfamily of ligand-inducible transcription factors, involved in glucose and lipid metabolism. Thiazolidinediones are PPAR ligands that improve insulin sensitivity, reduce lipid content in skeletal muscle and other non-adipose tissues, and reduce peripheral glucose levels in patients with Type 2 diabetes mellitus [162]. In a rat model of Type 2 diabetes, PPAR ligand treatment resulted in reduced body weight and intracellular lipid content, and increased insulin sensitivity [163]. One potential

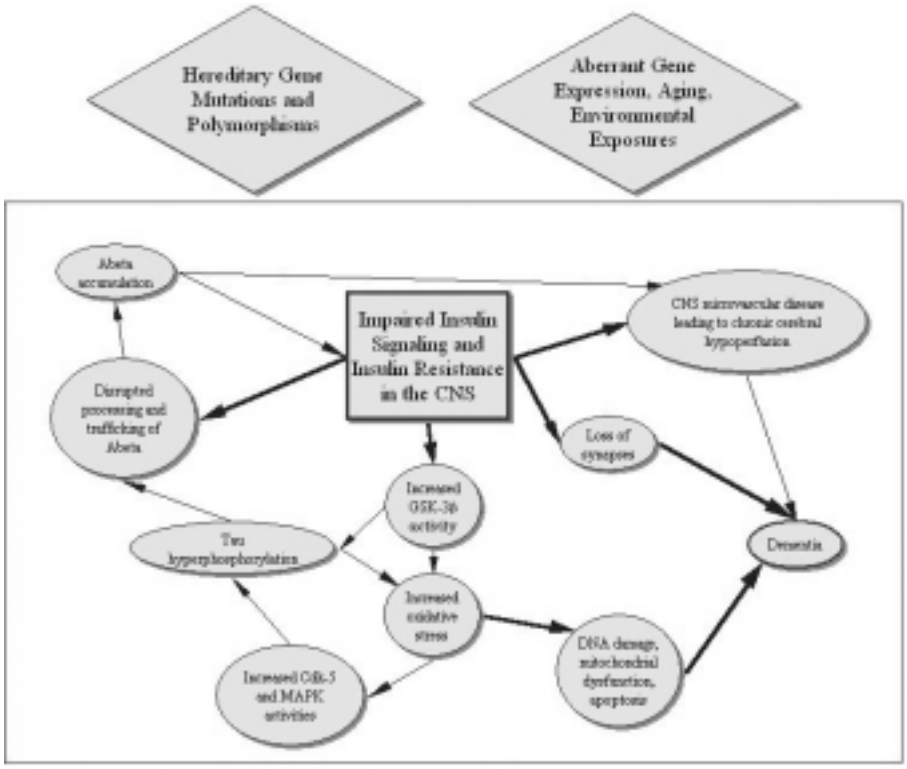

Fig. 1. Proposed Mechanisms Linking Impaired Insulin Signaling and Insulin Resistance in the CNS to the Pathogenesis of AD. Our proposed model puts insulin resistance and impaired insulin (and probably IGF-1) signaling in the CNS at the core of the disease process leading to neurodegeneration. A major consequence of impaired insulin signaling is increased GSK-3β activity which results in both increased oxidative stress and *tau* hyper-phosphorylation. Oxidative stress can result in increased Cdk-5 and MAPK activities, which also contribute to *tau* hyper-phosphorylation. Severe or sustained oxidative injury leads to mitochondrial DNA damage, mitochondrial dysfunction, and apoptosis, and the attendant cell loss and impaired neuronal function lead to dementia. Abeta accumulation and toxicity can be linked to cellular metabolic disturbances caused by *tau* hyper-phosphorylation or impaired insulin signaling which disrupts processing and secretion of Abeta. Abeta accumulation can inhibit insulin actions and binding, and can also be neurotoxic. CNS microvascular disease caused by impaired insulin signaling or insulin resistance (either related to peripheral diabetes mellitus or intrinsic CNS disease), Abeta deposition, or systemic hypertension, could contribute to AD dementia due to chronic cerebral hypoperfusion and micro-ischemic injury. Finally, since insulin actions contribute to synapse formation and synaptic function, impaired insulin signaling could result in loss of synapses and thereby contribute to dementia. Hereditary gene mutations and polymorphisms can serve as primary transducers at various points within this cascade, but these factors account for a relatively small percentage of the cases. In sporadic disease which accounts for the vast majority of cases, aberrant expression of genes that impair insulin signaling in the CNS, aging, environmental exposures, and systemic disease, e.g. Type 2 diabetes or hypertension, are likely to have contributory or combined causal roles in the pathogenesis of AD. This model predicts that the most effective therapeutic intervention would be produced by alleviating the CNS insulin/IGF resistance and impaired insulin/IGF signaling rather than at the level of any single or particular downstream abnormality.

mechanism of impaired energy metabolism and glucose utilization in AD is insulin resistance secondary to altered PPAR expression. In this regard, genetic analysis identified two polymorphisms located in Exon 5 and Intron 7 of the PPAR-α gene. These polymorphisms have already been investigated for their possible association with AD and for their effect in carriers of an insulin gene polymorphism (INS-1). The PPAR-α C $\rightarrow$ G polymorphism in Exon 5 (L162V) was associated with AD, in that the V-allele was more frequent in AD patients than in healthy subjects. Further analysis revealed an increased risk for AD among carriers of the PPAR-α L162V V-allele or INS-1 allele [143].

12. Hypothesis (Fig. 1)

Epidemiological and clinicopathological studies designed to demonstrate a role for Type 2 diabetes in AD or vascular dementia have generated data that are largely conflicting and inconclusive. On the other hand, the finding that peripheral administration of glucose or insulin can improve memory and cognition argues com-

pellingly in favor of insulin resistance having a causal role in AD/vascular dementia. Certainly the abundant expression of insulin, IGF-1, and IGF-II receptors, together with the molecular machinery required to transmit the corresponding growth factor signals in the CNS correlate with the experimental evidence that neuronal, glial, and vascular cells are responsive to insulin, IGF-1, and IGF-II stimulation. Although it is widely accepted that IGF-1 and IGF-II are expressed in the brain, controversy remains over the source of insulin. To settle this issue, we performed real time RT-PCR studies of brain tissue and cultured neuronal cells and clearly demonstrated that mRNA transcripts corresponding to all 3 growth factors and their receptors are in fact expressed in the brain (See Steen, et al. 2005). Local CNS synthesis of insulin is actually not as surprising as it may seem since all other pancreatic and gut neuroendocrine polypeptides are also expressed in the brain. Until now, the only exception was insulin. Although insulin and IGF-1 can be transported across the blood brain barrier, whether these molecules are actually taken up by CNS cells has not been established. Nonetheless, the extra-CNS sources of trophic factors could exert their effects by modifying the function of cerebral vessels and the choroid plexus. Correspondingly, withdrawal of insulin, hyperinsulinemia, or hyperglycemia could adversely affect the function of cerebral vessels and the choroid plexus. In this regard, empirical observations of postmortem human brains suggest that the major CNS abnormalities associated with Type 2 diabetes mellitus consist of cerebral microangiopathy and sclerosis of the choroid plexus, whereas AD-type neurodegeneration is not a frequent accompaniment of Type 2 diabetes mellitus. On the other hand, the strong evidence favoring a role for cerebrovascular disease and hypoxic/ischemic injury in the progression and pathogenesis of AD, suggests that cerebral micro-angiopathy caused by Type 2 diabetes and probably also systemic arterial hypertension contribute to the AD neurodegeneration cascade by causing chronic hypoperfusion and micro-infarcts. At the same time, it is important to realize that AD can and often does occur in the absence of significant cerebrovascular disease, and many patients with cerebrovascular disease have no evidence of AD.

Taking all of the information into account, including the potential sources of insulin, IGF-I, and IGF-II and the distributions of their receptors in the brain, we propose that AD-type neurodegeneration and attendant cognitive impairment are fundamentally mediated by CNS insulin/IGF depletion and secondary loss of cells that are responsive to and dependent upon these growth factors. As a separate and independent component, insulin resistance that occurs in Type 2 diabetes impairs cerebral microvascular and choroid plexus functions, leading to chronic hypoperfusion and compromise of the blood-brain and blood-CSF barriers. Both processes could simultaneously and differentially contribute to the AD neurodegeneration cascade, including all of the classical histopathological lesions.

The relationship between impaired insulin signaling and the typical AD-associated neuropathology could be explained as follows. Impaired insulin signaling in the brain caused by reduced ligand binding to its receptor, reduced local levels of insulin polypeptide, and/or reduced auto-phosphorylation and activation of insulin receptor tyrosine kinase could lead to increased levels of GSK-3β activity and oxidative stress. Increased activation of GSK-3β, as well as other kinases, including Cdk-5 and MAPKs that respond to oxidative stress and phosphorylate *tau*, promote hyper-phosphorylation and intracellular accumulation of *tau* in the form of paired helical filaments. Ubiquitination of these highly insoluble protease resistant fibrils results in further oxidative stress and eventual activation of cell death cascades. Since insulin is neurotropic and supports both neuronal viability and nascent synapse formation, impaired insulin signaling with inhibition of the PI3 Kinase-Akt survival and growth pathways would lead to reduced viability of neurons and retraction of neurites, i.e. loss of synapses.

Insulin or IGF-1 activation of Erk MAPK promotes physiological processing and trafficking of betaAPP/Abeta to the plasma membrane. Oxidative stress caused by increased GSK-3β activity and intracellular accumulation of hyper-phosphorylated *tau*, can disrupt physiological processing and intracellular trafficking of betaAPP/Abeta, resulting in local accumulations of Abeta. Impaired function of the CNS microvasculature can contribute to the defects in Abeta disposal and exacerbate its steady accumulation in the brain. High levels of Abeta cause neurotoxic cell death, and also interfere with insulin signaling mechanisms by impairing insulin binding and insulin receptor auto-phosphorylation.

The underlying basis for AD-associated disruptions in insulin signaling mechanisms is not known. However, from the above discussion, one might predict that the *tau*pathies, presenilin gene mutations, and excess brain accumulations of Abeta that occur in different familial forms of AD could precipitate or propagate neurodegeneration cascades by promoting oxidative stress and/or inhibiting insulin actions within the CNS. Similarly, other abnormalities in gene expression that re-

sult in impaired insulin stimulated signaling in neurons, such as those reported with respect to the Alzheimer-associated neuronal thread protein [164], in addition to various environmental exposures, may have important roles in precipitating the cascade of neurodegeneration in sporadic AD, which accounts for the vast majority of cases. It is not known why, in these circumstances, the AD phenotype is not clearly manifested until 50–70 years after birth. One potential explanation for this phenomenon is that in young brains, neuroprotective signaling mechanisms are ample and redundant, whereas with aging, the scaffolding deteriorates due to progressive mitochondrial DNA damage and attendant mitochondrial dysfunction. With regard to sporadic AD, which accounts for the vast majority of cases, the identification and characterization of underlying abnormalities that render the brains more sensitive to impairments in insulin signaling require further study.

This critical review of the literature helps to delineate the existence of two fundamental aging associated problems related to impaired insulin signaling in AD: one problem is centered on the cerebral microvasculature, while the other primarily pertains to CNS neuronal function. The importance of this concept is that the treatment of Type 2 diabetes will not likely prevent AD, although it may help to slow its progression via therapeutic actions on the CNS microvasculature. Instead, the use of insulin and IGF-1 sensitizers (preferably ones that are CNS-specific) would probably provide the best form of therapeutic rescue in the early and intermediate stages of disease. While treatment with GSK-3β inhibitors may seem attractive for preventing *tau* hyper-phosphorylation [107,165], this approach may impair the function of vital physiological targets of GSK-3β, and it also ignores many of the other critical components of the neurodegeneration cascade that would be unaffected by this type of therapy. Alternatively, the use of stem cells as vehicles for delivering insulin, IGF-I, and possibly other neurotropic factors may prove to be beneficial for enhancing neuronal survival and reducing oxidative stress in the CNS targets of neurodegeneration, although safety and efficacy issues must first be resolved.

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