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The nature, significance, and glucagon-like peptide-1 analog treatment of brain insulin resistance in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is an age-related neurodegenerative disease leading over the course of decades to the most common form of dementia. Many of its pathologic features and cognitive deficits may be due in part to brain insulin resistance recently demonstrated in the insulin receptor→insulin receptor substrate-1 (IRS-1) signaling pathway. The proximal cause of such resistance in AD dementia and amnesic mild cognitive impairment (aMCI) appears to be serine inhibition of IRS-1, a phenomenon likely due to microglial release of inflammatory cytokines triggered by oligomeric A β . Studies on animal models of AD and on human brain tissue from MCI cases at high risk of AD dementia have shown that brain insulin resistance and many other pathologic features and symptoms of AD may be greatly reduced or even reversed by treatment with FDA-approved glucagon-like peptide-1 (GLP-1) analogs such as liraglutide (Victoza). These findings call attention to the need for further basic, translational, and clinical studies on GLP-1 analogs as promising AD therapeutics.

Keywords

Alzheimer's disease; Glucagon-like peptide-1; Inflammation; Insulin receptor; Insulin receptor substrate-1; Insulin signaling; Hippocampus; Liraglutide; Streptozotocin; Type 3 diabetes

1. Introduction

Until recently, Alzheimer's disease (AD) was considered synonymous with a type of neurodegenerative dementia associated with abnormally high densities of amyloid β (A β) plaques and neurofibrillary tangles in the forebrain. Today, however, AD is more broadly defined to include the underlying pathophysiologic processes that gradually lead to dementia [1,2]. Over the course of decades, AD pathology develops gradually in three phases [3,4]: (a) a preclinical period beginning with asymptomatic accumulation of A β leading to early

neurodegeneration and then to subtle cognitive symptoms [2,5]; (b) a prodromal period known as mild cognitive impairment (MCI) due to AD in which the first clear, but not incapacitating, clinical symptoms emerge [6,7]; and (c) dementia due to AD [4,8] This final phase of the disorder commonly manifests at 65 years of age, but can emerge as early as age 30 years in relatively rare familial cases [3]. The personal impact of this last phase is devastating, ultimately robbing its victims of their identity, their capacity to care for themselves, and their ability to recognize or communicate with others.

AD dementia, the most common of all neurodegenerative dementias, is of special concern to society as a whole because it poses a clear public health risk of epidemic proportions worldwide [9] and because we lack effective treatments for it. Although >100 pharmacologic treatments for AD have been proposed and tested, most seeking to reduce brain levels of A β , none has proven more than minimally effective [10] for more than about a year after diagnosis [11]. If this situation persists, it is expected that at least 13.8 million Americans will be afflicted with AD dementia by the year 2050, with healthcare costs for them costing \$1.2 trillion [3].

There is consequently an urgent need for development of novel treatments of AD within the next decade [12]. Among the most promising of those now in development target brain insulin resistance (i.e., reduced neuronal responsiveness to extracellular insulin), which is an early, common, and major feature in AD cases with and without diabetes [13,14]. After outlining the history of research behind the discovery of brain insulin resistance, we discuss its nature, significance, probable cause, and promising treatments with GLP-1 analogs.

2. Discovery of brain insulin resistance in AD

Brain insulin resistance in AD was first proposed nearly 20 years ago by Siegfried Hoyer and colleagues [15,16], who hypothesized that desensitization of neuronal insulin receptors (IRs) may explain reduced brain glucose metabolism in this disorder. Although some [14,17], but not all [18], studies reported decreased IR sensitivity in the neocortex and/or hippocampus of AD cases, its relationship to reduced brain glucose metabolism in such cases remains uncertain, because it has been established that insulin by itself has no effect on neuronal glucose uptake in the forebrain [14,19] and also because Hoyer and coworkers relied on intracerebroventricular (ICV) streptozotocin (STZ) in rodents to test their hypothesis.

Since ICV STZ is often used to create animal models of brain insulin resistance [20] and AD [21], it must be explained why this drug treatment was insufficient to test the plausibility of brain insulin resistance in AD. The few studies that have directly tested the effect of STZ on insulin responsiveness were not conducted on the brain, but instead upon liver and muscle tissue after peripheral administration of the drug in rodents. The results of these studies were inconsistent, showing that STZ either increases [22], does not affect [23], or decreases [24,25] insulin responsiveness in the tissues tested. The studies used to justify using ICV STZ to model brain insulin resistance were naturally those reporting STZ-induced inhibition of insulin responsiveness [24,25], but the mechanism for this claimed effect does not apply to the brain, as indicated in the following considerations. The depressive effect on insulin

responsiveness is not produced by direct action on liver or muscle, but by the drug's rapid reduction of plasma insulin given that normal insulin responsiveness in STZ-treated animals can be restored by simply raising plasma insulin [24,25]. The ability of STZ, a glucose analog, to lower plasma insulin depends on its cellular uptake by glucose transporter 2 (GLUT2), which binds the glucose moiety of STZ [26]. Such uptake occurs preferentially in insulin-secreting pancreatic β cells, because they are among the few cell types in the rodent rich in GLUT2. Once taken up by the β cells, the *N*-methyl-*N*-nitrosurea moiety of STZ exerts its cytotoxic effects leading to cell death, and thus loss of pancreatic insulin secretion [26]. In the brain, however, GLUT2 is expressed at relatively low levels by few, if any, neurons expressing insulin, especially pyramidal neurons of the neocortex and hippocampus (CA1-3) [27–29]. This may explain why ICV STZ has not been shown to affect protein levels of brain insulin [30] and why this drug treatment has inconsistent effects on gene and/or protein expression of upstream versus downstream insulin signaling molecules within and across brain structures [31–33].

At present, then, there is no reason to expect (and no clear demonstration) that ICV STZ preferentially targets insulin signaling in the brain and, consequently, no reason to expect that it preferentially models brain insulin resistance. It is more likely that ICV STZ affects brain insulin signaling along with many other brain processes simply by inducing oxidative stress, glial inflammatory responses [33–35], and toxic effects on GLUT2 cells in the brain, including those in the hypothalamus and brainstem regulating autonomic control of pancreatic insulin and glucagon release, which disrupts systems ensuring sufficient glucose flux to the brain [36].

Pursuing evidence that brain insulin signaling was actually reduced in AD, Hoyer's group initiated study of insulin signaling molecules in postmortem cases of this disorder. As reported in 1998 by Frölich et al. [18], Hoyer's group found that normal humans exhibit significant reductions with age in neocortical levels of insulin and in insulin binding of neocortical IRs, but that AD cases were not significantly different in these respects from controls of similar age [18]. In 2005, Suzanne de la Monte and colleagues reported that gene and (less quantitatively assessed) protein expression of insulin and IR, as well as other insulin signaling molecules and IR insulin binding, were markedly lower in forebrains of AD cases compared to controls of unstated age [17,37]. Additional postmortem studies by other groups between 2005 and 2011 established that protein levels of insulin-signaling molecules do occur in AD cases when compared with age-matched controls [38–41]. The postmortem studies reported by 2011 nevertheless disagreed in many respects on the specific signaling molecules affected and whether the affected molecules were decreased or increased in AD [13,17,37–39].

By 2011, however, one consistent feature of AD brains had been identified, namely high serine phosphorylation of insulin receptor substrate-1 (IRS-1 pS) discovered by our group in the hippocampus [39] and confirmed there and in temporal neocortex by others [40,41]. Such phosphorylation inhibits IRS-1 and its ability to transmit IR signals to more downstream molecules [42]. Because elevated IRS-1 pS in adipose and muscle tissue is often associated with insulin resistance in type 2 diabetes (T2D) [42,43], we considered it plausible that T2D-induced IRS-1 pS elevation in the brain may help explain why T2D is a

risk factor for AD [44]. We also considered it likely that this phenomenon may be a general feature of AD not dependent on T2D, but rather on A β -induced inflammatory processes, as explained in Section 6. We were aware that elevated IRS-1 pS by itself cannot prove insulin resistance in the AD brain. That requires demonstrating reduced insulin responsiveness in such tissue. Therefore, our group studied insulin responsiveness in AD brain tissue using an ex vivo stimulation protocol. As described in what follows, that study [14] supplied the first direct demonstration of insulin resistance in the AD brain, specifically in the hippocampal formation (HF = hippocampus + dentate gyrus + subiculum) and, to a lesser degree, in the cerebellum of AD cases.

3. Brain insulin resistance in AD shown by ex vivo stimulation

3.1. Testing the IR→IRS-1→PI3K→Akt pathway for insulin resistance in the brain

Although not affecting neuronal glucose uptake, brain insulin resistance in AD is similar to muscle insulin resistance in T2D [14,43]. In both disorders, insulin's ability to activate a specific signaling pathway is weaker than normal. In this signaling pathway (Figs. 1 and 2), insulin binding of the IR at the cell surface activates IRS-1 intracellularly, which in turn activates phosphatidylinositol-3-kinase (PI3K) and then Akt, which inhibits many downstream molecules, including the apoptosis-inducing molecules BAD and caspase-9, forkhead box-containing transcription factors of the O family (FoxO1 and FoxO3), glycogen synthase kinase-3 (GSK-3), and the mammalian target-of-rapamycin complex 1 (mTORC1) [45,46]. Because IRS-1 in the brain is primarily neuronal [14,47], brain insulin resistance involving that signaling molecule would be mainly a neuronal phenomenon. Another IRS isoform (IRS-2) is abundant in the brain, but it does not mediate insulin signaling at or near physiologic doses of insulin [14]. IRS-2 instead mediates signaling by insulin-like growth factor-1 (IGF-1) at physiologic levels of that hormone [14].

Insulin signaling is regulated by serine kinase inhibition of IRS-1 [42]. As shown in Figure 2, IRS-1 interactions with the IR and/or PI3K are inhibited via feedback inhibition by GSK-3, mTOR/S6K1, and PKC, as well as via feed-forward inhibition by ERK2 (extracellular signal-regulated kinase 2), IKK (inhibitor of kappa B kinase), and JNK (c-Jun N-terminal kinase). These kinases phosphorylate IRS-1 at multiple sites, including S312 (IKK, JNK, mTOR/S6K1), S323 (ERK2 and PKC), S337/341 (GSK-3), S616 (ERK2, JNK, mTOR/S6K1, and PKC), and S636/639 (ERK2, mTOR/S6K1, and PKC). At least two of these sites (S616 and S636/639) are heavily phosphorylated in AD [14] and may be candidate biomarkers of brain insulin resistance in that disorder as explained in Section 6.

3.2. Ex vivo insulin responses mediated by IRS-1 are reduced in the AD brain

To test brain insulin resistance in AD, we studied ex vivo insulin responses by brain tissue from AD dementia patients and healthy controls of the same gender and similar age who had died within about 6 hours of autopsy [14,48]. Tests were run with both physiologic (1 nmol/L) and supraphysiologic (10 nmol/L) concentrations of insulin. To exclude the possibility that the results were due to diabetic conditions, we excluded cases with a history of diabetes.

Insulin induced significantly less activation of the IR→IRS-1→PI3K→Akt pathway in AD compared to healthy tissue in all brain areas our group has studied (HF, prefrontal cortex, and cerebellar cortex [14,48]). In the HF of AD cases, 1 nmol/L insulin induced 29%–34% less activation of the IR (i.e., tyrosine phosphorylation, pY), 90% less activation of IRS-1 (pY), 96% less recruitment of PI3K to IRS-1, 89% less activation of Akt (pS), and 74% less activation of mTOR (pS) [14] (Fig. 3A–F). IRS-1 was thus the first molecule in this signaling pathway showing severe dysfunction, which, accordingly, seems to be a central factor in brain insulin resistance. Increasing the insulin concentration to 10 nmol/L, which may be higher than safely achieved with intranasal insulin, did not significantly increase tissue responsiveness in the AD dementia cases.

Our subsequent ex vivo stimulation studies have shown lower, but significant brain insulin resistance in the HF from nondiabetic individuals with MCI (H.-Y. Wang et al., manuscript in preparation), which often progresses to AD dementia [49]. For this reason, brain insulin resistance can develop early in AD, even in the absence of diabetes. This also appears to be a common feature of the disorder because a very high percentage of MCI and AD dementia cases show elevated levels of candidate biomarkers of brain insulin resistance in the HF (i.e., IRS-1 pS616 and IRS-1 pS636/639) [14].

3.3. Ex vivo insulin responsiveness is not always evident from basal levels of insulin-signaling molecules in the AD brain

The responsiveness of all insulin-signaling molecules we tested [14] in the HF of AD versus age- and gender-matched controls is shown in Figure 2. As indicated earlier, 1 nmol/L insulin induced less activation or suppression of all insulin-signaling molecules studied in the AD cases (Figs. 2A and 3). In contrast, basal (i.e., not insulin-stimulated) levels of activated or suppressed forms of those molecules below the IR were all increased in the same AD cases (Fig. 2B). For example, AD cases showed increased basal levels of IRS-1 pY and Akt pS, which are commonly assumed to indicate increased insulin signaling. Because insulin nevertheless induced much smaller increases in IRS-1 pY and Akt pS in the AD cases than in controls, perhaps because IRS-1 and Akt pS are already near maximal phosphorylation in AD [14], basal levels of activated or suppressed insulin-signaling molecules below the IR does not necessarily reflect insulin responsiveness or levels of insulin signaling.

3.4. Ex vivo IGF-1 responses mediated by IRS-2 also reduced in the AD brain

According to our ex vivo stimulation studies on the HF, brain insulin resistance in AD is also accompanied by brain IGF-1 resistance (Fig. 3G–J). In particular, 1 nmol/L IGF-1 in AD cases was less effective in activating the IGF-1 receptor (IGF-1R) and IRS-2 and less effective in inducing PI3K recruitment to IRS-2, which is the IRS isoform mediating IGF-1 signaling in the brain [14]. Unlike insulin resistance, IGF-1 resistance was severe, even at the level of the hormone receptor. The significance of this phenomenon remains to be determined.

4. Brain insulin resistance in AD is not type 3 diabetes

Some regard evidence of reduced brain insulin signaling in AD, especially when coupled with claims of altered CSF insulin in that disorder, as proof that AD is a brain form of T2D commonly called type 3 diabetes (T3D) [37,50–52]. This is misleading for five reasons. First, the term “diabetes” is inappropriate: the key diagnostic feature shared by type 1 and type 2 diabetes is not insulin resistance, but hyperglycemia, which is not evident in the cerebrospinal fluid (CSF) of AD patients [53,54] as expected, since the glucose transporter present in cerebral vasculature (GLUT1 55 kDa) is reduced in AD [55,56]. Second, as noted previously, brain insulin resistance occurs even in AD cases without diabetes [14,15], that is, in cases without peripheral hyperglycemia. Third, although brain glucose metabolism is markedly reduced in AD [57], this is not directly related to brain insulin resistance, because such resistance does not affect insulin-induced neuronal glucose uptake [14,19]. Reduced glucose metabolism in AD may instead be a consequence of reduced postsynaptic neurotransmission (a potential effect of reduced insulin signaling in the brain [58]), given that glutamate and other depolarizing agents trigger glucose uptake in the brain [14,59] and that the potency of this effect is reduced in AD [14]. Fourth, it has not been established that the AD brain is commonly insulin-deficient. Although one group found that forebrain insulin content is reduced in AD dementia [37], another group found it was normal when compared with age-matched controls [18]. Similarly, whereas one group found that CSF insulin is reduced in mild AD dementia [60], other groups found that it was reduced only in moderate to severe AD dementia [61], or that it was normal [62] or even elevated [53] in AD dementia generally. Finally, unlike T1D or T2D, there is no evidence that loss of insulin secreting cells in the pancreas or brain is a precipitating factor in AD.

Brain insulin resistance in AD is more aptly described as a neuronal form of Reaven's insulin resistance syndrome [63], a condition characterized by insulin resistance and at least a subset of its associated conditions in other tissues (e.g., inflammation, dyslipidemia, and endothelial dysfunction). This syndrome, which is a core element of the metabolic syndrome, is not a separate medical disorder, but rather an aspect of diverse disorders, including T2D, cardiovascular disease, and essential hypertension [63]. Our work [14] suggests that a neuronal insulin resistance syndrome is characteristic of AD.

5. Significance of brain insulin resistance in AD

Insulin is best known as a pancreatic β -cell hormone secreted in response to elevated plasma glucose after meals. Its classic functions are stimulation of glucose uptake by adipose and muscle tissue and inhibition of no longer needed free fatty acid release by adipose tissue and glucose production by the liver. However, insulin is also synthesized in brain neurons [64], including many pyramidal and granule cells in adult cerebral cortex and hippocampus [28,29], where the density of insulin receptors is appreciable [65]. Although pancreatic insulin is transported in small amounts across the blood–brain barrier in many brain regions and exerts effects on brain function, especially in the hypothalamus [66], most insulin in the brain outside the hypothalamus seems locally derived because vascular hypo- and hyperinsulinemia has little, if any, effect on total brain insulin [67]. Thus, it seems likely that

outside the hypothalamus insulin resistance in the brain largely reflects reduced responsiveness to endogenous, not pancreatic, insulin.

As noted previously, insulin in the brain does not control cellular uptake of glucose [14,19]. But insulin has many other functions. In the brain, it normally performs many of the functions disrupted in AD, such as regulating apoptosis; lipid metabolism; cerebral blood flow; glial inflammatory responses (see Section 6); oxidative stress; A β clearance; tau phosphorylation; α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and gamma-aminobutyric acid, subclass A (GABA_A) receptor trafficking; synaptic plasticity; and memory formation [58,68]. Consequently, brain insulin resistance has the potential to affect wide range of AD-related pathology and symptoms [68,69]. The rate at which insulin resistance develops in the brain may thus play a major role in determining the rate at which AD progresses.

It is important to recognize, however, that the actual consequences of brain insulin resistance in AD are not certain when based only on studies of animal models of AD or on tests of basal, as opposed to insulin-induced, levels of insulin-signaling molecules. Our study of the hippocampus in AD cases [14] calls attention to what is and is not affected by brain insulin resistance in the disorder. Contrary to expectations based on indirect tests of neuronal glucose uptake in animal models of AD [16,50,70], we found that brain insulin resistance in AD does not affect neuronal glucose uptake [14], consistent with the failure of insulin to affect such uptake in normal rodent or human brains [14,19]. Contrary to expectations based on basal levels of suppressed GSK-3 in animal models of AD and in AD itself [69–71], brain insulin resistance in this disorder was not found to disinhibit GSK-3 and thereby raise levels of hyperphosphorylated tau in neurofibrillary tangles [14]. Indeed, we found that basal levels of suppressed GSK-3 in AD were positively, not negatively, correlated with density of neurofibrillary tangles [14].

What our study did support as a major consequence of brain insulin resistance in AD was cognitive decline. We studied 30 normal, 29 MCI, and 30 AD dementia patients from the Religious Orders Study for whom neurocognitive data and postmortem HF were available. HF field CA1 from the MCI and AD dementia cases showed elevated levels of candidate biomarkers for brain insulin resistance identified in our ex vivo insulin tests (i.e., IRS-1 pS616 and IRS-1 pS636/639). Linear regression analyses showed that basal activation states of insulin-signaling molecules in CA1 of all the cases combined (normal, MCI, and AD dementia) were highly correlated with scores for those cases on global cognition, working memory, and especially episodic memory [14] (Fig. 4). The association was positive for basal levels of molecules driving insulin signaling and negative for those inhibiting such signaling (Table 1).

The strongest cognitive relationship found in this study [14] was a negative association between levels of a candidate biomarker of brain insulin resistance (IRS-1 pS616) in CA1 and episodic memory (Table 1 and Fig. 4C). CA1 levels of that candidate biomarker accounted for almost 47% of the variance in episodic memory scores, which is among the highest such percentage of all pathologic variables associated with cognition in AD. Whereas A β can elevate levels of IRS-1 pS (see Section 6), the association of episodic

memory and IRS-1 pS616 proved statistically independent of A β plaques, suggesting that brain insulin resistance is mechanistically closer than A β plaques to the molecular causes of cognitive decline in AD.

6. Search for causes of brain insulin resistance

Many causes have been proposed to explain evidence of decreased insulin signaling in AD brains. Among the most often cited causes are: (a) reduced extracellular insulin deduced from CSF assays [60,61]; (b) reduced total [37] or cell surface [40,72] IR expression; and (c) reduced IR affinity for insulin [17]. There are, however, reasons to doubt that these are major factors in reduced brain insulin signaling in AD. Deficient extracellular insulin in the AD brain is uncertain given the conflicting evidence [18,37,53,60–62] described earlier (see Section 4). Deficiencies in total IR content of the AD brain are highly unlikely, given that they are not found in many studies explicitly using age-matched controls [13,14,18,40,73], nor are deficiencies in cell surface IR levels confirmed in cell fractionation studies on AD brain samples [14].

Although insulin binding of the IR may [17] or may not [18] be lower in AD brain tissue, insulin still activates the catalytic domain of the IR at 71%–74% of normal levels in the HF of AD dementia cases [14]. In contrast, far greater reductions in insulin responsiveness are seen below the IR in the AD brain, as noted previously, beginning with IRS-1, which is activated by insulin at only 10% of normal levels in the HF [14].

Insulin resistance associated with dysfunctional IRS-1 is thus the most likely proximal cause of reduced brain insulin signaling in AD. This probably reflects A β -induced glial secretion of proinflammatory cytokines (Fig. 5). Among the earliest abnormalities seen in AD is an elevation in soluble A β oligomers [74], which can assemble later into fibrils forming amyloid plaques or into amylospheroids [75,76]. Also occurring early in AD [77], A β oligomers and nascent fibrils (i.e., protofibrils) activate microglia, resulting in their secretion of proinflammatory cytokines, such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor- α (TNF- α) [78]. Such microglial activation may be critical in AD pathogenesis, because knockout of a gene encoding an intracellular microglial receptor NLRP3 (NOD-like receptor protein 3, part of the NLRP3 inflammasome) sensing extracellular pathogenic agents [79], including A β [80], prevents development of AD pathology and cognitive deficits normally occurring in an animal model of AD [81]. Via neuronal receptors, IL-1, IL-6, and TNF- α can activate the IRS-1 serine kinases IKK (inhibitor of kappaB kinase), JNK (c-Jun N-terminal kinase), and Erk2 (extracellular signal-regulated kinase 2) [41,42,82]. A β oligomers delivered to neuronal cultures or cerebral ventricles do, in fact, elevate IRS-1 serine phosphorylation (IRS-1 pS) at multiple sites, namely S312, S616, and/or S636/639 (S307, S612, and S632/635 in rodents) [41,82,83] (Fig. 5).

Elevated IRS-1 pS is prominent in the cerebral cortex and HF of AD dementia cases and appears to be a major cause of IRS-1 dysfunction in such cases [14,40,82,83]. Because such phosphorylation inhibits transmission of insulin-induced receptor activation to more downstream molecules as noted earlier, it is understandably an established cause of insulin resistance in peripheral tissues [42], especially muscle [43]. The same appears to be true in

AD brains, where insulin-induced IRS-1 activation is consistently reduced in tissues with significantly elevated levels of IRS-1 pS616 and IRS-1 pS636/639, which are thus potential biomarkers of brain insulin resistance [14]. As expected, levels of these candidate biomarkers are positively correlated with oligomeric A β plaque loads and negatively with cognitive status [14], as described in Section 5.

Chronically elevated neuronal IRS-1 pS resulting from A β -induced proinflammatory processes is thus the most likely explanation for brain insulin resistance in AD dementia and for its contribution to cognitive decline. The same appears to be true for amnesic MCI, but brain insulin resistance in non-amnesic MCI may be independent of elevated IRS-1 pS (H.-Y. Wang et al., manuscript in preparation). The mechanistic model presented here for AD may help explain why peripheral insulin resistance due to obesity and/or T2D appears to exacerbate brain insulin resistance in AD [13] and in animal models of AD [84,85]. Obesity and T2D are both risk factors for AD [86], and both have been associated with elevated vascular proinflammatory cytokines [87,88]. Especially in AD, where cerebral vasculature is damaged, cytokines can cross the blood–brain barrier [89] and activate IRS-1 serine kinases in a manner similar to microglial cytokines. Peripheral insulin resistance can also raise brain IRS-1 pS by decreasing clearance of brain A β , because insulin facilitates hepatic clearance of plasma A β [90], interference with which impairs brain clearance of that peptide [91].

7. Slowing age-related increases in brain insulin resistance

Between the ages of 40 and 64 years, the prevalence of pre-diabetes [92] and T2D [93] in the USA rises steeply, indicating a steep rise in peripheral insulin resistance starting in midlife. For the reasons described in Section 6, this phenomenon could promote brain insulin resistance, a view supported by our finding that brain levels of IRS-1 pS616 increase significantly from middle to old age, even in those without T2D. It is consequently important, especially from middle age onward, to continue (or to adopt) a lifestyle known to lower peripheral insulin resistance and reduce the risk of progressing to MCI, which elevates risk of AD dementia [49] as mentioned earlier. The most effective lifestyle changes for these goals are loss of excess weight, regular physical exercise, and adherence to a Mediterranean diet, particularly that of Estruch et al. [94], supplemented with nutrients in other diets that lower peripheral insulin resistance, reduce A β pathology in the brain, including IRS-1 pS levels, and improve cognition [95]. These additional nutrients include flavonoids in blueberries and green tea, curcumin in the spice turmeric, and the ω -3 fatty acid docosahexaenoic acid (DHA) enriched in fatty fish such as salmon [95].

8. Treating brain insulin resistance in AD

Although weight loss, exercise, and better diets may slow progression to clinical stages of AD and even mitigate symptom severity in MCI [96–99], randomized clinical trials have not yet provided consistent evidence that such lifestyle changes initiated after diagnosis of MCI or AD dementia markedly slow cognitive decline [96,100]. At those stages of AD, simply reducing peripheral insulin resistance is ineffective, as indicated by the failure of many T2D treatments to reduce AD risk or improve cognition in AD dementia, specifically treatments with peripherally administered insulin, metformin, sulfonylureas, and thiazolidinediones,

such as rosiglitazone and pioglitazone [101,102]. The thiazolidinediones are also clinically compromised by their elevation of heart failure risk in those with prediabetes or T2D [103].

Despite the failure of the antidiabetic treatments just named to reduce AD risk or cognitive impairment, the demonstrated ability of intranasal insulin to improve cognition in MCI and early AD dementia cases [104,105] shows the promise of augmenting brain insulin signaling in treating AD. Intranasal insulin administration by itself, however, is unlikely to overcome the levels of brain insulin resistance seen in AD dementia, as noted earlier. The antidiabetic agents specified may have failed as AD treatments for a number of reasons, such as rapid degradation, poor penetrance of the blood–brain barrier, and/or ineffectiveness in reducing neuronal insulin resistance in vivo.

Fortunately, antidiabetic agents called glucagon-like peptide-1 GLP-1 analogs or mimetics, do not have these limitations and are priority candidates among marketed drugs for development as AD therapeutic agents [11]. GLP-1 is one of two incretin peptides that are so named because their secretion by the intestines in response to food increases glucose-stimulated pancreatic insulin release [106]. Like insulin, it is produced in the brain, specifically by autonomic brainstem neurons [107] and by cerebrocortical and hippocampal microglia [108]. Also, like insulin, it has many functions outside the pancreas, including neuroprotection [109,110], promotion of neurogenesis [110,111], and potentiation of insulin signaling [112,113].

Because GLP-1 is quickly metabolized, degradation-resistant analogs have been developed for use in treating T2D. Two of those approved by the U.S. Food and Drug Administration (FDA) are exenatide (synthetic form of exendin-4, marketed as Byetta) and liraglutide (marketed as Victoza). Both effectively reduce peripheral insulin resistance [113,114] and have excellent safety profiles with a low incidence of hypoglycemia [115,116] as expected given that GLP-1 increases glucose-stimulated, not basal, pancreatic insulin secretion. Pancreatitis has occurred in a very small number of those taking GLP-1 analogs, which may reflect the fact that the drug is prescribed for diabetes, which is a risk factor for pancreatitis [115,116]. A recent meta-analysis, however, showed no evidence that GLP-1 analogs increase risk of pancreatitis [117].

Peripherally administered GLP-1 analogs, including exendin-4 and liraglutide, cross the blood–brain barrier [111,118] and are thus able to bind GLP-1 receptors found widely in the brain, including pyramidal cells of the cerebral cortex and HF [119]. The GLP-1 analogs have a remarkable number of beneficial effects on neurons, many of which may derive from their ability to block A β -induced neuronal insulin resistance [82]. In mouse models of AD, including aged animals, these drugs reduce A β plaque loads, block A β -stimulated inflammatory responses, and promote neurogenesis, neuronal survival, and synaptic integrity; restore long-term potentiation; and reduce cognitive deficits [82,109–111,120,121]. Given that elevated IRS-1 pS in the brain may be the primary cause of brain insulin resistance in AD, it is noteworthy that exendin-4 and liraglutide were found to reduce levels of IRS-1 pS616 and IRS-1 pS636 in the APP/PS1 mouse model of AD [82,122].

Our group has recently shown that liraglutide essentially restores brain insulin sensitivity in APP/PS1 mice [123]. Using ex vivo stimulation, we found that the HF in such mice is as insulin-resistant at 7.5 months as the same brain area in elderly AD cases. We also showed that 2 months of daily liraglutide administration (25 nmol/kg intraperitoneally) starting at 5 months virtually restored normal HF responses to insulin in the IR→IRS-1→PI3K→Akt pathway. The same drug treatment restores long-term potentiation in the HF and greatly improves cognition in the APP/PS1 mouse model of AD [120,121].

Our most recent work suggests that liraglutide could be very potent in reducing brain insulin resistance in the HF of MCI cases (H.-Y. Wang et al., in preparation). As noted earlier (see Subsection 3.2), such tissue in MCI cases is insulin-resistant to a lesser degree than the same brain area from AD dementia cases. After exposure to 100 nmol/L liraglutide for 1 hour, the HF of MCI patients was found to be much more responsive to 1 nmol/L insulin. Indeed, this treatment resulted in virtually normal insulin responsiveness in tissue from non-amnestic MCI cases and substantially improved insulin responsiveness in tissue from amnestic MCI cases. The same treatment also significantly improved insulin responsiveness in the HF of AD dementia patients, but left such responsiveness far from normal.

GLP-1 analogs thus emerge as very promising therapeutic agents in AD at an early clinical stage before extensive, irreversible neurodegeneration occurs. This puts a premium on early diagnosis of MCI due to AD, which is becoming possible with current methods to image A β plaque levels in the brain with positron emission tomography [124] and in the retina with optical imaging after curcumin ingestion [125]. The results of the first clinical trials of GLP-1 analogs on MCI subjects in the USA and the UK [126] are eagerly anticipated. Hopes are raised by the reversal of cognitive decline observed recently in the first clinical trial of a GLP-1 analog (exenatide) on a neurodegenerative disorder, namely Parkinson's disease [127]. As in AD, dementia in that disorder is associated with peripheral insulin resistance [128].

Like AD and Parkinson's disease, a wide range of neurological disorders (e.g., stroke, Creutzfeld-Jakob disease, and multiple sclerosis) are associated with elevated brain levels of the same proinflammatory cytokines [129] that appear to induce brain insulin resistance in AD (Fig. 5). In animal models of stroke, Parkinson's disease, and multiple sclerosis, GLP-1 analogues ameliorate the pathology and symptoms of those disorders [126], potentially by relieving brain insulin resistance and thereby restoring insulin's potent neuroprotective effects [58]. We believe, therefore, that GLP-1 and dual incretin analogues [130] should be aggressively pursued for their therapeutic effects on multiple neurological conditions.

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Fig. 1.

Schematic of the IRS-1→PI3K→Akt insulin signaling pathway. Insulin binding of the α chains in insulin receptor-A (IR-A, the type of insulin receptor found in the brain) triggers phosphorylation at tyrosine sites (pY) on the intracellular IR β chains. When activated, IR-A binds and tyrosine phosphorylates insulin receptor substrate 1 (IRS-1). This leads to activation of PI3K, promoting generation of membrane PIP3 that binds Akt (and PKC λ/ζ ; see Fig. 2) and thereby brings them into contact with the membrane-associated enzymes phosphoinositide-dependent kinase 1 and 2 (PDK 1 and 2), which activates Akt by phosphorylating it at T308 and S473 (and PKC λ/ζ by phosphorylating it at T403 and T410). Activated Akt has many cell functions that are mediated by inhibiting a wide range of substrates. Those substrates most relevant to adult neurons are shown here (BAD, caspase-9, FoxO1, FoxO3, GSK-3, and mTORC1). Some consequences of inhibiting these Akt substrates in adult neurons are listed in the shaded boxes.

Fig. 2.

Schematic summary of hippocampal formation alterations in the IR→IRS-1→PI3K→Akt→mTOR signaling pathway of AD dementia cases versus age- and gender-matched controls. Total protein levels of the signaling molecules IR, IRS-1, Akt, GSK-3, mTOR, and PKCλ/ζ, as well as the insulin signaling regulators ERK2, IKK, and JNK (see Table 1 for abbreviations) were normal in AD. In contrast, activated or suppressed forms of these molecules (identified in Table 1) were altered in AD. (A) AD alterations in 1-nmol/L insulin-stimulated responses normally activating or suppressing insulin signaling molecules, including ERK2, which is activated by insulin via Grb2, not IRS-1. Note that insulin responsiveness in AD is reduced at all levels of the signaling pathway studied and is associated with reduced binding of IRS-1 to the IR and to a regulatory subunit of PI3K (p85α). (B) AD alterations in activated or suppressed forms of insulin signaling or regulating molecules without insulin stimulation. All these molecules are activated forms, except for IRS-1 pS and GSK-3 pS, which are suppressed forms. The question mark (?) reflects uncertainty regarding IR pY1150/1151 stemming from data based on an antibody recognizing these phosphorylation sites on both IR and IGF-1R. IRS-1 inhibition by serine (S) phosphorylation 312, 616, and 636/639 results from elevated basal activation of Akt, ERK2, IKK, JNK, mTOR, and PKCλ/ζ. Decreased insulin-induced phosphorylation of molecules below the IR probably reflects their high basal levels of phosphorylation shown in (A), which limits further phosphorylation upon insulin stimulation. (Adapted from Talbot et al. [14]).

Fig. 3.

Graphs showing the magnitude of decreased insulin responsiveness in the IR→IRS-1→PI3K→Akt→mTOR signaling pathway in the hippocampal formation of AD cases compared to age- and gender-matched controls. Cases with low postmortem intervals (about 6 hours) were used to test responses to 1- and 10-nmol/L (nM) insulin in this pathway and to the same doses of IGF-1 in the IGF-1→IRS-2 pathway. Each bar in the graphs shows the percent increase above nonstimulated levels of activation or recruitment of a signaling molecule in the same diagnostic group. Reduced insulin responsiveness was seen in activation of the IR (A) and recruitment of IRS-1 to the IR (B), but the magnitude of the reduction was much less than in total IRS-1 activation (C), recruitment of the regulatory subunit of PI3K (p85α) to IRS-1 (D), Akt activation via its S473 site (E), and mTOR activation via its S2448 site (F). Reduced IGF-1 responsiveness was seen in the same hippocampal formation samples from AD cases, specifically in IGF-1R activation (G),

IRS-2 recruited to IGF-1R (H), IRS-2 activation (I), and recruitment of PI3K p85 to IRS-2 (J). * $P < .05$; * $P < .01$; * $P < .0001$. (Adapted from Talbot et al. [14]).

Fig. 4.

Human cognition is negatively associated with levels of a candidate biomarker of brain insulin resistance (IRS-1 pS616) in the hippocampus. Linear regression plots of combined data from 30 normal, 29 MCI, and 30 AD dementia cases in the Religious Orders Study showed highly negative associations between density of CA1 neurons with elevated IRS-1 pS616 (i.e., those with detectable cytoplasmic IRS-1 pS616) and composite scores on tests of global cognition (A), working memory (B), and especially episodic memory (C). The strength of the negative associations is emphasized by the very large percentage of the variance (R^2) in the memory scores accounted for by the density of neurons with elevated IRS-1 pS616. (Adapted from Talbot et al. [14]).

Fig. 5.

Diagram of a likely mechanism causing brain insulin resistance in AD. Circled numbers indicate the sequence of steps hypothesized to culminate in impaired neuronal insulin signaling. Soluble Aβ oligomers (and Aβ protofibrils) activate microglial cells (1), which consequently secrete proinflammatory cytokines, including IL-1β, IL-6, and TNF-α (2). Via neuronal receptors, each of these cytokines activates one or more of three IRS-1 serine kinases, namely JNK, IKK, and ERK (3). Each of these kinases phosphorylate one or more IRS-1 sites, specifically S312, S616, and/or S636/639, using numbering for the human molecule (4). Such phosphorylation, which is abnormally high in AD, inhibits IRS-1 interactions with the IR upstream (5) and PI3K downstream (6), thereby inhibiting transmission of insulin signals from reaching downstream targets. Inactivation of IRS-1 leads to its removal from signaling domains and its degradation (7). Steps 5–7, along with reduced insulin responsiveness of the IR, produce insulin resistance [14,41,42,82], which is known to promote formation of Aβ oligomers [131] (8). The mechanism outlined applies to amnesic MCI and AD dementia, but may not apply to non-amnesic MCI in which brain insulin resistance may be independent of elevated IRS-1 pS (Wang et al., manuscript in preparation).

Linear regression prediction of episodic memory scores from levels of molecules driving or inhibiting insulin signaling in the human hippocampal field CA1^{*}

Table 1

Independent variable	N	R ² for model	F statistic, P value for model	Parameter estimate	t-score	P value
Age, gender, and education only	89	0.11	$F[4, 85] = 3.36, P = .02$			
Variables driving insulin signaling:						
IR/IGF-1R pY [†]	85	0.20	$F[5, 80] = 4.88, P = .0014$	0.0019	3.41	.001
IRβ pY [‡]	87	0.18	$F[5, 82] = 4.46, P = .0026$	0.0017	2.73	.0078
PIP3	85	0.32	$F[5, 85] = 9.27, P < 1 \times 10^{-6}$	0.017	4.92	4×10^{-6}
Variables inhibiting insulin signaling:						
IRS-1 pY [‡]	84	0.40	$F[5, 79] = 13.29, P < 1 \times 10^{-6}$	-0.053	-6.44	$< 1 \times 10^{-6}$
IRS-1 pY [‡]	81	0.36	$F[5, 76] = 10.84, P < 1 \times 10^{-6}$	-0.049	-5.80	$< 1 \times 10^{-6}$
IRS-1 pS [‡]	88	0.34	$F[5, 83] = 10.70, P < 1 \times 10^{-6}$	-0.086	-5.38	1×10^{-6}
IRS-1 pS [‡]	88	0.47	$F[5, 83] = 18.04, P < 1 \times 10^{-6}$	-0.137	-7.42	$< 1 \times 10^{-6}$
IRS-1 pS [‡]	88	0.29	$F[5, 83] = 8.51, P = 2 \times 10^{-6}$	-0.038	-4.67	1.1×10^{-5}
PKC/λ pT [‡]	84	0.36	$F[5, 79] = 10.94, P < 1 \times 10^{-6}$	-0.056	-5.61	$< 1 \times 10^{-6}$
GSK-3α/β pS [‡]	65	0.33	$F[5, 59] = 7.44, P = 1.8 \times 10^{-5}$	-0.00026	-4.44	4×10^{-5}
mTOR pS [‡]	89	0.37	$F[5, 84] = 12.33, P < 1 \times 10^{-6}$	-0.031	-5.93	$< 1 \times 10^{-6}$
IKKα/β pS [‡]	87	0.25	$F[5, 82] = 6.91, P = 2 \times 10^{-5}$	-0.043	-3.85	.0002
JNK 1/2 pT [‡]	87	0.27	$F[5, 82] = 7.53, P = 7 \times 10^{-6}$	-0.025	-4.34	4×10^{-5}

Abbreviations: GSK-3α/β, glycogen synthase kinase-3, isoforms α and β; IKKα/β, inhibitor of kappaB kinase, isoforms α and β; IR, insulin receptor; IR/IGF-1R, IR + insulin-like growth factor-1 receptor; IRS-1, insulin receptor substrate-1; JNK, c-Jun N-terminal kinase; mTOR, mammalian target of rapamycin; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PKC/λ, protein kinase C, λ/ζ isoforms; pS, serine phosphorylated; pT, threonine phosphorylated; pY, tyrosine phosphorylated.

^{*} Associations between episodic memory scores and basal levels of the variables listed were calculated using a combined set of normal (N = 30), MCI (N = 29), and AD dementia (N = 31) cases in the Religious Orders Study. Data were adjusted for age, gender, and years of education. IR/IGF-1R = insulin + insulin-like growth factor receptor. Adapted from Talbot et al. [14].

[†] Activated form of this molecule. Why elevated basal levels of IRS-1 pY is expected to inhibit insulin signaling is explained by Talbot et al. [14].

[‡] Suppressed form of this molecule.