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The incretin hormones glucagonlike peptide 1 and glucose-dependent insulinotropic polypeptide are neuroprotective in mouse models of Alzheimer's disease

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Abstract The incretin hormones glucagonlike peptide 1 and glucose-dependent insulinotropic polypeptide (GIP) have been developed to treat type 2 diabetes and also act as growth factors. We have tested several long-acting incretin mimetics in the amyloid precursor protein $(APP)_{Swe}$/presenilin 1 $(PS1)_{\Delta E9}$ model of Alzheimer's disease (AD). We found that liraglutide, lixisenatide, and D-Ala2-GIP cross the blood–brain barrier and prevent the impairment in memory formation and synaptic plasticity, increase synapse numbers, reduce amyloid plaque load and soluble amyloid-β levels, reduce oxidative stress and the chronic inflammation response in the brain, enhance the proliferation of neuronal progenitor cells, and increase neurogenesis in the dentate gyrus. In an ^{18}fluorodeoxyglucoe positron emission tomographic/computed tomographic imaging study in PLB1-triple mice, a mouse model that expresses human mutated APP, PS1, and tau proteins, glucose metabolism was found to be normalized in forebrain areas after liraglutide treatment, demonstrating that neuronal metabolic activity was normalized. A clinical trial testing liraglutide in patients with AD is currently ongoing.

Keywords: Alzheimer's disease; Parkinson's disease; Growth factors; GLP-1; Liraglutide; Lixisenatide

1. Introduction

Alzheimer's disease (AD) is a sporadic disease with very few risk gene links associated with it. The genetically induced inherited forms of AD are very rare—about 1% of all patients. Therefore, it is very difficult to assess what the initial causes are that trigger the onset of this disease. Because AD can be diagnosed only when it has already developed, very little is known what those initial processes are that initiate the sequence of events that eventually lead to neurodegeneration. Therefore, a useful approach to investigate potential contributing factors is to determine the correlation with other factors that increase the risk of developing AD. Several such risk factors have been identified, and type 2 diabetes is one of them. In type 2 diabetes mellitus (T2DM), insulin signaling is impaired, often caused by a desensitization of the insulin response. The previous reviews in this special issue by Steculorum and colleagues [1] and Talbot [2] have outlined the importance of insulin signaling in the brain. Insulin is a growth factor, and a reduction of growth factor signaling will have repercussions on gene expression, neuronal repair, synaptic activity, and cell metabolism [3]. Insulin is crucial for cell growth and survival. Neurons also carry insulin receptors, and activating them induces dendritic sprouting, neuronal stem cell activation, and general cell growth, repair, and neuroprotection [4–8]. In diabetes, research into other signaling pathways that support insulin signaling is ongoing. The use of incretins, a class of peptide hormones that helps to normalize insulin signaling, has proved to be very successful [9,10].

2. The incretins hormones: glucagonlike peptide 1 and glucose-dependent insulinotropic polypeptide

Because insulin signaling is already desensitized in T2DM, and injection of insulin loses its effectiveness over time, researchers of diabetes are investigating different

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strategies of how to improve blood glucose levels. Different signaling pathways exist that also modulate blood glucose levels and activate a similar, second messenger signaling cascade—in particular, glucagonlike polypeptide 1 (GLP-1), but others are under investigation as well, such as GLP-2, oxyntomodulin or glucose-dependent insulinotropic polypeptide (GIP) [11].

GLP-1 is an endogenous 30-amino acid peptide hormone. It has several physiological roles in different tissues to control cell metabolism [10]. The GLP-1 receptor (GLP-1R) belongs to the class B family of G-protein coupled receptors. The receptors for glucagon, GLP-2 and GIP, also belong to this group. Activation of the receptor activates an adenylate cyclase, increases inositol triphosphate (IP3) levels and gene expression, and has a range of other downstream effects [10,12]. GLP-1R stimulation enhances β-cell proliferation in the pancreas by activating stem cell proliferation, facilitates glucose-dependent insulin secretion, and lowers blood glucose in patients with T2DM [13,14].

GIP is a 42-amino acid incretin hormone that activates pancreatic islets to enhance insulin secretion and to help reduce hyperglycemia, similar to GLP-1 [15]. GIP also has been shown to promote pancreatic β-cell growth, differentiation, proliferation, and cell survival, documenting its growth hormone properties [15]. Therefore, research is ongoing to develop GIP as a therapeutic tool for T2DM treatment [16].

3. Incretins play important roles in the brain

GLP-1Rs are found on neurons in the brain of rodents and humans [17,18]. They are expressed predominately on large neurons, on the cell bodies, and also on dendrites, indicating they are located on the synapse [19]. As described by Greig [20], GLP-1R activation with the long-lasting GLP-1R agonist exendin 4 increases cell growth, proliferation, and repair; and inhibits apoptosis. In neurons, exendin 4 induced neurite outgrowth to protect against excitotoxic cell death and oxidative injury in cultured neuronal cells [17,21]. In addition to this, mice that overexpress GLP-1Rs in the hippocampus showed increased neurite outgrowth and improved spatial learning. Enhanced progenitor cell proliferation in the brain was also found in that study [22]. In contrast, the elimination of the GLP-1R in a knockout (KO) mouse model impaired learning abilities severely and also reduced synaptic plasticity greatly [23].

Apart from exendin 4, several other protease-resistant long-acting GLP-1 analogs have been developed to treat diabetes. The novel GLP-1 mimetics liraglutide and lixisenatide also cross the blood–brain barrier (BBB) when injected peripherally, and they increase the division of neuronal progenitor cells in the brain and enhance neurogenesis [24,25].

Interestingly, amyloid-β (Aβ) fragments can affect synaptic transmission directly and impair the use-dependent upregulation of synaptic transmission (long-term potentiation [LTP]). Because such a mechanism could be a used for storing information in the brain [27], this amyloid-induced block of LTP may be responsible in part for impaired memory formation in patients with AD [28,29]. As described in the review by de Felice [30], soluble Aβ fragments bind directly to insulin receptors on neuronal dendrites and impair synaptic activity [31,32]. This may be a mechanism of how insulin signaling in the brain becomes impaired in people with AD.

Additional studies showed that direct injection of GLP-1 or long-lasting GLP-1 analogs into the brain enhanced markedly LTP in the hippocampus, a brain area that is involved in memory formation. Agonists such as Val^8-GLP-1 showed a clear upregulation of LTP, whereas the selective GLP-1 antagonist exendin (9-36) blocked LTP [33]. The novel GLP-1 analog liraglutide that has been released onto the market as a treatment for T2DM also upregulated LTP [34]. More important, GLP-1 analogs were able to prevent the impairment of LTP induced by Aβ fragments in vivo [33–35].

GIP receptors are also expressed in the brain and are found on larger neurons such as the pyramidal cortical neurons [36], which is very similar to the pattern of expression of GLP-Rs [19]. The peptide GIP is also expressed in neurons and serves as a neuronal transmitter [37]. Stable analogs such as D-ala^2-GIP or N-glyc-GIP facilitate synaptic plasticity in the hippocampus, whereas the antagonist Pro^3-GIP impairs LTP. More impressive, GIP analogs can prevent the LTP impairment that Aβ fragments induce on synaptic transmission in the brain [38]. In a GIP receptor KO mouse strain, LTP was also much reduced, and paired-pulse facilitation showed an effect on presynaptic activity, indicating that the release of synaptic vesicles is reduced [39].

Protease-resistant GIP analogs such as D-Ala^2-GIP, with a longer half-life in the blood, cross the BBB and also enhance neuronal stem cell proliferation in the brain [25,39]. Furthermore, GIP analogs have clear effects on memory formation, with the GIP receptor agonist D-Ala^2-GIP facilitating memory, and the GIP receptor antagonist Pro^3-GIP impairing memory [39]. GIP analogs also have effects on synaptic plasticity in the brain. They enhance LTP in the hippocampus, and GIP analogs protect synapses from the detrimental effects of Aβ (25–35) [38]. In another study, intracerebroventrical infusion of Abeta1-40 in mice produced impairments in a water maze test, and the infusion of GIP intracerebroventricularly (icv) prevented amyloid-induced impairment in spatial learning [40].

4. Novel incretin analogs have neuroprotective effects in mouse models of AD

4.1. GLP-1

As an important preclinical test, novel analogs of GLP-1 have displayed neuroprotective properties in mouse models of AD. In one study, the GLP-1 analog Val^8-GLP-1 had

neuroprotective effects in a transgenic (tg) mouse model that overexpresses the human Swedish mutated form of amyloid precursor protein (APP) and a human mutated form of presenilin 1 (PS1). The mice develop large numbers of Aβ plaques in the cortex and hippocampus starting at 3 months of age [41]. When injecting Val^8-GLP-1 chronically intraperitoneally (ip) at a dose of 25 nmol/kg ip Once daily for 3 weeks, synaptic plasticity in the hippocampus was protected from the effects of plaque formation and did not differ from littermate wild-type control mice. LTP was protected completely, even at 18 months of age. In addition, the number of Congo Red-positive dense-core amyloid plaques in the brain was reduced. LTP was also improved in 18-month-old wild-type mice compared with controls, indicating that GLP-1 analogs also protect the brain, to some degree, from age-related synaptic degenerative processes [38, 42].

In another study, the novel GLP-1 analog liraglutide, which is also on the market as a T2DM treatment, enhanced memory formation and synaptic plasticity in the brain of 9-month-old APP_{Swe}/PS1ΔE9 mice after intraperitoneal injection (25 nmol/kg bw, once daily) for 8 weeks at a dose that is comparable with the dose given to patients with T2DM (0.9–1.8 mg subcutaneously once daily). The learning impairments observed in untreated AD mice were prevented by liraglutide, and the impairment of hippocampal synaptic plasticity that develops over time in the tg mice was also prevented. More important, the amyloid plaque load was reduced to 50%, and the formation of Congo Red-positive dense-core amyloid plaques was reduced to 30%. In addition, the inflammation response (activated microglia) was also reduced by 50%. Furthermore, increased neurogenesis was observed in the dentate gyrus of these mice, normalizing the number of young neurons when compared with wild-type controls (Fig. 1). The levels of APP and of soluble amyloid oligomers was also greatly reduced [24]. This study demonstrates clearly that GLP-1R activation by the long-acting analog liraglutide has effects on a range of parameters that are involved in AD, including a reduction of amyloid levels and plaque load; a reduction in the inflammation response, but more crucially in the loss of synapses; a reduction in the loss of synaptic transmission and plasticity; and a reduction in the impairment of forming new memories. The mice were at an age when AD symptoms were starting to be visible, comparable with patients who have been diagnosed recently with AD. This suggests that the drug may be helpful in preventing further development of the disease.

In a follow-up study, we investigated whether liraglutide would have restorative effects in late-stage progression of AD symptoms in these mice. Accordingly, 16-month-old APP/PS1 mice were tested after having been injected with liraglutide (25 nmol/kg bw) ip for 2 months. Spatial memory and synaptic plasticity were improved by drug treatment, and synapse numbers were increased. The amyloid plaque load was reduced by 33%, the inflammation response was reduced by 30%, and neuronal progenitor cell count in the dentate gyrus was increased by 50%. Total brain APP and soluble Aβ oligomer levels were reduced in liraglutide-treated APP/PS1 mice [43]. These results demonstrate that liraglutide not only has preventive properties, but can reverse some of the key pathological hallmarks of AD. It is interesting to note that synapse number increased even at such a late stage, suggesting that synaptogenesis had occurred, which is part of the GLP-1 growth factor range of physiological effects. This synaptogenesis could also be a result of an increased release of brain-derived neurotrophic factor (BDNF), as others have shown that GLP-1R activation facilitates BDNF in the brain [44].

One of the physiological effects of reduced insulin signaling is the reduced uptake of glucose, and impaired energy use and metabolism in neurons. As described by Edison and Brooks [45] in this special issue, energy metabolism in the cortex of patients with AD is indeed reduced in correlation with the advance of the disease, as shown in a diminished positron emission tomographic (PET) signal of the labeled fluorodeoxyglucose (FDG) tracer ^{18}F-FDG PET. We therefore studied the effect of liraglutide in a PET imaging study in 12-month-old PLB1-triple mice, a mouse model that expresses human mutated APP, PS1, and tau proteins [46], glucose metabolism (measured via ^{18}F-FDG PET/computed tomographic imaging) was measured in forebrain areas after liraglutide treatment.

Based on the very encouraging results in these preclinical studies, a phase 2 clinical trial with liraglutide in 206 patients with mild cognitive impairment has started. The trial has a randomized and placebo-controlled double-blind design and will analyze FDG-PET signal changes in neuronal metabolism and cortical activation, among other biomarkers [47]. See also the review by Edison and Brooks [45] in this special issue for more details.

4.2. GIP

The long-lasting GIP analog D-Ala^2-GIP also had neuroprotective effects in the APP_{Swe}/PS1ΔE9 mouse model of AD used to test liraglutide. In 12-month-old mice, synaptic plasticity in area CA1 of the hippocampus, and spatial memory formation, was protected by D-Ala^2-GIP in APP/PS1 mice. The reduction in synaptic numbers observed in saline-treated tg mice was prevented in the drug group. In addition, the amyloid plaque load was much reduced, as was the inflammation response as measured by microglia activation. The number of neuronal progenitor cells in the dentate gyrus was also increased by D-Ala^2-GIP [48]. The drug also had protective effects on synaptic transmission in 19-month-old tg mice, increased synaptic numbers, and reduced plaque load and inflammation response, but failed to rescue memory

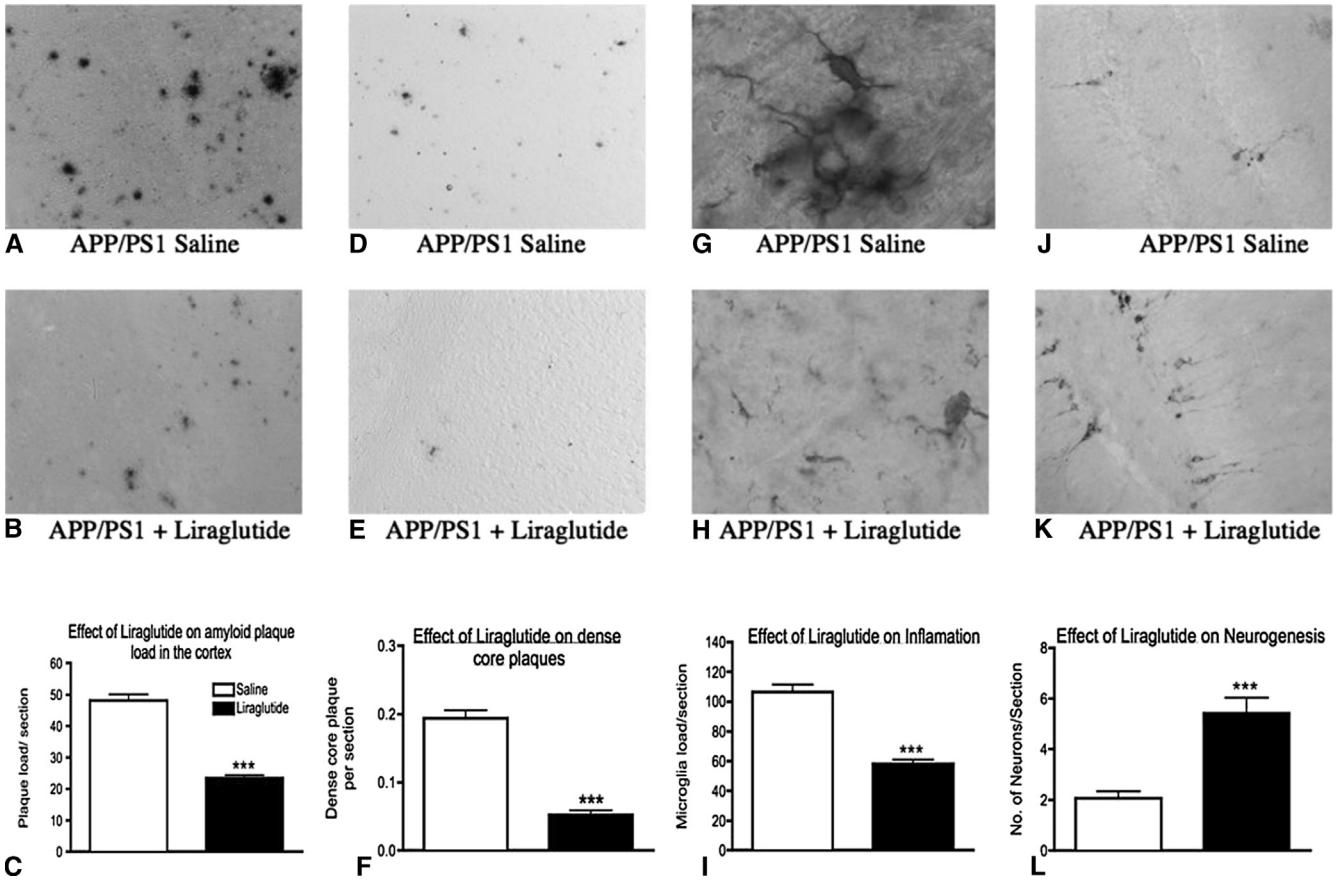

Fig. 1. (A–C) Histologic hallmarks of Alzheimer's disease are improved with liraglutide. Histologic analysis of liraglutide-injected amyloid precursor protein (APP)/presenilin 1 (PS1) mice showed a reduction in the number of amyloid plaques in the cortex and hippocampus of liraglutide-treated APP/PS1 mice was halved. (D–F) The number of Congo Red-positive dense core plaques was reduced to 25%. (G–I) The inflammatory response, as shown by activated glia (IBA-1 antibody stain to identify activated microglia), was also halved. (J–L) Mice treated with liraglutide also had a significant increase in neurogenesis (doublecortin-positive cells to identify young neurons) compared with saline treated animals. Sample micrographs show saline-treated (top), liraglutide (below), and overall quantification (bottom). *P < .001 [24]. The novel glucagonlike peptide 1 receptor agonist lixisenatide had very similar effects in this mouse model (unpublished data).

formation [49]. Overall oxidative stress and astrogliosis were also reduced by D-Ala^2-GIP in these mice [50]. In a separate study, the injection of the GIP peptide ip had protective effects on spatial learning in memory tasks, and also reduced plaque formation and amyloid load in an AD mouse model [40].

This suggests that GIP analogs have very similar neuroprotective properties in AD as GLP-1 analogs have, and protect synapses from the detrimental effects of Aβ, even at the advanced stage of amyloid accumulation in the brain. The receptor distribution in the brain and also the effects of analogs on LTP are very similar when comparing GLP-1 analogs with GIP analogs. This would suggest that the physiological roles of these incretins may also be very similar. However, the clear results in impairing LTP (and learning) in the GLP-1R KO [23] or GIP receptor KO mice [39] show that one incretin cannot compensate the block or receptor loss of the other. This suggests that both incretins play distinctive roles in the brain that cannot be compensated for, but that both incretins also activate similar growth factor-like effects that have neuroprotective effects in the brain of AD tg mice.

5. Growth factors show neuroprotective effects

As described in previous reviews [3,51] and in this special issue, insulin is a growth factor, and a reduction of growth factor signaling can have detrimental effects on neuronal repair, glucose use and cell metabolism, and inhibition of apoptosis. Fig. 2 [52] shows the cellular signaling pathway that is activated by the activation of GLP-1Rs. These pathways are also activated by other growth factors, enabling the incretin analogs to compensate for the desensitization of insulin and insulin-like growth factor signaling. Other growth factors have been shown to be neuroprotective. For example, BDNF has been shown to protect synapses in mouse models of AD. Injecting BDNF icv improved cognition, prevented impairments of LTP, and led to an enhancement of hippocampal synaptic densities in mouse models of AD [53]. Increasing BDNF production in the brain by gene delivery vectors also has protective effects on synapses. An increase in BDNF levels, when administered after disease onset, reversed synapse loss, improved synaptic plasticity, and restored learning abilities in a mouse model of AD

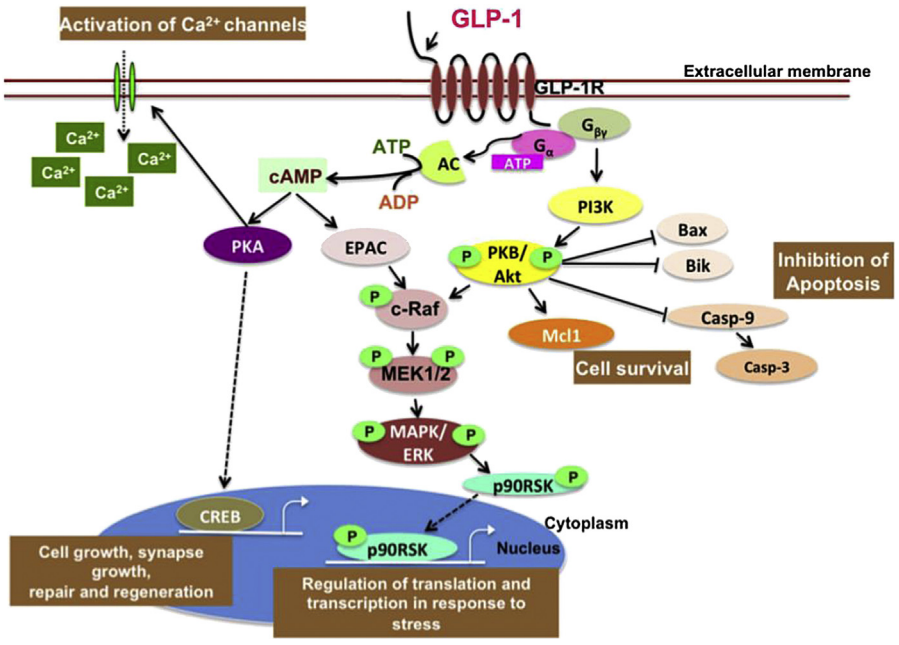

Fig. 2. Growth-factor related cell signaling activated by glucagonlike peptide 1 (GLP-1) receptors. Diagrammatic representation of the neuroprotective effects of the long-lasting GLP-1 analog liraglutide, mediated by protein kinase B (PKB)/Akt and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways. liraglutide stimulates GLP-1 receptor (GLP-1R), resulting in an increase in the cyclic adenosine monophosphate (cAMP), leading to additional intracellular events such as cell survival; inhibition of apoptosis; activation of Ca21 channels; cell growth, repair, and regeneration; and regulation of translation/transcription in response to stress. AC, adenylate cyclase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Bax, Bcl2-associated X protein; Bik, Bcl2-interacting killer; Casp-3, caspase 3; Casp-9, caspase 9; c-Raf, cellular Raf gene (rapidly accelerated fibrosarcoma); CREB, cyclic AMP response element binding protein; EPAC, exchange proteins directly activated by cAMP; Mcl1, myeloid cell leukemia protein 1; MEK1/2, MAPKs or ERKs; p90RSK, ribosomal S6 kinase; PI3 K, phosphoinositide 3 kinase; PKA, protein kinase A. For details see [52].

[54,55]. Clearly, the effects of BDNF are very similar to those of the GLP-1 and GIP analogs tested in our lab. This suggests that growth factors have common modes of action. There is, however, one vital difference: BDNF does not cross the BBB, and therefore a gene delivery system to the brain would need to be used if BDNF was to be used as a drug treatment [56,57]. This clearly limits the application of BDNF as a treatment for AD or Parkinson's disease (PD).

A different growth factor that has shown great promise as a treatment for neurodegenerative disorders is nerve growth factor (NGF). Again, NGF was found to protect synapses, LTP, and learning abilities in AD mouse models or in nonprimate monkeys without affecting amyloid plaque load [58–60]. However, NGF does not cross the BBB either, limiting the use of the growth factor. Clinical trials to get NGF into the brain or to increase NGF production in the central nervous system have not shown protective effects so far [57,59]. Still, clinical trials are ongoing to test the effects of NGF delivery into the brains of patients [61].

Other growth factors have similar protective effects on neurons in AD models, such as insulin-like growth factor 1 [62,63], vascular endothelial growth factor [64], and glial cell line-derived growth factor (GDNF) [65]. In one study, vascular endothelial growth factor decreased the levels of amyloid in the brain, and improved memory formation and progenitor cell proliferation in the brain of an AD mouse model [64]. These growth factors have shown promising results in protecting neurons from the effects of Aβ, promoting cell repair, reducing inflammation, and protecting synaptic functions and cognitive performance. Some of these growth factors have additional, unwelcome effects, such as the development of hyperalgesia by NGF [66], or enhancement of angiogenesis [64]. A major problem for some of these growth factors is the fact that they do not readily cross the BBB. This poses a technical challenge that so far has not been met [67–69]. In PD, the neuroprotective effects of GDNF have been investigated in detail [70,71]. A clinical trial that tested GDNF infusion into the brain of a PD patient showed the first positive effects [72]. Other trials are currently ongoing, see [73]. These first positive results support the concept of growth factors as a treatment for neurodegenerative disorders.

Because there is some redundancy in cell signaling activated by growth factors [3], the lack or loss of one growth factor signaling pathway (e.g., insulin) can be compensated for by other growth factors. For example, in a cell culture study, GLP-1 was able to compensate to some degree for a lack of NGF signaling [21]. An additional mode of action may be that the release of one growth factor is enhanced

by another. For example, GLP-1 analogs can increase the levels of BDNF in the brain, thereby increasing the synaptoprotective effects of BDNF signaling [44].

6. Incretin analogs are promising neuroprotective agents

The incretins GLP-1 and GIP have a number of cardinal properties that make them promising drug candidates for treating neurodegenerative disorders. Most incretin mimetics cross the BBB readily [24,26,49,74] Also, three GLP-1 mimetics are already on the market as a T2DM treatment and show few side effects in chronic use [14,75]. This made it straightforward to take these drugs into clinical trials for PD or AD.

The first clinical studies of nasal application of insulin in patients with AD showed positive and encouraging results that demonstrate that the hypothesis of insulin desensitization as an underlying mechanism for the neurodegenerative processes in AD has validity. The effects of liraglutide in reversing the insulin desensitization in the brains of patients with AD ex vivo also supports the incretin strategy of compensating for insulin desensitization. The pilot study of exendin 4 in patients with PD showed promising first results (see the review by Foltynie [76] in this special issue), again demonstrating a proof of principle. Additional trials are ongoing, such as a larger scale trial testing exendin 4 in patients with AD (see the review by Greig [20] this special issue) and a clinical trial of liraglutide in patients with AD (see the review by Edison and Brooks [45] in this special issue). Additional trials of liraglutide and lixisenatide in patients with PD are in the planning stages.

Acknowledgments

CH is a named inventor on a patent application by Ulster University that lists GLP-1 analogs as a potential treatment for neurodegenerative diseases.

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