Brain insulin resistance: role in neurodegenerative disease and potential for targeting

Prof. Christian Hölscher, PhD

Second hospital, Neurology department, Shanxi medical University, Taiyuan 030001, Shanxi province, PR China
Research and Experimental Center, Henan University of Chinese Medicine, Zhengzhou 450046, Henan province, PR China

Email: c.holscher@hactcm.edu.cn
Phone: +86-18339962156

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Abstract

Introduction: This review evaluates the novel strategy of treating Alzheimer’s and Parkinson’s disease (AD and PD) with drugs that initially have been developed to treat type 2 diabetes. As insulin signalling has been found to be de-sensitized in the brains of patients with these diseases, drugs that can re-sensitize insulin signalling have been tested to evaluate if this strategy can alter disease progression.

Areas covered: The review will give an overview of preclinical and clinical tests in AD and PD of drugs activating insulin receptors, glucagon-like peptide -1 (GLP-1) receptors, and glucose-dependent insulinotropic polypeptide (GIP) receptors.

Expert opinion: Insulin, GLP-1 and GIP receptor agonists have shown good effects in preclinical studies. First clinical trials in MCI/AD patients have shown that insulin can improve on key pathological symptoms of AD such as memory impairment, brain activity, neuronal energy utilization, and inflammation markers. The improvements are still visible months after the trial has stopped. A GLP-1 receptor agonist has shown disease-modifying effects in PD patients, and first pilot studies have shown encouraging effects of a GLP-1 receptor agonist in AD patients. The results are proof of concept that demonstrate that enhancing insulin signalling in the brain does have positive effects on disease progression that are long lasting. Novel dual GLP-1/GIP receptor agonists that cross the blood brain barrier at an enhanced rate show superior neuroprotective effects compared to single GLP-1 or GIP receptor agonists, and show great promise as novel treatments of AD and PD.

Highlights:
- drugs that lower amyloid levels have failed to stop Alzheimer’s disease progression
- growth factors such as insulin, GLP-1 or GIP have neuroprotective effects in AD and PD models
- first clinical trials show improvements in AD patients by nasal application of insulin
- a GLP-1 receptor agonist showed good protective effects in PD patients
- novel dual GLP-1/GIP receptor agonists have been developed that can cross the BBB
- dual GLP-1/GIP receptor agonists show improved protection in preclinical studies in AD and PD

Keywords
Alzheimer’s disease; growth factor; GLP-1; inflammation; insulin; mitochondria; Parkinson’s disease
1. Introduction

Chronic neurodegenerative disorders such as Alzheimer’s disease (AD) or Parkinson’s disease (PD) are a major burden to the health systems. Unfortunately, no disease-modifying treatments are available that can limit or stop disease progression. Currently, the main treatment is the administration of the precursor of dopamine, L-DOPA, which offers some improvement of the symptoms to PD patients [1], but the improvement is short lived and only lasts as long as the drug is present in the system. Other treatments such as dopamine receptor agonists do not fare better. In addition, the effect of L-DOPA fades over time as the neurons in the substantia nigra that metabolize it to dopamine continue to die. Furthermore, long-term users of L-DOPA can develop serious side effects such as dyskinesia/dystonia [2]. There are only two drug types available to treat AD, both have very limited effects on the symptoms and have no disease-modifying properties. Acetylcholine esterase inhibitors can provide slight improvement of cognitive symptoms, and the NMDA glutamate receptor antagonist memantine has very limited effects on AD [3]. Hence, there is an urgent need to investigate new disease mechanisms and concepts that show promise to be more successful in the clinic.

2. A return to Physiology

Unfortunately, all clinical trials that were based on the amyloid hypothesis have failed or have shown only very minimal improvements that are not relevant in the clinic. Progressive neurodegenerative disorders such as AD and PD are complex syndromes that go through several stages over time and involve multiple physiological and pathological processes that include a range of cell types and organs. A monocausal explanation of such syndromes is unlikely to be correct and will not lead to a successful treatment of these syndromes. It is time to go back and have a closer look at the underlying pathology and physiology of these diseases in order to find more promising strategies to treat these illnesses.

2.1 Triggers for AD and PD
The initial triggers of diseases such as AD and PD are not completely known. Several mutations of risk genes have been identified that can lead to these syndromes, but the majority of patients do not carry these risk genes [4-8]. Environmental influences must play an important role in triggering these diseases. We know that in PD, chemicals such as pesticides can induce PD in people who have been
exposed to these chemicals for some time [9]. For example, the chemical 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can induce PD in humans [10, 11]. Detailed investigations found that the chemical can cross the blood-brain barrier (BBB)[12]. In dopaminergic neurons, MPTP is converted to MPP+ by the enzyme monoamine oxidase B (MAO-B) [13, 14]. This free radical can block the electron transport chain in mitochondria by blocking complex 1, impairing the neurons’ ability to produce ATP [15, 16]. Mitochondrial dysfunction is a key feature in PD and can lead to neuronal death [12, 17, 18]. A range of other chemicals and stressors have been shown to have similar properties that lead to a PD phenotype in humans [8, 19]. Virus infections that lead to chronic inflammation responses and eventually lead to neurodegenerative progressive syndromes have been proposed as another trigger for AD [20] as have bacterial infections [21, 22]. It is likely that the triggers for AD and PD development are manifold and include genetic risk genes, virus and bacterial infections, exposure to heavy metals or specific chemicals, the development of an auto-immune response, and more. A combination of stressors may be required for the full development of the disease over time, as discussed here [8].

2.2 Risk factors of AD and PD: diabetes
As the initial triggers for these slowly-developing progressive diseases are not easy to identify, a look at the list of risk factors and risk genes may give us a clue what the underlying mechanisms are that drive such diseases. The list includes a range of factors that increase stress on the brain, such as high blood pressure, smoking, head injury, and high cholesterol levels [23]. It is easy to see how these risk factors can stress neurons in the CNS and increase the chance of developing AD or PD. However, there are other risk factors that do not appear to be linked to neurodegenerative disorders in any way. In several studies, type II diabetes mellitus (T2DM) has been identified as a risk factor for AD and PD [24-35]. Indications that impaired brain metabolism is causally linked to the development of dementia had already been observed 50 years ago [36, 37]. T2DM and progressive neurodegenerative disorders are very different conditions, and few people would associate those two conditions. However, unexpected associations of this nature can potentially uncover previously unknown mechanisms that drive these diseases.

2.3 Insulin is a growth factor
Insulin is well known as a hormone that regulated blood glucose levels. However, insulin plays a lot more roles in physiology. It is a key growth factor that regulates cell energy utilization, and the uptake of glucose from the blood is part of that role. Insulin can activate cell growth, cell repair, gene
expression, mitochondrial activity and energy utilisation, autophagy and protein synthesis. The insulin receptor activates key second messenger cascades that activate kinases including phosphoinositide 3 kinase (PI3k), protein kinase B (Akt/PKB), peroxisome proliferator-activated receptor (PPAR)γ/δ, mammalian target or rapamycin (mTOR), and activate transcription factors such as nuclear respiratory factor 1/2 (NRF1 and NRF2). This promotes cell metabolism, mitogenesis, glucose utilization, synaptic activity, gene expression that can deal with oxidative stress, and all genes required for cell growth and repair [38-44], see figure 1 for details. Insulin crosses the BBB [45] and the insulin receptor is expressed on neurons [38, 41, 46, 47].

2.4 Insulin signalling is impaired in the brains of AD and PD patients

Preclinical studies have shown that animal models of AD and PD show impaired insulin signalling and a range of downstream effects that contribute to the pathology [34]. An important finding in the recent years was that insulin signalling in the brains of AD and PD patients is de-sensitized, too. When analysing brain tissue from patients, it was found that the insulin and insulin-like growth factor 1 (IGF-1) receptor along with the insulin receptor substrates 1 and 2 (IRS1/2) and key second messenger kinases such as Akt and mTOR were inactivated, similar to what is observed peripherally in diabetes [48-50]. In fact, one group termed AD ‘type 3 diabetes’ [48]. However, insulin desensitization in diabetes is driven by high glucose and insulin levels, and insulin desensitization in the brain of AD patients was observed even in people that did not have diabetes [50]. Instead, insulin desensitization is most likely driven by the chronic inflammation response in the brain in AD and PD. Pro-inflammatory cytokines such as tumour necrosis factor (TNF) will block growth factor signalling such as that of insulin or IGF-1 [51-53]. Considering that insulin is important for the growth and repair of neurons, it is easy to see how desensitization can put neurons at risk from damage over time. As neurons are not replaced in the brain (neurogenesis in the cortex is negligible [54]), damage to neurons may accumulate over time and present itself as neurodegeneration at the final stage.

3. The insulin hypothesis: Normalizing insulin signalling as a viable strategy for treating AD and PD

Insulin signalling impairment has been observed in the brains of AD patients, even in patients that were not diabetic [48, 50]. In addition, insulin signalling has been found to be compromised in PD patients, too [55, 56]. It is likely that chronic inflammation in the brain is the main driving force
behind this, as pro-inflammatory cytokines inhibit growth factor signalling [39, 51]. The observation that insulin signalling is impaired in the brains of AD and PD patients laid the foundation of the theory that improving insulin signalling may be beneficial and can improve the pathology. The research group of Suzanne Craft and colleagues have conducted a series of key clinical trials to test this theory in MCI/AD patients. In order to test if improving insulin signalling has any beneficial effects in the brain, the group employed a method of applying insulin to people who are not diabetic. As insulin lowers blood sugar levels, it would not be safe to apply insulin via intravenous injection. Therefore, a novel nasal application technique had been developed [57]. This way, insulin enters the brain by absorption via the nasal epithelium while peripheral insulin levels in the blood increase very little [40, 58].

3.1 Clinical trials of intranasal insulin application in MCI/AD patients

Clinical studies have been conducted in patients with mild cognitive impairment (MCI), a condition that can develop further into AD. A pilot study testing intranasal application of insulin in MCI/AD patients had been conducted on memory-impaired subjects in a double-blind placebo controlled study. Insulin treatment improved recall of verbal memory without affecting blood glucose levels in the periphery. These improvements were stronger in memory-impaired in non-apolipoprotein E epsilon 4 (APOEε4) carriers than in memory-impaired APOEε4 carriers and control subjects. Interestingly, memory-impaired APOEε4 carriers showed poorer recall following insulin administration. These positive results demonstrate that intranasal insulin can indeed improve memory impairments in AD/MCI patients. The results also demonstrate that the APOEε4 allele has an effect on insulin treatment efficacy [59]. In a second study in AD/MCI patients, insulin treatment improved recall of verbal memory in memory-impaired non-APOEε4 carriers. In contrast, memory-impaired APOEε4 carriers showed a deterioration of verbal memory. These results confirmed the previous pilot study and further demonstrate that APOE affects the insulin effect in a complex interaction [60]. In a follow-up pilot study, insulin treatment showed improvements in recalling verbal information after a delay and other cognitive processes (e.g. orientation, judgment, social interactions, home activities, personal care, speech/language) as rated by care givers. Insulin treatment also increased the Abeta 40/42 ratio, which is considered to be an improvement of these AD biomarkers [61].

After these small studies that showed encouraging effects, a larger study with a longer 4 month treatment regime had been conducted [62]. The study was randomized, double-blind, and placebo-controlled. 104 patients with either MCI or mild to moderate AD were included. Patients received
either placebo, 20 IU of insulin, or 40 IU of insulin intranasally for 4 months. Primary measures consisted of delayed story recall score and the Dementia Severity Rating Scale score, and secondary measures included the Alzheimer Disease's Assessment Scale-cognitive subscale (ADAS-cog) score and the Alzheimer's Disease Cooperative Study-activities of daily living (ADCS-ADL) scale. CSF samples were taken in 23 patients, and $^18$FDG-PET brain scans were conducted in 40 patients before and after treatment. $^18$FDG is a radiolabelled modified glucose molecule that is taken up by neurons but is not metabolized. This brain scan can measure brain activity and energy utilization. As brain activity is reduced in AD patients and correlates well with cognition [63], $^18$FDG-PET brain scans are ideal for investigating if insulin treatment does indeed enhance insulin signalling and neuronal activity in the brain. The results showed that treatment with 20 IU of insulin improved memory, and both doses of insulin (20 and 40 IU) improved caregiver-rated daily living abilities. Both insulin doses also preserved general cognition as assessed by the ADAS-cog score and functional abilities as assessed by the ADCS-ADL scale. Importantly, placebo-treated participants showed decreased $^18$FDG-PET uptake in the key cortical regions, and insulin minimized this disease progression. The decrease in $^18$FDG was not observed in drug-treated patients, demonstrating that the progressive degeneration of brain activity and energy utilization of neurons had been stopped. In follow-up tests, the improvements in episodic memory were still present two months after treatment had been stopped [64]. Importantly, key biomarkers were analysed in this study from exosomes found in patient blood plasma. Exosomes are vesicles that are released from cells and contain a range of peptides that originate from the cells of origin. Neuron-specific membrane standing markers such as NCAM can be used to identify which exosomes originate from the brain. Analysing the content of these exosomes showed that in MCI patients treated with 20 IU insulin, biomarkers of insulin resistance (pS312-IRS-1, pY-IRS-1) were improved and showed strong positive correlations with ADAS-Cog changes, especially in ApoEε4 non-carriers [62].

This chronic study testing a larger group of patients and also assessing AD biomarkers and glucose utilization in the brain supports the concept that impairment of insulin signalling is at least one crucial parameter in the disease progression of AD, and that improving insulin signalling has indeed beneficial effects on cognition and brain activity as shown in the $^18$FDG-PET scans. The exosome study confirms the PET scan results and show that the reduced insulin signalling in the brains of AD patients has been improved. The effect was still visible two months after treatment, which suggests that the insulin effect is not just an acute improvement of neuronal and cognitive function and is not permanent, but that over time, neuronal cell metabolism and neuronal activity and cognition is improved on a structural level that is permanent.
A follow-up study had been conducted to compare detemir with native insulin. Three groups of AD/MCI patients were recruited, placebo (N=13), insulin (n=12), and detemir (n=12). A cognitive battery test was given at baseline and after two and four months of treatment. MRI brain scans were taken at baseline and after four months. The primary outcome was change from baseline to four months in memory tests. Secondary outcomes included global cognition (ADASC), daily functioning, MRI brain volumes and amyloid biomarkers in CSF. Native insulin but not detemir improved memory formation at both 2 and 4 month time points. In MRI brain scans, insulin reduced the observed shrinkage in the placebo group. In CSF analysis, a reduction in the tau-P181/Alβ42 ratio was observed, which is interpreted as an improvement [65].

In conclusion, these clinical trials have shown a clear proof of concept that the improvement of insulin signalling shows improvement on key pathological markers of AD such as cognition, memory, brain activity and energy utilization as shown in 18FDG-PET scans, brain volume as shown in MRI scans, an improvement in amyloid and tau AD biomarkers, and an improvement in insulin cell signalling in neurons as shown in the exosome study. Contrary to the clinical trials testing drugs that reduce amyloid levels in the brain, these clinical trials with sometimes even small patient numbers and short exposure to insulin treatment consistently showed improvements, demonstrating that this type of treatment does affect key mechanisms underlying the pathology of AD. The insulin hypothesis is therefore the only current hypothesis that has shown clinical evidence of disease modification, and all efforts should be made to develop novel treatments that build on it.

4. Novel strategies to treat AD and PD: The incretin hormone glucagon-like peptide -1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)

While the studies with insulin showed good results, insulin is not an ideal drug to be developed as a major treatment for AD and PD. Insulin is used as a treatment for T2DM in the clinic. However, the long term prognosis in diabetes is not good for treatment with insulin, as higher insulin levels progress insulin desensitization further, to the point where insulin is no longer effective [66]. The mixed result from the Detemir study, in which the drug improved cognition in APOEε4 carriers, but non-carriers got worse compared with placebo, point in that direction. Importantly, higher peripheral insulin resistance at baseline correlated with better performance, while lower peripheral insulin resistance correlated with worsening of memory formation after treatment with Detemir. As a consequence of this detrimental property of insulin, research of novel drug treatments for diabetes has moved away from insulin analogues to new peptide hormones that have similar effects, in
particular the incretin hormones (GLP-1 and GIP) [67, 68]. These drugs do not enhance insulin desensitization as they do not activate insulin receptors. Instead, they can re-sensitize insulin signalling [68-71]. In addition, GLP-1 analogues do not affect blood glucose levels in normoglycemic people [72], and therefore can be safely given to non-diabetic AD or PD patients. The side-effects are very mild and include nausea and loss of appetite.

4.1 GLP-1 analogues as treatments for T2DM
Agonists of the incretin hormone GLP-1 receptor are effective and well tolerated drug treatments for T2DM [73-75]. GLP-1 is part of the peptide growth-factor family and activates a glucagon-type seven membrane spanning G-protein coupled receptor [76, 77]. The downstream signalling effects on cell growth and repair are comparable to those of insulin (see figure 2 for details). GLP-1 receptors are expressed in neurons of rodents, primates and humans [78-81]. Nigel Greig and colleagues were the first to test GLP-1 analogues in preclinical studies to discover that they have neuroprotective properties [82, 83]. Further work from my lab showed that a range of GLP-1 receptor agonists such as liraglutide, lixisenatide and semaglutide that are on the market to treat T2DM have neuroprotective effects in animal models of AD and PD and re-sensitize insulin signalling [70, 84, 85]. Importantly, some of these GLP-1 receptor agonists can cross the BBB and act in the CNS in a protective way [86-92].

4.2 GLP-1 mimetics show neuroprotective effects in animal models of AD
In a range of mouse models of AD, GLP-1 receptor agonists were found to be neuroprotective. The GLP-1 receptor agonists exendin-4 showed good neuroprotective effects in a triple transgenic mouse model of AD that expresses APP, PS1, tau human mutated genes related to early-onset AD and FTD [93]. Currently, exendin-4 is on the market as a treatment for T2DM (Byetta®, Bydureon®). Importantly, Exendin-4 showed neuroprotective effects in several animal models of neurodegeneration such as PD and traumatic brain injury [94-97]. Liraglutide (Victoza®), another drug that is on the market to treat T2DM [98], showed clear neuroprotective effects in the APP/PS1 transgenic mouse model of AD. Memory loss, impaired synaptic transmission (LTP) in the hippocampus, synapse loss, chronic inflammation in the brain, and the amyloid plaque load and amyloid levels in the cortex were much reduced [89]. Liraglutide treatment showed protective effects in aged 14-16 months old APP/PS1 mice, suggesting that treatment even at more progressed stages of AD may still have benefits [99]. When treating APP/PS1 mice chronically for 8 months, liraglutide stopped disease progression and therefore has the potential to be used as a prophylactic
The GLP-1 receptor agonist lixisenatide (Lyxumia®) had similar neuroprotective effects in the APP/PS1 mouse model [101]. Liraglutide furthermore showed protective effects in the triple APP/PS1/tau tg mouse model [102]. These protective effects of liraglutide in APP/PS1 mice were repeated in several independent studies [103, 104]. One study failed to find such protective effects of liraglutide in two AD mouse models. However, this study contains several technical shortfalls and misinterpretations. For example, the authors chose a tg mouse model that expresses the London APP mutation. This mutation produces predominately intracellular amyloid deposits and very few extracellular plaques [105]. Yet, the authors measured only amyloid plaques in this model and found that liraglutide did not lower the very small number of plaques found in the brain [106]. In a separate study, liraglutide showed protective effects in the human P301L mutated tau gene -expressing mouse, a model of fronto-temporal lobe dementia (FTD). Liraglutide reduced motor impairments and the amount of tangles and hyperphosphorylated tau in the brain [107]. In the accelerated senescence SAMP8 mouse model of AD, liraglutide improved memory formation and reduced neuronal loss in the hippocampus [108]. Liraglutide furthermore improved insulin desensitization and chronic inflammation in the brain induced by the injection of amyloid oligomers into the cortex of cynomologous monkeys. The level of synaptic markers was also protected from the effects of amyloid in the brain, indicating that synaptic loss was prevented [109, 110]. As a further demonstration of its growth-factor properties, GLP-1 mimetics are able to normalize neuronal progenitor cell proliferation and neurogenesis in the brains of mouse models of AD and of diabetes [86, 89, 93, 111-115]. They furthermore protected against ER stress toxicity and autophagy reduction [116, 117]. Importantly, GLP-1 receptor agonists have anti-inflammatory effects in the brain, thereby addressing not just growth-factor re-sensitization but also reduction of chronic inflammation in the brain [89, 101, 118, 119]. The activation of microglia and astrocytes and the elevated expression of pro-inflammatory cytokines was much reduced by liraglutide in an X-ray exposure model of brain inflammation [120]. Chronic inflammation is a key driver of AD pathology, and pro-inflammatory cytokines can block growth-factor signalling, thereby facilitating insulin desensitization and contributing to disease progression [52, 121, 122].

4.3 GLP-1 mimetics show protective effects in animal models of PD

The GLP-1 mimetic Exendin-4 showed good protective effects in several animal models of PD. In the 6-hydroxydopamine (6-OHDA) lesion model in the rat, the drug protected dopaminergic neurons and improved motor activity [123, 124]. Exendin-4 had similar protective effects in the MPTP mouse model of PD [125]. We showed that liraglutide and lixisenatide are protective while exendin-4
showed only minor protection in comparison in the MPTP mouse model. Motor coordination was normalised in part, and neurons in the substantia nigra were protected by both drugs. Pro-apoptotic mitochondrial BAX/BAD levels were reduced, while insulin related second messenger signalling was normalised [126]. These encouraging preclinical results suggest that GLP-1 analogues are a viable strategy to treat PD [84, 127-129].

4.4 Clinical trials in AD and PD patients
Currently, several GLP-1 mimetics are on the market as treatments for T2DM that show good effects and are well tolerated. Based on the promising results from animal studies, clinical trials are on the way that investigate the neuroprotective effects of exendin-4 or liraglutide in PD or AD patients. First results from clinical trials are already available.

4.4.1 Parkinson’s disease
A first pilot trial of exendin-4 in PD patients had been conducted. This clinical trial tested exendin-4 in an open label trial in 45 non-diabetic patients. The average time since diagnosis was 10 years, which indicates that PD had progressed quite far already. The drug was administered twice daily for 12 months, and were re-tested 2 months after the trial had stopped. At the end of the trial, drug treated patients showed an improvement of 2.7 points on the MDS-UPDRS test battery of motor activity, while control patients declined 2.2 points. The neurologists that assessed the patients were blind to treatment. In addition, patients were assessed in the Mattis DRS-2 cognitive test, as late stage PD patients often develop cognitive impairments, too. There was a clear improvement in the drug group, while the control group deteriorated rapidly [130]. Patients were tested again 12 months after the trial was finished. The drug group did not deteriorate in motor skill tests and in the cognitive assessment, while the control group had deteriorated further as expected for PD patients at that stage of disease progression. This suggests that the drug effect was not short-lived, but improved functionality in particular on cognitive measures [131]. A follow-up phase II double-blind, placebo-controlled trial had been conducted to test if the outcome is reliable and reproducible and not dependent on the placebo effect. The patients were not as progressed in PD and the period since diagnosis was around 6 years. The cognitive performance of patients in the placebo group did not deteriorate during the duration of the trial, so no drug effect on cognition could be measured between groups as there was no impairment to protect from. In the MDS-UPDRS part 3 test battery, the drug group scored 4.3 points better after 48 weeks compared with the placebo group. 12 weeks after the trial had stopped, patients were re-tested, and, the difference between groups was still 3.5 points. The
outcome confirmed the first pilot study. It showed disease-modification by the drug treatment, as improvements were still visible even when the drug was no longer present in the body. Tests of CSF samples demonstrated that the drug is able to enter the brain, and that 12 weeks after the trial had finished, no drug remained in the system [91]. In order to investigate the underlying mechanism of action, exosomes were analysed from blood plasma. These exosomes originate from neurons as they contain neuron-specific cell markers. The content of the exosomes showed that drug treatment normalised insulin signalling in neurons, as predicted from preclinical studies. When analysing the levels of the insulin receptor activated second messenger cascade by measuring phosphorylated IRS-1, Akt and mTOR, it was shown the insulin-desensitisation was much reduced by the drug treatment [56]. This is a proof of concept that GLP-1 mimetics can normalise insulin signalling in the brain and modify disease progression.

Three other clinical trials are currently ongoing, testing the drugs lixisenatide (clinical trials identifier NCT03439943), liraglutide (NCT02953665), or semaglutide (NCT03659682) in PD patients, underscoring the rising importance of this drug discovery field.

4.4.2 Alzheimer's disease
A pilot study tested the effects of liraglutide in AD patients. This double-blind, randomized, placebo-controlled trial included memory tests and ^18^FDG-PET brain imaging, and PIB-PET imaging to estimate amyloid plaque load [132]. The low number of 38 Patients in this trial meant that the trial was underpowered for the memory tests. Additionally, drug treatment only lasted for 6 months, which proved too short for the placebo control group to deteriorate enough to show a drug effect on disease progression. However, the ^18^FDG-PET brain scans showed an effect. While the placebo control group showed a up to 20% of reduced ^18^FDG-PET activity, the drug group did not show any reduction. This demonstrates that glucose utilization and neuronal activity in the cortex did not deteriorate [133]. This result is exactly what one would predict based on the outcomes of animal studies. Another double-blind placebo controlled pilot study testing liraglutide in cognitively impaired patients showed a drug effect in fMRI brain scans after one year of treatment. Brain activity and the connection between different active brain areas was reduced in placebo treated subjects, but not in the drug treated patients, suggesting that the drug prevented disease progression [134]. Another pilot study tested cognitive performance in people with T2DM. One group received liraglutide while the other group received diet and exercise treatment to control for blood glucose levels. The drug group was better in short term memory (mean Digit Span Z score) and memory composite z-score compared to the non-drug intervention group [135].
A larger, placebo controlled double blind phase II clinical trial testing liraglutide in over 200 MCI/AD patients is currently ongoing. It analyses 18F-DG-PET activity, markers of microglia in PET scans, MRI brain scan changes, CSF samples to test levels of inflammation and AD biomarkers, and a range of cognitive tests [136]. The trial will report results in mid-2020 (NCT01843075).

4.5. Glucose-dependent insulino-tropic polypeptide (GIP)

GIP is the ‘sister’ incretin hormone of GLP-1 and their physiological roles are closely related [137, 138]. It is a 42 amino acid long peptide hormone that expressed in a range of cells, including neurons [139]. The GIP receptor is a seven membrane spanning G-protein coupled receptor of the glucagon-like family that enhances cAMP levels just like GLP-1 [140]. GIP receptor expression has been found in large neurons such as the pyramidal neurons in the cortex and hippocampus, granule neurons in the dentate gyrus, Purkinje cells in the cerebellum, and basal brain areas [141-143].

4.6 GIP analogues are protective in animal models of Alzheimer’s disease

We have tested protease-resistant long-acting GIP analogues in the APP/PS1 mouse model of AD. D-Ala2GIP protected learning and memory in 12 month-old APP/PS1 mice. Synapses loss was reduced, and synaptic plasticity in the hippocampus was protected in electrophysiology studies, while control tg mice showed extensive loss of synapses and impaired synaptic plasticity. The amyloid plaque load was also reduced by the GIP analogue. The activation of microglia and astrocytes in the chronic inflammation response in the brain was diminished by drug treatment, as was oxidative stress and DNA damage [144, 145]. In aged 19 month-old APP/PS1 mice, D-Ala2GIP was still able to reduce synaptic loss and inflammation in APP/PS1 mice and even in wild-type control animals. Furthermore, the drug was able to enhance synaptic plasticity in the hippocampus of aged APP/PS1 and wild-type mice, suggesting that loss of synapses can be reversed by enhancing synaptogenesis [146]. Oxidative stress and DNA damage was also reduced by the drug [144]. Direct infusion of native GIP was also effective and prevented memory impairments induced by icv. injection of amyloid [147]. These and other results suggest that GIP receptor activation has similar protective properties as GLP-1 receptor activation, and that improving GIP signalling in the brain may be protective in AD [148].

4.7 GIP analogues show neuroprotective effects in animal models of PD

Since GLP-1 and GIP analogues showed good effects in AD models, and GLP-1 receptor agonists showed protective effects in PD also, we tested long-acting GIP analogues in animal models of PD.
When testing GIP analogues in the MPTP mouse model of PD, it was found that D-Ala2-GIP-glu-PAL showed good neuroprotective effects. Motor coordination and grip strength was normalized by the drug, as was TH expression in dopaminergic neurons in the SN. Synapse numbers were protected from MPTP toxicity, too. MPTP treatment induced a chronic inflammation response and activated microglia and astrocytes and increased levels of pro-inflammatory cytokines in the brain. Drug treatment reduced the inflammation response, and normalized cAMP/PKA/CREB second messenger signalling in the SN, indicating that growth factor signalling had been restored [149]. After chronic MPTP treatment, a model that is considered to be more realistic, D-Ala2-GIP-glu-PAL was able to improve motor activity and protected dopaminergic neurons, too, and additionally reduced the increased alpha-synuclein levels in the brain. MPTP treatment leads to a much-increased expression of this protein. Moreover, Drug treatment reduced the chronic inflammation response in the brain, lowered oxidative stress and lipid peroxidation, and increased the levels of brain-derived neurotrophic factor (BDNF) [150]. BDNF can protect synapses in a range of neurodegenerative disorders [151-153]. Other research groups found very similar effects of D-Ala2-GIP in this mouse model of PD. Again, motor activity and dopaminergic neurons were protected from MPTP toxicity. The drug effect was blocked by the GIP receptor partial antagonist (Pro3)GIP. D-Ala2GIP additionally reduced the levels of oxidative stress, lowered malondialdehyde levels and increased glutathione levels in the brain. D-Ala2-GIP was able to normalize dopamine levels in the striatum [154]. Another animal model of PD is the 6-OHDAlesion rat model. Continuous infusion of GIP by an osmotic minipump reduced the 6-OHDA toxicity, and motor impairments were brought back to normal levels [155].

5. Novel dual GLP-1/GIP receptor agonists

As GIP and GLP-1 both have their protective effects and work together in cell signalling, novel GLP-1 and GIP receptor dual agonists are being developed as novel treatment for T2DM. Studies in diabetic animals have shown an added benefit if GIP analogues are added to GLP-1 analogues [156]. Several dual agonists have already been tested in clinical trials in patients with T2DM and show superior performance compared to liraglutide [157, 158]. We have tested 5 different GLP-1/GIP dual agonists that we have named DA1-DA5 [84]. DA1-JC (NNC0090-2746) is a dual agonist that has been lipidated with a C16 fatty acid to enhance the biological half-life in the blood [157, 158]. DA2 is the same dual agonists peptide that has been pegylated (40kDa) to increase the biological half-life in the blood [157]. DA3-CH is the dual agonist without any modifications [157, 159]. In addition, we have developed two novel dual receptor agonists that have been modified for enhanced BBB
We found that neuroprotective effects of these drugs correlated directly with their ability to cross the BBB. Using fluorescent labelled peptides, we were able to give an estimate of the ability of these drugs to enter the brain. DA5-CH was the most effective, followed by DA4-JC, DA3-CH and exendin-4 showed lower BBB penetration, lipidated peptides such as liraglutide and DA1-JC showed even lower penetration, and pegylated peptides did not cross the BBB in this assay [92, 160]. Using the \(^{125}\text{I}\)-labelled peptide technique confirmed these results and showed that DA1-JC entered the brain only in limited amounts, while the pegylated DA2 did not enter it at all. In comparison, DA3-CH showed enhanced BBB penetration, but DA4-JC crossed the BBB at the highest level (Salameh et al., manuscript submitted).

5.1 Novel dual GLP-1/GIP receptor agonists are protective in animal models of PD

When comparing the efficacy of GLP-1 analogues, GIP analogues, oxyntomodulin and DA1-JC, we observed that the GLP-1/GIP dual receptor agonist was most effective in protecting SH-SY5Y cells from rotenone stress, a pesticide that can induce PD in humans [161, 162]. This result confirms the finding the GIP can add to the protective effects of GLP-1 receptor activation. DA1-JC showed only limited protective effects in the MPTP mouse model of PD. MPTP-induced motor impairments were reduced, and synapse numbers were normalized. Neurons in the SN were protected from MPTP toxicity. Chronic inflammation in the brain was improved by the drug. BDNF levels in the brain were enhanced [163, 164]. Compared with single GIP or GLP-1 receptor agonists, DA1-JC did not show improved effects, though [126, 149]. We also tested DA1-JC in the 6-OHDA lesion model of PD. Motor activity was normalized to some extent. Dopaminergic neurons in the SN are killed by 6-OHDA, and DA1-JC was able to rescue some of them. Dopamine levels in the basal ganglia were found to be improved compared to the 6-OHDA lesion group, but did not reach the levels seen in saline control rats. The levels of glial-derived neurotrophic factor (GDNF), a neuroprotective growth factor [165], were enhanced by the drug. In addition, Akt and CREB second messenger cell signaling had been improved. Furthermore, autophagy was normalized by the drug [166]. DA3-CH was found to be more effective than liraglutide at equal doses in the MPTP mouse model of PD. DA3-CH was superior to liraglutide in motor tests and in protecting dopaminergic neurons. Inflammation in the brain was reduced by the drug, too [167]. DA1-JC, DA4-JC, DA5-CH and liraglutide had been tested at equal doses in a direct comparison in the MPTP model of PD. Importantly, the dual agonists DA4-JC and DA5-CH that are best at crossing the BBB showed the highest neuroprotection. In motor tasks and grip strength tests, DA5-CH was the most effective drug. Dopaminergic neurons in the SN
were also most protected by DA5-CH. Levels of pro-inflammatory cytokines were reduced most by DA5-CH, while levels of GDNF found in the brain were increased the most by DA4-JC treatment. Synapse protection was highest with DA4-JC and DA5-CH treatment, and both DA1-JC and liraglutide were not as effective [168]. In a separate study, DA5-CH was more effective than exendin-4 in a direct comparison in the MPTP mouse model of PD. When comparing DA5-CH with liraglutide, DA5-CH was more effective in reducing lipid peroxidation, inhibiting the apoptosis pathway (TUNEL assay) and in normalizing autophagy in the SN and striatum as well as in protecting mitochondrial activity by reducing the Bax/Bcl-2 ratio [92].

5.2 Dual GLP-1/GIP receptor agonists are protective in AD animal models

DA3-CH showed good protective effects in the APP/PS1 mouse model of AD. DA3-CH rescued learning and memory in the water maze. The amyloid plaque load in the cortex was lowered, too. Endoplasmic-reticulum stress and autophagy was improved by DA3-CH also [169]. In a direct comparison with liraglutide, DA4-JC was superior in protecting memory formation and synaptic plasticity (LTP) in the hippocampus of APP/PS1 mice. Additionally, DA4-JC was more effective in lowering the amyloid plaque levels and reduced microglia activation as well as lowered the levels of pro-inflammatory cytokines (Maskery et al., manuscript submitted). The streptozocin (STZ) icv treated rat model of insulin desensitization in the brain is considered to be a model of AD [48-50, 170]. Increasing GLP-1 levels has shown good effects in reversing insulin desensitization in this model [171]. DA4-JC treatment rescued memory formation and decreased the levels of phosphorylated tau in the brain. DA4-JC reduced the chronic inflammation response in the brain found in this model, too. Apoptosis and mitophagy was reduced, and insulin signaling in the brain was re-sensitized as shown by reduced levels of phospho-IRS1Ser1101 levels and elevated phospho-AktSer473 levels [172]. DA5 showed good neuroprotective effects in the STZ model, too. Tau phosphorylation was reduced, insulin signaling rescued and inflammation reduced. In studies of EEG recordings, STZ icv. injection reduced theta rhythm, and treatment with DA5-CH helped to normalize this impairment [160]. DA5-CH was effective in the APP/PS1 mouse model, too. Drug treatment improved working memory and spatial memory, and additionally lowered the amyloid plaque load and phosphorylated tau protein levels in the brain. DA5-CH was able to reverse the impairment of synaptic plasticity in the hippocampus. Additionally, DA5-CH normalized insulin signaling and improved PI3K and AKT activity [173].

- Table 1 somewhere here -
5.3 Triple GLP-1/Glucacon/GIP receptor agonists

Recently, a triple GLP-1/Glucacon/GIP receptor agonist has been developed as a potential treatment for T2DM [174]. It was hoped that the addition of glucagon would be add value to the physiological action of this drug. We have tested this triple agonist in two animal models of AD with good effects. In the APP/PS1 mouse model of AD, memory deficits in the spatial water maze were reduced. Moreover, the drug reduced levels of the mitochondrial pro-apoptotic signalling molecule BAX, increased the anti-apoptotic signalling molecule Bcl-2 and enhanced the levels of BDNF, a key growth factor that protects synaptic function. Furthermore, neurogenesis in the dentate gyrus was furthermore enhanced as shown in the increase of doublecortin positive cells, and drug treatment reduced the level of beta-amyloid, reduced neuroinflammation (activated microglia and astrocytes), and oxidative stress in the cortex and hippocampus [175]. In the triple tg mouse that expresses human APP, PSN1 and tau genes with mutations, the triple agonist reduced memory impairment, normalized long-term potentiation (LTP) in the CA1 region of hippocampus, reduced the amyloid-beta plaque load and the amount of phosphorylated tau aggregates, and normalized CREB and CAMKII signalling in the hippocampus [176]. In a patch clamp study of the same 3xtg mouse strain, the drug increased spontaneous excitatory synaptic activities, differentially modulated voltage- and chemically-gated Ca\(^{2+}\) flux, and reduced the over-excitation of pyramidal neurons in hippocampal slices [177]. While the effects on the AD pathology are impressive, they were not greater than those observed with the GLP-1/GIP dual agonists. It appears that adding the glucagon receptor agonist did not actually enhance the physiological protective effects in the brain. Previous studies in GLP-1R KO mice have shown that oxyntomodulin, a GLP-1/glucagon dual receptor agonist, shows no effects in the brain [178], indicating that the activation of the glucagon receptor in the brain did not contribute to the oxyntomodulin neurophysiological activity. We have tested oxyntomodulin in different animal models of disease previously, and also found that the neuroprotective effects did not differ from single GLP-1 receptor agonists [179-181].

6. Conclusion

The results presented here show that improving insulin signalling in the brains of AD or PD patients has clear disease-modifying effects. Not only are symptoms such as attention, memory impairments or other cognitive impairments much reduced in AD and PD patients, and motor coordination improved in PD patients, but the improvements long outlast drug treatment duration. \(^{18}\)FDG-PET and
fMRI brain scans demonstrated a lasting improvement in brain activity and energy utilization, and analysing the biochemical changes in the brain using the exosome technique proved that insulin signalling in the brain had indeed been re-sensitized. This is a proof of concept that the insulin hypothesis does affect disease progression and has the potential to be a disease-modifying treatment that can halt the progression. Larger clinical trials and tests of newer and more potent drugs are required to develop viable treatments for AD and PD.

7. Expert opinion

7.1 The death of the amyloid hypothesis

In AD, the main theory has been that the aggregation of beta-amyloid in the brain followed by aggregation of phosphorylated tau is the main driver of the disease [182]. Recently, that concept had been extended to soluble ‘amyloid oligomers’ that supposedly cause toxic effects in the brain [183, 184]. Drugs designed to lower the levels of amyloid in the brain had been developed, and numerous clinical trials tested either antibodies directed against amyloid or inhibitors of the proteases that cleave the amyloid precursor protein, gamma and beta secretases, showed very limited effects or even made the patients worse. The first clinical trial took place in 2000, testing active immunization in AD patients by the company ELAN. The trial had to be stopped due to auto-immune responses that made patients worse. Amyloid was reduced in the brain, but the disease progression continued in all patients [185, 186]. Elan and Wyeth tested the antibody called Bapinezumab in several phase 3 trials. In 2012, the companies stopped trials as the AD patients did not improve. Interestingly, the antibody did lower amyloid levels in the brain as expected [224]. Merck tested the antibody Verubecestat in phase 3 trials without success [225]. In late 2016, Eli Lilly tested Solanezumab without success [226]. In 2018, Eli Lilly and AstraZeneca stopped phase 3 trials with their antibody lanabecestat as there was no likelihood of success [227]. In January 2019, Roche terminated their phase 3 trials testing the antibody crenezumab after a futility study determined the drug ineffective [228]. In March 2019, BIOGEN and Esai stopped their drug discovery program after their antibody aducanumab failed in two phase III clinical trials after a futility analysis. However, in October 2019, BIOGEN announced that when re-analysing their clinical trial data that tested their antibody aducanumab, and including patient data that had not been included in the futility analysis that stopped the trial, they found a small improvement in cognitive tests. After 6 and 12 months of
treatment, patients in the high dose group supposedly improved, but only three of 16 scales at 12 months were significant at p<0.05, unadjusted for multiple comparisons. More worrying, brain oedema (ARIA-E), were observed in about 40% of APOE ε4 allele carriers, and nearly half of these patients discontinued treatment. Therefore, a significant number of APOE ε4 carriers discontinued treatment in the drug group, while none discontinued in the placebo group. It had been noted that the placebo group showed a faster deterioration as one would expect, which may be due to this fact. In addition, the high incidence of ARIAs lead to an unblinding of a large subset of the drug treated patient cohort, in particular in APOE ε4 carriers. This suggests that the placebo effect may well play a role in this trial. Finally, even if one accepted the outcome as valid, the improvement in the CDR-SB tests was only 0.39 points on an 18 point scale, making it questionable whether or not this difference actually is of any clinical importance. Therefore, it appears that the BIOGEN claim is overstated, and that new clinical trials with balanced patient cohorts would need to be conducted in order to substantiate their claim that the drug had worked. For a detailed critique, please read [187].

In any case, even if such a very small effect turns out to be real, it is not sufficient to rescue the amyloid theory, which claims that amyloid is the main driver of the disease, and a removal will stop disease progression completely.

Recently, a failure of testing two antibodies in AD patients with gene mutations has been reported in the DIAN-TU study. In that study, Roche’s gantenerumab and Lilly’s solanezumab were tested. Neither antibody showed an effect in primary cognitive readouts [229]. This is particularly worrying as these people have the inherited form of AD, which previously was thought to be triggered by the production and accumulation of mutated amyloid. The fact that even in the familial forms of AD there is no benefit from lowering the amount of amyloid in the brain tells us that the role of amyloid in this disease has been misjudged all along.

Inhibition of gamma-secretase was not a successful strategy either. Semagacestat (LY-450139) was tested in a phase III trial. 1500 patients were treated for 21 months. In 2010, the trial was halted as it showed a worsening of dementia in patients compared to placebo controls [230]. Inhibitor of beta-secretase (BACE1) did not fare any better. In June 2018, Lilly and AstraZeneca stopped a Phase 2/3 trial of 2,219 people with AD testing the BACE1 inhibitor Lanabecestat. In July 2018, Janssen stopped a Phase 2b/3 trial and a Phase 2 trial of its inhibitor Atabecestat due to liver toxicity. Nov 2018: Merck announced that in a Phase 3 trial, people with AD who took the inhibitor verubecestat scored worse on cognitive tests than those on placebo. Novartis/Amgen’s BACE inhibitor CNP520 (Umibecestat) was tested in two Phase 2/3 trials. In July 2019 the company announced that the trials have been stopped due to patients getting worse. Participants taking umibecestat declined in the
RBANS cognitive composite tests, had more brain atrophy, and lost more weight than people on placebo did. In September 2019, BIOGEN and Eisai halted two phase 3 trials on the basis of safety concerns caused by their drug Elenbecestat. For details on these trials please see [231].

It should be clear from this extended list of failures that the original concept of the amyloid hypothesis is not valid. While several of the drugs tested did indeed lower amyloid levels in the brain in a dose-dependent manner, no such improvement was found in cognitive tests. The only rationale conclusion from this is that amyloid is not the main driver in AD development. Clearly, focussing on a single protein that ‘misfolds’ and becomes toxic when aggregating was not a successful strategy for drug development. Unfortunately, the main concepts of this ‘proteinopathy’ have been copied by scientists that are investigating Parkinson’s disease (PD) and try to develop novel treatments. Here, the protein that supposedly ‘misfolds’ and forms toxic aggregates or soluble oligomers is called alpha-synuclein [188]. Researchers follow the same ideas and use the same techniques to test the effects of oligomers on cell culture and animal models, ignoring the fact that those techniques produced a long series of false positives in AD research. Clinical trials testing antibodies directed against these alpha-synuclein oligomers are ongoing and will most likely end the same way that the amyloid antibody tests did. The persistent failure to develop effective treatments for these diseases suggests that the concepts that underly drug discovery are not correct. It is therefore important to rethink the basic concepts of disease development and progression, and to look for new ideas that show promise to be successful in the clinic. It is time to go back to take a long hard look at the pathology and physiology that underlies progressive neurodegenerative disorders.

7.2 Returning to Neurophysiology

The amyloid hypothesis focused mostly on protein misfolding and aggregation without analysing the actual neuronal physiological and pathological developments in the brain. However, AD and PD are complex syndromes that go through different stages over time and that involve a range of processes, cell types and tissues. For example, it is well established that the chronic inflammation response plays a key role in AD and PD. Pro-inflammatory cytokines that are release by glia have degenerative effects on neuronal metabolism by impairing energy utilization, increasing oxidative stress and affecting synaptic activity [51, 189, 190]. Any treatment strategy that will have any chance of success has to address the chronic inflammation response in the brain. In addition, key growth factors in the brain such as NGF, GDNF, BDNF, IGF-1, insulin and others lose their effectiveness [49, 191-193]. The consequences of growth factor loss have been known for a long time, and a range
of growth factors have shown clear neuroprotective effects in various animal models of disease. Importantly, the improvements of growth factor treatments are long-lasting and halt disease progression, indicating that they act on the underlying processes that drive these diseases and enable neurons to repair and regain their functions [43, 151, 153, 194, 195]. So far, the development of successful treatments have been hampered by the fact that the growth factors chosen did not cross the BBB [196]. Growth factors such as insulin, GLP-1 and GIP can cross the BBB and offer a promising treatment strategy [87, 91, 92, 197]. The advantage of such an approach is that it can improve a range of pathological processes observed in AD and PD, not just one parameter. They reduce the chronic inflammation response in the brain via receptors expressed in glia [198-200], thereby reducing levels of pro-inflammatory cytokines [119, 120]. The drugs furthermore can restore the expression of key growth factors such as NGF, BDNF and GDNF [42, 163, 201, 202], which can explain the protective effects on synapses, dopaminergic neurons, and pyramidal neurons. Energy utilization is restored in the brain [64, 133, 203]. Synaptic activity and plasticity is brought back [101, 145, 173, 204], memory formation and brain functionality is improved [101, 134, 145, 205], mitochondrial function and mitogenesis is normalized [161, 175, 206, 207], autophagy is brought back [161, 169], and apoptosis is reduced [149, 206, 208, 209].

This demonstrates that a range of key physiological processes that are affected by disease are improved by the normalization of growth-factor signalling and the reduction of chronic inflammation.

7.3 The future is bright
The treatment strategy of normalizing growth factor signalling in the brain has the potential to stop disease progression and to address the underlying disease mechanisms rather than just focusing on a single disease parameter or on symptom management. The first encouraging results from clinical trials in AD and PD patients demonstrate that the preclinical results translate to the clinic and that this approach has the potential to reduce or stop neurodegenerative disorders such as AD and PD. Single GLP-1 receptor agonists or dual GLP-1/GIP receptor agonists have the potential to reverse a range of pathological parameters such as loss of energy utilization, mitochondrial dysfunction, reduced growth factor signalling, loss of synaptic activity, enhanced apoptosis, reduced autophagy, and chronic inflammation in the brain.

Conflict of interest declaration
The author is a named inventor on patents and patent application that cover the use of GLP-1, GIP and dual GLP-1/GIP receptor agonists as treatments for neurodegenerative disorders. The patents are owned by Ulster University and Lancaster University, UK. He is the CSO of the company Kariya Pharmaceuticals.

References:


**Pioneering study that analyses exosomes from patients' blood to show insulin re-sensitization in the brain**


** a proof of concept clinical trial that shows that improving insulin signalling in the brain reduces AD disease progression


*First study to show that activating GLP-1 receptors is neuroprotective*
*First study to show that liraglutide has neuroprotective properties in AD*
**Proof of concept clinical trial that demonstrates that GLP-1 receptor activation is disease modifying in PD**


* first clinical trial to show that liraglutide can normalize energy utilization in the brain in AD


* first study to show that GIP receptor activation is neuroprotective in a PD animal model


* first study to show that dual agonists that can cross the BBB are superior to single agonists


177. Li, T., et al., A GLP-1/GIP/Gcg receptor triagonist alleviates memory impairments in 3xTg-AD mice via improving synaptic transmission, neuronal excitability and Ca2+ homeostasis. Neuropharmacol., 2020. in press.


224. https://www.alzforum.org/therapeutics/bapineuzumab

227. https://www.alzforum.org/therapeutics/azd3293
Insulin signalling plays important roles in neuronal growth, synaptic development, energy utilization, mitogenesis, inhibition of apoptosis and more. Insulin binds to the $\alpha$-subunit of the receptor. This activates the tyrosine kinase phosphorylation of the $\beta$-subunit. Subsequently, several second messenger pathways can be activated:

1. Activation of the insulin receptor-Shc-MAP kinase pathway activates gene expression for proteins that are required for cell growth, synapse growth, and for cell repair and maintenance [210, 211].

2. Insulin receptor activation has a direct effect on neurotransmission, and primes synapses for induction of long-term potentiation of neuronal transmission (LTP) [212-214]. Modulation of neurotransmission will influence memory formation, information processing, and cognitive processes [40].

3. Insulin receptors furthermore modulate neurotransmission directly by altering glutamatergic and GABAergic receptor activity. NMDA glutamate receptors can be phosphorylated to increase the opening of the associated Ca$^{2+}$ channel [215]. IR activation also affects GABA transmission by recruiting functional GABA receptors to the postsynaptic site [216].

4. As a growth factor, insulin also increases energy utilization and suppresses the induction of apoptosis. This pathway involves stimulation of PI3K binding to IRS-1 and -2, activation of PI3K, PDK, and protein kinase B (Akt/PKB), which suppresses the induction of apoptosis and thereby protects neurons [213, 217-219]. Modified from [220].

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Akt/PKB = protein kinase B complex; CPD3B = cyclic phosphodiesterase 3 beta; Grb2/SOS = Growth factor receptor binding protein 2 / son of sevenless protein; IRS = insulin receptor substrates that get phosphorylated after activation, MAPK = mitogen activated protein kinase; mTOR = mammalian target of rapamycin; NRF1 = nuclear respiratory factor-1; PGC-1$\alpha$ = peroxisome proliferator-activated receptor $\gamma$ coactivator 1-$\alpha$; PDK = phosphatidylinositol-dependent kinase; PI3K = phosphatidylinositol 3 kinase; PPAR = peroxisome proliferator-activated receptor family; Raf = regulation of alpha-fetoprotein; Ras = rat sarcoma virus peptide; Shc = Src homology collagen peptide
Fig. 2: GLP-1 and GIP-induced signalling and the second messenger cell signalling pathways that are activated by the GLP-1 and GIP receptors. Second messenger signalling controls energy utilization, mitogenesis, gene expression, autophagy, inhibition of apoptosis, modulation of ion channels, cell growth and repair, and the cellular response to oxidative stress, similar to insulin signalling [76, 77, 116]. GLP-1 and GIP receptors can form dimers which show enhanced cAMP production and can activate other G-protein linked enzymes such as PLC [77, 137, 221, 222].

Abbreviations: GLP-1R= GLP-1 receptor; PKA= protein kinase A; PLC= phospholipase C; PI3K= phosphoinositide 3 kinase; PKB= protein kinase B; AC= adenylate cyclase; EPAC= exchange proteins directly activated by cAMP; MAPK= mitogen-activated protein kinase; mTOR = mammalian target of rapamycin; ERK= extracellular signal-regulated kinase; CREB= cyclic AMP response element binding protein; P90RSK= ribosomal S6 kinase; PPAR= peroxisome proliferator-activated receptor family; K+ = potassium ions; MEK1/2= MAPK or Erk kinases; PGC-1α = peroxisome proliferator-activated receptor γ coactivator 1-α; c-Raf= cellular Raf gene (Rapidly accelerated fibrosarcoma); McI1= myeloid cell leukemia protein-1; Casp-9= caspase 9; Casp-3= caspase 3; Bax, Bik= Bcl2-interacting killer; Ca2+= calcium ions.
<table>
<thead>
<tr>
<th>Animal model</th>
<th>drug tested</th>
<th>result</th>
<th>publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPTP mouse model of PD</td>
<td>DA1-JC</td>
<td>improvement in motor tasks, protection of dopaminergic neurons, enhanced release of BDNF, Pi3k activity was enhanced, pro-apoptotic signalling reduced</td>
<td>[163]</td>
</tr>
<tr>
<td>MPTP mouse model of PD</td>
<td>DA1-JC</td>
<td>improvement in motor tasks, protection of dopaminergic neurons, reduced inflammation in the brain, enhanced synaptic protein levels</td>
<td>[164]</td>
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<tr>
<td>SH-SY5Y cell culture treated with rotenone</td>
<td>DA1-JC</td>
<td>DA1-JC is the most effective drug compared to single GLP-1 or GIP analogues or to oxyntomodulin. Protection of cell viability, reduction of apoptotic signalling, improvement of autophagy and Pi3k signalling</td>
<td>[161]</td>
</tr>
<tr>
<td>6-OHDA rat model of PD</td>
<td>DA1-JC</td>
<td>motor activity was improved, dopaminergic neuronal loss was reduced, levels of GDNF were increased and pAkt/CREB signalling normalized</td>
<td>[166]</td>
</tr>
<tr>
<td>icv. STZ rat model of AD</td>
<td>DA4-JC</td>
<td>memory formation was improved, tau phosphorylation reduced, insulin signalling improved, reduced apoptosis and inflammation in the brain</td>
<td>[206]</td>
</tr>
<tr>
<td>MPTP mouse model of PD</td>
<td>DA3-JC</td>
<td>DA3-CH is more effective than liraglutide in improving motor acidity, protecting dopaminergic neurons, reducing inflammation, levels of GDNF are increased</td>
<td>[159]</td>
</tr>
<tr>
<td>MPTP mouse model of PD</td>
<td>DA1-JC, DA4-JC, DA5-CH</td>
<td>DA4-JC and DA5-CH are more effective in protecting the brain than liraglutide and DA1-JC. Motor activity was improved, dopaminergic neurons protected, levels of pro-inflammatory cytokines reduced, GDNF levels were increased</td>
<td>[168]</td>
</tr>
<tr>
<td>APP/PS1 mouse model of AD</td>
<td>DA5-CH</td>
<td>memory formation was protected, synaptic plasticity (LTP) preserved and tau phosphorylation reduced, PI3k and Akt activity normalized</td>
<td>[173]</td>
</tr>
<tr>
<td>APP/PS1 mouse model of AD</td>
<td>DA3-CH</td>
<td>DA3-CH improved memory formation, normalized autophagy, reduced ER stress and apoptotic signalling, reduced amyloid plaque load in the brain</td>
<td>[169]</td>
</tr>
<tr>
<td>icv. amyloid(31-35) AD model</td>
<td>DA1-JC</td>
<td>memory formation was improved and disturbance of circadian rhythm improved</td>
<td>[223]</td>
</tr>
<tr>
<td>Model</td>
<td>Type</td>
<td>Outcome</td>
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<tr>
<td>APP/PS1 AD mouse model</td>
<td>DA1-JC</td>
<td>DA1-JC was more effective than liraglutide in a slow release formulation in improving memory formation, reducing inflammation and reducing oxidative stress</td>
<td>[104]</td>
</tr>
<tr>
<td>icv. STZ rat model of AD</td>
<td>DA5-CH</td>
<td>memory formation is rescued, EEG theta rhythm normalized, tau phosphorylation is reduced, apoptosis signalling is reduced, CREB signalling is normalized, DA5-CH is superior to DA1-JC, liraglutide or exendin-4 in crossing the blood-brain barrier</td>
<td>[160]</td>
</tr>
<tr>
<td>MPTP mouse model of PD</td>
<td>DA5-CH</td>
<td>receptor binding study of DA5-CH showing selective binding to GLP-1 and GIP receptors. DA5-CH is superior to DA1-JC, DA2, DA3-CH, liraglutide or exendin-4 in crossing the blood-brain barrier. DA5-CH is superior to exendin-4 in a dose-response study. Motor activity is protected, inflammation is reduced, lipid oxidation is reduced, apoptosis is reduced, DA5-CH is superior to liraglutide</td>
<td>[92]</td>
</tr>
<tr>
<td>APP/PS1 AD mouse model</td>
<td>DA4-JC</td>
<td>DA4-JC receptor binding study shows selective binding. DA4-JC is superior to liraglutide in a dose-response study in reducing amyloid plaques. DA4-JC was more effective than liraglutide in reversing memory loss, enhancing synaptic plasticity (LTP) in the hippocampus, reducing amyloid plaques and lowering pro-inflammatory cytokine levels in the brain.</td>
<td>submitted</td>
</tr>
<tr>
<td>6-OHDA rat model of PD</td>
<td>DA5-CH</td>
<td>DA5-CH is more effective than exendin-4 in protecting motor activity, reducing α-synuclein levels and pro-inflammatory cytokine levels in the brain. It was also more effective than exendin-4 in reducing apoptotic signaling. Insulin desensitization was reversed and the levels of autophagy markers were normalized.</td>
<td>submitted</td>
</tr>
</tbody>
</table>

Table 1. Overview of studies testing GLP-1/GIP dual agonists in animal models of AD or PD.