Insulin and IGF-1 signalling: longevity, protein homoeostasis and Alzheimer’s disease

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Abstract
The quality control of protein homoeostasis deteriorates with aging, causing the accumulation of misfolded proteins and neurodegeneration. Thus, in AD (Alzheimer’s disease), soluble oligomers, protofibrils and fibrils of the Aβ (amyloid β-peptide) and tau protein accumulate in specific brain regions. This is associated with the progressive destruction of synaptic circuits controlling memory and higher mental function. The primary signalling mechanisms that (i) become defective in AD to alter the normal proteostasis of Aβ and tau, and (ii) initiate a pathophysiological response to cause cognitive decline, are unclear. The IIS [insulin/IGF-1 (insulin-like growth factor 1)-like signalling] pathway is mechanistically linked to longevity, protein homoeostasis, learning and memory, and is emerging to be central to both (i) and (ii). This pathway is aberrantly overactivated in AD brain at the level of increased activation of the serine/threonine kinase Akt and the phosphorylation of its downstream targets, including mTOR (mammalian target of rapamycin). Feedback inhibition of normal insulin/IGF activation of the pathway also occurs in AD due to inactivation of IRS-1 (insulin receptor substrate 1) and decreased IRS-1/2 levels. Pathogenic forms of Aβ may induce aberrant sustained activation of the PI3K (phosphoinositide 3-kinase)/Akt signal in AD, also causing non-responsive insulin and IGF-1 receptor, and altered tau phosphorylation, conformation and function. Reducing IIS activity in animal models by decreasing IGF-1R levels or inhibiting mTOR activity alters Aβ and tau protein homoeostasis towards less toxic protein conformations, improves cognitive function and extends healthy lifespan. Thus normalizing IIS dysfunction may be therapeutically relevant in abrogating Aβ and tau proteotoxicity, synaptic dysfunction and cognitive decline in AD.

AD (Alzheimer’s disease): a protein-folding disorder
AD and all of the age-related neurodegenerative disorders are fundamentally protein-folding disorders. In AD, excessive levels of a peptide, Aβ (amyloid β-peptide), and a protein, the MAPT (microtubule-associated protein tau), both of which are normally soluble, become misfolded and aggregate in β-pleated sheet amyloidogenic structures. Aβ fibrils accumulate in extracellular plaques and also in the cerebrovasculature and comprise peptides of 38–43 amino acids which emanate from β- and γ-secretase-induced proteolytic cleavage of the APP (amyloid precursor protein) [1–3]. Aβ is synthesized normally in the brain; however, Aβ deposition in plaques occurs at high levels in AD, and this also occurs in the brains of people with Down’s syndrome, due to triplication of the APP gene on chromosome 21. Mutations in APP, PS1 (a key component of the γ-secretase complex) and PS2 cause rare early age of onset familial forms of AD, and all APP and PS1/2 mutations cause increased levels and/or fibrillogenicity of Aβ peptides and plaque formation (for reviews, see [1–3]).

Aggregates of insoluble tau form structures called NFTs (neurofibrillary tangles), which build up within the neuronal cell soma, in the neuropil as neuropil threads and in neuritic components surrounding Aβ plaques in AD [4,5]. Tau is normally highly soluble and its best known function is the stabilization of microtubules in axons where it predominantly resides in the normal adult brain. The fibrillogenicity and insolubility of tau is a key feature of a number of neurodegenerative disorders known as tauopathies, many of which result in dementia. These include FTDP-17 (frontotemporal dementia with Parkinsonism on chromosome 17) a subgroup of which is actually caused by mutations in tau (MAPT) [6].

In recent years, it has emerged that a continuum of protein-folding states exist particularly for Aβ, where peptide/protein aggregation can include soluble ‘low-n oligomers’, including dimers, trimers, dodecamers and protofibrils and also insoluble β-sheet fibrils in mature neuritic plaques [2,7] (Figure 1). In the case of tau, the misbehaviour and self-aggregation of the protein in NFTs is closely linked with its excessive phosphorylation. Tau in NFTs has a high β-sheet composition and can be seen ultrastructurally as PHFs (paired helical filaments) [8]. Evidence also points to multiple aberrant protein-folding states for tau, including soluble oligomeric species and protofibrils [9]. Dense aggregates of

Key words: Alzheimer’s disease, amyloid, insulin/IGF-1-like signalling (IIS), protein misfolding, tau.

Abbreviations used: Aβ, amyloid β-peptide; AD, Alzheimer’s disease; APP, amyloid precursor protein; DAF2, decay-accelerating factor 2; FGFR, forkhead box O; GIP-1, glucagon-like peptide 1; GSK3, glycogen synthase kinase 3; HSF1, heat-shock factor 1; IGF-1, insulin-like growth factor 1; IRS, insulin receptor substrate; IRS-1, insulin/IGF-1 receptor; mTOR, mammalian target of rapamycin; MAPT, microtubule-associated protein tau; mTOR, mammalian target of rapamycin; NFT, neurofibrillary tangle; PS1, phosphoinositide 3-kinase; PS2, phosphatase and tensin homologue deleted on chromosome 10.

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Aβ in plaques and tau in NFTs are not innocuous; however, it is hypothesized that they may in part result from attempts to bundle up Aβ and tau into inert, less toxic species, with the more rampant neurotoxicity elicited by soluble conformationally altered Aβ and tau in non-β-sheet forms.

**Relationship between protein misfolding and the development of AD**

AD is a disease of slowly progressive synaptic collapse affecting areas of the brain underlying memory and higher mental function [10]. Synaptic loss in these brain areas relates directly to the progressive cognitive decline that characterizes AD. This is closely associated with the aberrant folding of tau and a hierarchical progressive build-up of tau pathology through connecting neuronal/synaptic circuits of the brain [5]. The relationship between the clinical progression and severity of AD and Aβ deposition in plaques is more complex [2,3,11] and the number or localization of plaques in the brain does not relate to cognitive decline or synaptic loss. This is because it is believed, but not totally established, that it is the early build-up of soluble Aβ oligomers/protofibrils, years before the appearance of cognitive effects, that initiates toxicity in AD [2,3,7,11].

The dynamic interplay between the different aberrant protein species of either Aβ or tau and their relative physiological function and potential brain-damaging effects are unclear. Combined evidence from genetic, neurobiological/molecular and behavioural studies suggests that increased production and/or decreased clearance of aberrant conformations of Aβ is the primary initiating trigger for AD [1,2,7]. However, the presence of human tau, and the emergence and progression of tau pathogenesis, is believed to be an essential mediator of Aβ pathophysiology and to be necessary for the progressive cognitive decline in AD (for reviews, see [3,11,12]). AD also has a clear inflammatory component through astrocytic and microglial responses, which is closely related to the aberrant protein folding and impaired clearance of Aβ and tau [13].

**IIS [insulin/IGF-1 (insulin-like growth factor 1)-like signalling]: major regulators of longevity and protein homeostasis with direct relevance to AD**

AD occurs randomly in the aging population and the underlying cause of protein misfolding, synaptic malfunction and associated cognitive decline remains elusive. Thus the primary signalling mechanisms that (i) become defective with age in AD to alter the normal protein homeostasis of Aβ and tau, and (ii) initiate a pathophysiological response to the altered Aβ and tau to cause cognitive decline/synaptic loss are unclear and much sought after.

One pathway that is emerging to be central to this is the IIS pathway. This system has been a subject of fascination in aging research, in understanding the regulation of lifespan and the maintenance of healthy protein networks with age. In essence, decreasing activity through the IIS pathway prolongs stress resistance and protein homeostasis with age, enabling a longer healthier lifespan [14–16]. The IIS pathway has a high degree of evolutionary conservation (Figure 2) and has been much studied in Caenorhabditis elegans where a single IGF-1 and IR (insulin receptor) exists called DAF2 (decay-accelerating factor 2) that responds to an insulin-like ligand. The IGF-1R/IR in mammalian species are 2α–2β-subunit tyrosine kinases receptors highly expressed in the nervous system. Ligand-induced activation triggers autophosphorylation and a multistep amplification cascade...
whose primary instigator occurs via activation of PI3K (phosphoinositide 3-kinase) and thereby the serine threonine kinase Akt [also known as PKB (protein kinase B)] [17]. Akt in turn phosphorylates a plethora of target protein, including GSK3β (glycogen synthase kinase 3β), mTOR (mammalian target of rapamycin), p70/S6 kinase, and inactivates the FOXO (forkhead box O) forkhead transcription factors. The primary negative regulator of this pathway is the lipid and protein phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10). The downstream protein networks targeted by Akt regulate a diverse array of vital biological processes. In the nervous system, these include survival, migration, neuronal polarity, inflammatory responses/stress resistance, protein translation, synaptic plasticity, learning and memory, autophagy, cell cycle, protein transport/trafficking, metabolism and myelination.

Reducing IIS pathway activity can allay aberrant Aβ and tau proteostasis and improve cognitive function

Keeping the IIS pathway at just the right level of activity is vital [17]. So although deletion of both copies of IGF-1R is lethal in mammalian species, deleting one copy of the IGF-1R gene creates mice that live an average of 26% longer and this is even more significant in female mice [18]. This observation agrees with extensive studies in C. elegans, where reducing signalling through DAF2 and several components of the downstream pathway increase longevity [14]. Although the reasons underlying the longevity-inducing effect of reducing IIS are complex, one of the major effects of this is facilitation of the activation of transcription factors, such as FOXO and HSF1 (heat-shock factor 1), which allow transcription of anti-stress genes that have a positive effect on protein homoeostasis and a healthy longer life [15,16]. In parallel with this, mTOR activity is down-regulated, permitting activation of autophagy pathways that clear misfolded proteins, in addition to the inhibition of cap-dependent and preponderance of cap-independent protein translation [19]. Together this leads to maintenance of protein health and quells protein misfolding. This is especially vital in the adult nervous system, where post-mitotic neurons cannot get rid of faulty proteins through division programmes.

Research shows that reducing activity of the IIS pathway (in worm, fly and mouse models) can alter Aβ and tau protein homoeostasis towards less toxic protein conformations and
Sustained activation of IIS in AD brain may be caused by Aβ and induce both conformational changes in tau and non-responsive insulin and IGF-1 receptors via aberrant downstream phosphorylation networks

Could the same situation extend to humans with age? Does having less IIS increase longevity? Are increased levels of IIS activity linked to the aberrant protein folding states of Aβ and tau and the neurodegenerative process in people with AD? Should we be considering treatments that attempt to reduce and/or normalize the IIS pathway in AD? Basically, the answer to all the above questions would appear to be yes.

Firstly, when considering human aging, functionally relevant IGF-1R mutations, with down-regulated IGF-1R activity, have been discovered in female centenarians [27]. In addition, low IGF-1, P13K and IRS-1 (insulin receptor substrate 1) correlate with prolonged lifespan [28,29], as do FOXO3a mutations [30]. When considering age-related neurodegeneration in AD, signalling through the IGF-1R and IR is patentely disturbed in the AD brain [31–33]. These defects describe both increased [32] and decreased levels of IGF-1R [33], with unchanged [32] or decreased levels of IR [33]. We performed a very detailed analysis of the levels and localization of IGF-1R, IR and IRS-1/2 proteins in the post-mortem temporal cortex of individuals who had AD [32]. The results show that overall levels of IGF-1R in the cerebral cortex are significantly increased in people with AD compared with people of the same age without the disease. Significantly increased IGF-1R levels localize to activated astrocytes and degenerating synapses and neurites within and surrounding Aβ plaques, but total neuronal IGF-1R levels are actually decreased in AD and their subcellular localization is altered particularly within neurons with NFTs.

IRs also show an altered localization, with internalization of surface IR within the neuronal cell soma and a loss of IRs from dendrites in AD [32]. Consistent with other findings [33,34], decreased levels of both IRS-1 and IRS-2 in AD neurons, and in addition very marked increased levels of major inactivating IRS-1 phosphorylation motifs at Ser312/616, are present in AD brain co-localizing closely within neurons with NFTs. In previous work, we discovered an excessive hyperactivation of the Akt signalling pathway in the same AD neurons and diminished levels and altered subcellular localization of PTEN, with an eventual loss of Akt/PTEN signalling neurons in AD [35]. Decreased levels and impairment of PTEN activity has been further reported in AD [36], as has increased activation of mTOR and p70/S6 kinase [37], often showing strong association with NFT formation. Together, these findings lead to the hypothesis that an excessive and inappropriate hyperactivation of Akt in AD neurons serves to both overactivate the IIS pathway while also inducing feedback inhibition and desensitization of IGF-1R and IR through inactivation of the key adaptor IRS proteins. In parallel, this can induce increased tau phosphorylation and thereby aberrant tau protein conformations and functions that link to cognitive decline and synaptic dysfunction in AD (Figure 3).

What could cause this sustained activation of IIS and desensitization of normal insulin and growth factor responses in the AD brain? Notably, diverse Aβ species have been described to bind to IRs [38,39] and Aβ oligomers induce IR internalization in mature primary neurons and remove IRs from dendrites, mimicking what is seen in AD brain [39,40]. Moreover, Aβ oligomers block IR activation in vitro [39], which can increase Akt activation [39,41] and also cause the inactivation of IRS-1 by phosphorylation at serine residues [34]. Aβ can hyperactivate mTOR [23–25], and some Aβ species can inactivate PTEN [42]. Together this indicates that soluble Aβ oligomeric conformations may derail and/or compete for the IGF-1R/IR signalling system in AD neurons through inappropriately increased activation of the IIS pathway and feedback shut-off of normal IIS activation. The mechanism by which Aβ causes sustained activation of the P13K/Akt pathway is unknown but may occur via Aβ-induced-inactivation of PTEN or other major brakes on the IIS pathway.

A sustained activation of IIS will have a direct effect on the hyperphosphorylation, conformation and function of tau, as many of the kinases and phosphatases implicated in tau hyperphosphorylation in AD are direct components of, or interact with, the IIS pathway. These include: Akt, GSK3β, mTOR p70/S6 kinase, MARK [MAP (microtubule-associated protein)-regulating kinase/microtubule affinity-regulating kinase], Cdk5 (cyclin-dependent kinase 5) and AMPK (AMP-activated protein kinase). Basically, Akt can directly phosphorylate tau at Ser394, an event closely linked to the detachment of tau from microtubules and possible initiation of tau pathogenesis (for discussion, see [35]). Increased mTOR-induced cell cycle activation can also increase neurodegeneration in a Drosophila tauopathy model [26] and blocking mTOR activation can decrease tau pathogenesis in vivo in the 3×Tg-AD model.

It is of strong relevance that fine-tuned regulation of the Akt/GSK/mTOR axis functions mechanistically in learning, memory and synaptic plasticity processes, including effective use-dependent protein translation at synapses. Specifically,
it has been shown that hyperactivation of PI3K/Akt by Aβ links to cognitive decline in animal models and that blocking this can alleviate cognitive decline [43], with similar findings shown for mTOR [24,25]. Controlled on/off signals through the PI3K/Akt/GSK3β/mTOR axis regulate key elements of LTD (long-term depression)/LTP (long-term potentiation) [44–47]. Moreover, sustained overactivation of mTOR is a major mechanism responsible for aberrantly increased protein translation at synapses [44,48]. This can severely impair higher mental function, such as in Fragile X disorder [44,48,49] and interestingly can cause the overtranslation of APP [50].

**Targeting IIS: therapeutic considerations for AD**

Maintaining appropriately responsive IGF-1R/IR signalling in neurons, but blocking the sustained hyperactivation of the Akt/mTOR arm of the IIS pathway, would appear to be crucial to protect against the instigation/progression of protein misfolding in AD, Aβ-induced synaptotoxicity and the spread of tau pathology. This is highlighted by in vitro findings showing that inhibition of IGF-1R and IR family tyrosine kinase activity selectively mimics the detrimental effects of Aβ oligomers on synaptic signalling [38] and that IGF-1R and IR family tyrosine kinase activity prevents the pathogenic binding of Aβ oligomers to neurons, thereby blocking their synaptotoxic effects [40].

Intranasal insulin therapy can directly target the CNS (central nervous system), but not peripheral IR, and is being currently tested in pilot clinical trials in individuals with MCI (mild cognitive impairment), showing some positive effects [51]. IGF-1 can also be protective in some contexts in AD animal models [52]. These IIS agonist treatments come with the caveat that they may induce eventual excessive activation and desensitization of IR/IGF-1R, serving to negate any beneficial effect. Moreover, the internalization of IR as seen in AD brain may limit insulin’s protective potential. A better approach may be the use of drugs used therapeutically to treat deficits in insulin signalling in Type 2 diabetes mellitus, in particular the long-lasting incretin hormones [GLP-1 (glucagon like peptide-1)] analogues, exendin-4 and liraglutide. These drugs cross the blood–brain barrier, can reduce levels of Aβ and prevent Aβ-induced neurotoxic effects, including inflammation, and improve indicators of learning and memory [53,54].

Overactivation of the PI3K/Akt/mTOR pathway is a key pathogenic signal driving cancer, thus many of the approaches used to block this excessive kinase activation in cancer may have an application in diminishing AD pathogenesis if targeted to the brain. This is highlighted by results showing that inhibiting mTOR activation is protective in preclinical models of AD [24,25]. Other more indirect treatments targeting mTOR, for example, using mGLUR (metabotropic glutamate receptor) antagonists, as is used in the treatment of fragile X disorder may be worthy of consideration [49].

In summary, aberrant protein folding of Aβ and tau in AD and the detrimental effect of this on cognition and synaptic function in brain integrates on several levels with heightened and pathologocal activation of the PI3K/Akt/mTOR component of the IIS pathway in the disease. Targeting this pathway to abrogate overactivation of the PI3K/Akt/mTOR arm may be a viable therapeutic strategy, possibly in conjunction with promotion of normal insulin/IGF-1 signalling either directly through IR/IGF-1R or via parallel signalling receptor pathways, such as GLP1R.
(GLP-1 receptor), or approaches such as exercise and/or caloric restriction. Meanwhile, improving the understanding of the regulation of IIS in normal aging and in AD is an area deserving significant attention.

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**References**


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