Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases

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Short title: Role of GLP-1 in Neuroprotection

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Abstract

The incretin hormone GLP-1 has many effects in the body. It is best known for the
‘incretin effect’, facilitating insulin release from the pancreas in hyperglycaemic
conditions. Building on this, GLP-1 mimetics have been developed as a treatment for
type 2 diabetes. In the course of monitoring of patients, it has become apparent that
GLP-1 mimetics have a range of other physiological effects in the body. In preclinical
trials, a substantial body of evidence has been built that these mimetics have
neuroprotective and anti-inflammatory effects. GLP-1 also has very similar growth-
factor like properties as insulin, which presumable is the underlying basis of the
neuroprotective effects. In preclinical studies of Alzheimer’s disease (AD),
Parkinson’s disease (PD), stroke and other neurodegenerative disorders, it has been
shown that most GLP-1 mimetics cross the blood brain barrier and show impressive
neuroprotective effects in numerous studies. In animal models of Alzheimer’s disease,
GLP-1 mimetics such as exendin-4, liraglutide, and lixisenatide have shown
protective effects in the CNS by reducing beta-amyloid plaques, preventing loss of
synapses and memory impairments, and reducing oxidative stress and the chronic
inflammation response in the brain. In animal models of PD, exendin-4 showed
protection of dopaminergic neurons in the substantia nigra and prevention of
dopamine loss in the basal ganglia while preserving motor control. These encouraging
findings have spawned several clinical trials, some of which have shown first
encouraging results. Therefore, GLP-1 mimetics show great promise as a novel
treatment for neurodegenerative conditions.

Keywords: Neurodegeneration, Alzheimer’s disease, Parkinson’s disease,
Amyothrophic lateral sclerosis, stroke, ischaemia, incretins, multiple sclerosis,
Glucagon-like peptide 1
1. Introduction

The main hallmark of type 2 diabetes mellitus (T2DM) is insulin desensitization. The discovery that the incretin hormone Glucagon-Like Peptide-1 (GLP-1) facilitates insulin release during episodes of hyperglycaemia and has several additional properties to overcome insulin desensitisation made GLP-1 an ideal candidate as a treatment for diabetes (see Bayliss and Starling 1902 and reviews in this special issue). Several drugs have been developed and brought onto the market as treatments, and therefore GLP-1 is primarily known in the context of diabetes. However, it has become apparent that GLP-1 has additional properties as well which have not been researched to such a high degree as its properties in maintaining normoglycaemia.

2. Insulin desensitisation can occur in the brain

Recent research has shown that insulin desensitisation can also occur in the brain. In several patient data base analyses, T2DM has been identified as a risk factor for AD and PD, indicating that insulin desensitization in the periphery may be a factor in initiating or accelerating the development of neurodegenerative processes (Aviles-Olmos, et al. 2012; Moroo, et al. 1994). Several epidemiological studies found a correlation between T2DM and an increased risk of developing AD or other neurodegenerative disorders at a later stage in life (Biessels, et al. 2006; Haan 2006; Luchsinger, et al. 2004; Ristow 2004; Strachan 2005). One study of the Mayo clinic showed a clear correlation between T2DM and AD. In this study, 85% of AD patients had either T2DM or increased fasting glucose levels, compared to 42% in the age matched non-demented control group (Leibson, et al. 1997). In a different study, T2DM had been identified as a risk factor that doubled the chance of developing AD (Janson, et al. 2004). In a longitudinal study, an oral glucose tolerance test showed an increased risk of developing AD in people with elevated 2-hour postload glucose levels (Ohara, et al. 2011). In general, reduced insulin sensitivity and efficacy is commonly observed in elderly people and contributes to the development of AD (Carro and Torres-Aleman 2004; Hoyer 2004).

Importantly, biochemical studies of brain tissue demonstrate that insulin signaling in the brain is desensitised in AD patients, and the brain tissue shows a very similar profile than in diabetic people in insulin signaling biomarkers of desensitisation in the periphery (Lester-Coll, et al. 2006; Steen, et al. 2005; Talbot, et al. 2012). In a first study, insulin receptor levels were found to be phosphorylated and expression levels downregulated in the brains of patients with AD (Steen et al. 2005). In a histological study of AD brain tissue, IGF-1 and insulin receptors were found to be internalised in neurons, and the second messengers IRS1 and IRS2 were reduced in total levels but had increased levels of phospho\textsuperscript{Ser312} (Moloney, et al. 2010). Furthermore, in a recent study, it was found that in brain tissue of AD patients, IGF-1 and insulin signaling was strongly desensitized. Phosphorylation of the insulin receptor β chain was reduced at positions IRβ\textsuperscript{pY\textsuperscript{1150/1151}} and IRβ\textsuperscript{pY\textsuperscript{960}}, while the insulin receptor substrate 1 (IRS-1) was hyperphosphorylated at positions IRS-1\textsuperscript{pS\textsuperscript{616}} and IRS-1\textsuperscript{pS\textsuperscript{636}}, which deactivates IRS-1 signaling, and IRS-1 binding to PI3K p85α was also much reduced. In addition, it was found in a AD brain tissue incubation study that treating brain tissue with insulin induced a reduced downstream second messenger activation (Talbot et al. 2012). The observed biochemical changes were very pronounced, and
also occurred in AD patients that did were not diabetic. This type of CNS insulin
signaling desensitisation is not dependent on glucose levels.

Initial studies of patients with PD found similar biochemical changes in insulin
signaling in brain regions that are relevant to this disease. It was found that the levels
of insulin receptor phosphorylation were increased in the basal ganglia and the
substantia nigra (Moroo et al. 1994). Furthermore, Increased IRS-2 phosphorylation, a
marker of IGF-1 resistance, was found in the basal ganglia of the 6-hydroxydopamine
lesion rat model of PD (Morris, et al. 2008). Animal studies show similar changes. In
a high fat mouse model of T2DM, learning and memory and synaptic plasticity in the
brain was impaired (Porter, et al. 2010b). In a high-fat diet rat model of early-stage
T2DM, insulin resistance was observed while dopamine release was attenuated and
dopamine clearance was diminished in the basal ganglia, suggesting that
dopaminergic signaling is compromised in T2DM (Morris, et al. 2011).

This unexpected connection between T2DM and AD /PD opened up novel research
avenues to investigate what the underlying mechanisms for this may be. Insulin is a
hormone that has a range of functions in the body. Its general physiological profile is
that of a growth factor (see fig. 1). As a growth factor, insulin plays an important role
in cell growth and survival. Neurons also carry insulin receptors, and activating these
induces dendritic sprouting, neuronal stem cell activation, and general cell growth,
repair and neuroprotection (Holscher 2005; Hoyer 2004; Li and Hölscher 2007;
Stockhorst, et al. 2004; van Dam and Aleman 2004). In addition, insulin has potent
neuroprotective effects against stressors (Carro and Torres 2004; Li and Hölscher
2007). Insulin enhances brain functions such as attention, memory formation and
cognition in humans (Okereke, et al. 2008; Reger, et al. 2008a; Watson and Craft
2004; Zhao, et al. 2004). When applied by nasal application where it enters the brain
more directly, insulin improved attention and memory formation (Craft 2007; Reger
et al. 2008a; Reger, et al. 2008b). Importantly, a phase II clinical trial showed that
nasal application of insulin improved cognition in patients with mild cognitive
impairments (MCI), considered the early phase of AD. It further improved the
amyloid1-40/1-42 ratio in CSF, increased the cortical activation as seen in FDG-PET
scans, and also showed improvement in mental tasks (Craft 2010). Several follow-up
clinical studies have started (eg. 
k=1). For a review, see (Freiherr, et al. 2013).

One possibility for the development of neurodegenerative disorders is an impairment
in growth factor signaling such as insulin and IGF-1. The desensitization would
reduce vital gene expression for cell repair and growth and could put neurons at an
increased risk over time if additional stressors occur (Holscher 2011). Neurons do not
divide and cannot be replaced, and most of them live for the duration of the person’s
lifetime. The amount of actual neurogenesis is by far insufficient to compensate for
the loss. This means neurons are exposed to stressors over a long time frame, and
damage may accumulate over decades and will finally result in synaptic loss and
neuronal dysfunction and ultimately, neuronal death (Holscher 2011).

3. GLP-1 mimetics have neuroprotective properties

AS T2DM had been identified as a risk factor for AD, the concept developed that
drugs that can treat T2DM successfully may also have neuroprotective properties. In
diabetes, a range of drugs are on the market or under development which could be
tested for potential neuroprotective properties. As described in this special issue,
mimetics of the incretin GLP-1 are a successful strategy to treat T2DM (Campbell
and Drucker 2013; Drucker and Nauck 2006; Holst 2004). Not only have a range of
effective and long-lasting mimetics been developed and tested, three of these have
received approval as treatments for T2DM (Elkinson and Keating 2013; Madsbad,
et al. 2011). In the brain, GLP-1 receptors are expressed by neurons, in particular on
pyramidal neurons in the hippocampus and neocortex, and Purkinje cells in the
cerebellum (During, et al. 2003; Hamilton and Holscher 2009; Perry, et al. 2007). Glia
cells were not found to express this receptor, but induce expression when activated in
an inflammation response (Iwai, et al. 2006). In initial studies of synaptic plasticity in
the hippocampus, we found that novel GLP-1 analogues such as Val8GLP-1,
liraglutide, or exendin-4, which are DPP-IV protease resistant and have a much
enhanced biological half life in the body (Holst 2004), have profound effects on
memory formation and on synaptic plasticity in the brain (Holscher 2010). In
addition, GLP-1 mimetics can protect synapses from the detrimental effects that beta-
amyloid has on synaptic plasticity in the hippocampus (Gault and Holscher 2008).
Most of these novel mimetics can cross the blood brain barrier (Hunter and Holscher
McGovern, et al. 2012), a property that is of vital importance if they are to be
used to treat neurodegenerative disorders of the CNS.

GLP-1 is a growth factor, and the neuroprotective effects are most likely due to
classic growth factor effects such as increased gene expression of genes that are
linked to cell growth and repair and replacement, increase of cell metabolism,
inhibition of apoptosis and reduction of inflammation responses (see fig. 2 for
details on the underlying molecular mechanisms). Other growth factors have shown
similar neuroprotective properties (Bradbury 2005; Kuipers and Bramham
2006). However, most, neuroprotective growth factors such as NGF and BDNF do
not cross the blood brain barrier, and therefore have no protective effect in the
CNS when injected peripherally (Holscher 2011).

Progressive neurodegenerative diseases as well as stroke induce a chronic
inflammation response in the brain. This secondary down-stream process causes
further neurodegenerative effects via the activation of immune cells such as microglia
in the brain. These cells release pro-inflammatory cytokines and free radicals such as
nitric oxide (NO), which is neurotoxic (Ayasolla, et al. 2004). The degenerative
effects of chronic inflammation in the brain are extensive (Arnon and Aharoni
2009), and intense research for anti-inflammatory drugs for such conditions are
It is therefore of great interest to note that GLP-1 mimetics are not only
neuroprotective, but also have anti-inflammatory properties. One study demonstrated
that both activated microglia and activated astrocytes, which take part in the
immune/inflammation response, induce GLP-1 receptor expression. GLP-1 treatment
prevents an endotoxin (LPS) induced release of the cytokine IL-1β by these cells
(Iwai et al. 2006). IL-1β is pro-inflammatory and reduces neuronal transmission
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while increasing apoptosis-related signaling. Furthermore, exendin-4 can reduce monocyte adhesion to aortic endothelium in an inflammation response in atherosclerosis, and also prevents lipopolysaccharide (LPS)-induced cytokine and chemokine release in both human and mouse monocytes (Arakawa, et al. 2010) and an increase in microvascular permeability (Dozier, et al. 2009). LPS activates a systemic inflammation response, as bacterial walls contain this molecule class. In our study of chronic treatment the APP/PS1 mouse model of AD with liraglutide injected ip., the numbers of activated microglia in the brain was much reduced (McClean et al. 2011). As this effect may be due to the reduction of amyloid plaque, we followed up this study with a second study that measured the effects of liraglutide on inflammation only. X-ray exposure is known to induce an inflammation response. We found that the main pro-inflammatory cytokines and nitric oxide syntheses in the brains of x-ray exposed mice (6 Gray) after 30 days of ip. once-daily injection of liraglutide was significantly reduced (Parsarathy and Holscher 2011). These data indicate that GLP-1 mimetics may be useful in treating the chronic inflammation response seen in neurodegenerative disorders.

Alzheimer’s disease

In preclinical studies of established animal models of AD, neuroprotective effects were observed. In the APP/PS1 transgenic mouse model of AD, which expresses the human Swedish mutated form of amyloid precursor protein (APP) and a mutated human form of presenilin-1 (PS-1), both mutations which lead to AD, we found that chronic ip. injection of the GLP-1 mimetic Val(8)GLP-1 blocked synaptic degradation that is observed in this AD mouse model and rescued synaptic plasticity in the hippocampus (Gengler, et al. 2012). In rats that received icv. injections of beta-amyloid, a protein that accumulates in the brains of AD patients, it was found that Val(8)GLP-1 prevented the block of synaptic plasticity in the brain, and prevented the impairment of spatial learning (Wang, et al. 2010). In a separate study, the GLP-1 mimetic liraglutide also protected synapses from the detrimental effects of beta-amyloid, and rescued memory formation (Han, et al. 2013; McClean et al. 2011). Liraglutide (Victoza®) is on the market as a treatment for T2DM (Courreges, et al. 2008). When tested in the APP/PS1 mouse model of AD, once daily injections ip. had clear neuroprotective effects. Liraglutide prevented the memory impairment that is usually observed in aging APP/PS1 mice, protected the synapses in the hippocampus from degradation, and furthermore protected synaptic plasticity. Importantly, the beta amyloid plaque load and the total amount of beta-amyloid in the brain was much reduced. This is an important biomarker for AD, and a reduction in amyloid levels is an important beneficial property. Furthermore, the chronic inflammation response that is found in the brains of the AD mouse model was also much reduced (McClean et al. 2011). Exendin-4 also has been shown to reduce endogenous levels of beta-amyloid in the mouse brain (Perry, et al. 2003). This drug is also on the market as a treatment for T2DM under the name Byeatta®. Exendin-4 has a range of neuroprotective properties in transgenic mouse models of AD (Li, et al. 2010) and cell culture studies (Perry and Greig 2005). GLP-1 mimetics also have been shown to induce neurite outgrowth and to protect against excitotoxic cell death in cell cultures (Perry, et al. 2002; Perry et al. 2007). Furthermore, mice that overexpress GLP-1 receptors in the hippocampus showed increased neurite growth and improved learning (During et
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al. 2003). GLP-1 mimetics also normalise neuronal progenitor cell proliferation and neurogenesis, exendin-4 as well as liraglutide and lixisenatide. It has been demonstrated in several studies in mouse models of AD and of diabetes or wildtype mice that incretin analogues can increase or normalise neuronal progenitor cell proliferation in the CNS (During et al. 2003) (Hamilton, et al. 2011; Hunter and Holscher 2012; Li et al. 2010; McClean et al. 2011; Porter, et al. 2010a; Porter et al. 2010b).

In conclusion, GLP-1 mimetics show an impressive range of protective effects on synaptogenesis, neurogenesis, cell repair, and the reduction of the chronic inflammation response, and most importantly reduce the levels of amyloid plaques in the brain. These findings suggests that these drugs may be used as a novel treatment for AD (Azzouz, et al. 2004; Bradbury 2005; Cotman, et al. 2007; Gregory-Evans, et al. 2009; Holscher 2011).

Parkinson’s disease

There are several preclinical studies that have demonstrated neuroprotective effects of exendin-4 in animal models of PD. The protective effects of exendin-4 on neural stem/progenitor cells in the subventricular zone in the rat brain and the beneficial effects in an animal model of PD as well as in cell culture had been tested (Bertilsson, et al. 2008). Exendin-4 increased the number of neural stem/progenitor cells in cell culture experiments. Furthermore, in an in vivo experiment, ip. injection of exendin-4 enhanced the numbers of BrdU positive progenitor cells in the subventricular zone. Neuronal precursor cell counts were also increased, suggesting that new neurons form that may compensate for the loss of dopaminergic neurons in the substantia nigra (Bertilsson et al. 2008). Exendin-4 was injected ip to test its effect in the 6-hydroxy-dopamine (6-OHDA) PD animal model which demonstrates neuronal loss in the substantia nigra. Five weeks after unilateral 6-OHDA lesion, the rats were injected ip. for 3 weeks with exendin-4. In a functional test of the dopaminergic system, amphetamine was injected that enhances dopamine release in the basal ganglia. A reduction of rotations in the movement of the exendin-4 group demonstrated a reduced functional impairment in this group. The expression of dopamine synthesis related enzymes was also elevated in the drug group. This result demonstrates that exendin-4 has cellular and functional beneficial properties in protecting rodents from the loss of dopaminergic neurons and transmission induced by 6-OHDA (Bertilsson et al. 2008). This was confirmed in a second study which employed the 6-OHDA and the lipopolysaccaride (LPS) induced substantia nigra injection lesion model of PD, were used to test the effects of exendin-4. Seven days after inducing the pharmacological lesions, Exendin-4 was injected ip. After 7 days of treatment, amphetamine induced circling behaviour was reduced in the exendin-4 groups. The levels of dopamine measured in the basal ganglia were also increased. Histological markers also confirmed that dopamine production was increased compared to the lesion-only groups (Harkavyi, et al. 2008). An additional study tested exendin-4 in cultured dopaminergic rat neurons. These cells are vulnerable to 6-OHDA exposure. Exendin-4 protected the neurons taken from wild type mice, but not those taken from GLP-1 receptor knockout mice. In an in vivo study, Exendin-4 protected dopaminergic neurons and rescued motor function in the 1-methyl-4-
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phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion mouse model of PD (Li, et al. 2009).

Patient data analyses also confirmed that a higher percentage of PD patients were diabetically or glucose compared to age matched control subjects. It was found that 8–30% of PD patients are diabetic, a significantly higher percentage compared to age matched controls (Aviles-Olmos et al. 2012; Pressley, et al. 2003).

Based on the encouraging preclinical studies, a clinical trial testing exendin-4 in PD patients has been conducted (see below).

Amyothrophic lateral sclerosis (ALS)

A different progressive neurodegenerative disorder that may be treatable with GLP-1 mimetics is amyotrophic lateral sclerosis (ALS). The dominating symptom is increased motor neuron degeneration in the cortex, the brain stem and spinal cord. As a consequence, patients with ALS show fast developing paralysis and muscle wasting, and die within 3–5 years after diagnosis mainly due to respiratory failure (Kunst 2004). In order to test the effects of exendin-4 in this disease, the drug was tested in NSC-19 neuroblastoma cells and a mouse model of ALS (SOD1 G93A mutant mice). It was found that exendin-4 protected NSC-19 cells and elevated the biomarker for acetylcholine neurotransmission, choline acetyltransferase (ChAT) activity and also protected cells from hydrogen peroxide-induced stress. The SOD1 mouse model of ALS was treated with exendin-4 from 6 weeks of age onwards until the final stage of the disease progression. The drug treated SOD1 mice had near normal motor activity. In histological analysis, exendin-4 treatment mice had a reduced rate of motor neurons in the spinal cord. In immunohistochemical analysis, motor neuron markers such as ChAT were normalised (Li, et al. 2012). In a different approach, injection of cells that release GLP-1 into the brains of SOD1 mice, their survival was significantly extended and motor impairment and weight loss were much delayed. In motor activity analysis, an improvement of function was also observed. The chronic inflammation response in the CNS was also reduced (Knippenberg, et al. 2012). These neuroprotective effects of the GLP-1 mimetic exendin-4 furthermore supports the concept that GLP-1 signaling is neuroprotective and may be a treatment strategy for ALS.

Peripheral neuropathy

Peripheral neuropathy is a degeneration of the neurons of the peripheral nervous system and can be induced by a range of causes. In chronic T2DM, peripheral neuropathy is often observed. To test the potential neuroprotective effect of exendin-4, the drug was tested in the diabetic polyneuropathy that is found in the streptozotocin (STZ) animal model of diabetes. STZ is toxic to beta cells in the pancreas and reduces insulin production. The effect of GLP-1 (7-37) or exendin-4 was tested in cultured dorsal ganglion neurons from the peripheral nervous system. Both drugs accelerated the neurite outgrowth of cultured ganglion neurons. In the STZ induced diabetes mouse model, exendin-4 was injected ip. for 4 weeks. When testing the motor and sensory nerve conduction velocity of peripheral nerves, both GLP-1 (7-37) and exendin-4 protected the conduction of neurons. In behavioural tests, pain
perceptions and motor and sensory neuronal conduction was improved by exendin-4. In histological studies, the skin nerve fiber densities were also normalized by exendin-4 (Himeno, et al. 2011). Exendin-4 had been tested in a different model of peripheral neuropathy which was induced by Vitamin B6 overdose. In anatomical studies, axons sizes were normalized by GLP-1. In motor tasks, the rats were partially protected from the effects of high B6 doses (Perry et al. 2007).

Again, GLP-1 signaling has neuroprotective effects on peripheral neuropathy, and may be of use in treating patients with such conditions.

Ischemia and stroke

The anti-inflammatory properties and the neuroprotective effects of GLP-1 mimetics suggest that these drugs may be useful in treating stroke victims. Exendin-4 showed good neuroprotection in a transient middle cerebral artery occlusion stroke model in rats. It was found that Exendin-4 reduced the brain area that degenerated after the stroke had been induced. In a functional score of motor activity, the drug treated group performed better (Li et al. 2009). In a transient cerebral ischemia model in gerbils, the effect of exendin-4 treatment was measured in the hippocampal CA1 region. It was found that GLP-1 receptor expression was increased after one day, and GLP-1R immunoreactivity was found not only in pyramidal neurons but also in astrocytes and GABAergic interneurons. Exendin-4 reversed the ischemia-induced hyperactivity, reduced neuronal loss, and also reduced microglial inflammatory activation in a dose-dependent manner (Lee, et al. 2011). A further study tested the neuroprotective effect of exendin-4 injected iv. after a 60-minute focal cerebral ischemia induction. The drug reduced infarct volume and protected the mice from motor impairment. It also reduced oxidative stress, induction of an inflammation response, and neuronal death after reperfusion (Teramoto, et al. 2011). In a neuronal cell culture study, exendin-4 showed good neuroprotection under hypoxic conditions. This process was PKA dependent, the kinase that is activated by the GLP-1 receptor via adenylyl cyclase activation (Wang, et al. 2012).

Taken together, these preclinical studies demonstrate good efficiency of GLP-1 signaling in protecting neurons from stressors and in reducing the inflammation response in stroke and ischemia. GLP-1 mimetics therefore show promise in preventing some of the secondary damage that occurs after a stroke or an ischemic insult.

Multiple sclerosis

Considering that GLP-1 mimetics have anti-inflammatory properties and also protect synapses from stressors, it appears that these drugs may have beneficial effects in multiple sclerosis. The main hallmarks of this disease are continuous or intermittent inflammation responses that seem to be directed against the myelin sheath that insulates larger axons. A strong inflammation response that causes the loss of myelin sheaths, slow axonal signal conduction and also loss of synapses and finally neuronal loss and impairment of motor control (Arnon and Aharoni 2009; Rossi, et al. 2012). At present, no scientific publications are available on the effects of GLP-1 mimetics in animal models of multiple sclerosis. However, a patent is available that reports the
effects of exendin-4 in mouse models of multiple sclerosis (‘GLP-1 receptor agonists for treating autoimmune disorders’ (WO 2011/024110A2) by the companies Pfizer and Rinat).

In this patent, the experimental results of the effects of exendin-4 and GLP-1 in several mouse models of multiple sclerosis are reported. One mouse model named Experimental Autoimmune Encephalomyelitis (EAE) involves the active immunisation of mice with membrane components of myelin such as the myelin basic protein, proteolipid protein, and myelin oligodendrocyte glycoprotein (MOG). This induces an autoimmune response and develops ascending paralysis. The development of this autoimmune response can be acute or chronic, depending on the mouse strain and the myelin proteins used in the immunisation. Further hallmarks of EAE are a strong inflammation response followed by demyelination in the CNS, mainly induced lymphocytes and macrophages that infiltrate the brain (Aharoni, et al. 2011; Arnon and Aharoni 2009; Rossi et al. 2012).

The experimental studies analysed spinal cord sections for infiltrating cells, T cells, monocytes and microglia. The level of demyelinisation was also assessed. Citokine levels of IL-17 and IFN-gamma were measured. The motor activity of the mice was also scored, and survival times were quantified. The motor impairment in the mice was reduced by exendin-4, and life expectancy was increased. The main inflammatory responses such as T-cell proliferation and activation as well as the inflammatory cell invasion into the CNS were much reduced, as was the level de-myelinisation. In addition, the cytokine release in the spleen was reduced.

These preclinical results are encouraging and suggest that GLP-1 mimetics may have beneficial effects in patients with multiple sclerosis. It is important to note, however, that the scientific results presented in the patent have not been peer reviewed.

4. Clinical trials

The preclinical studies listed above show an impressive range of neuroprotective and anti-inflammatory effects of GLP-1 mimetics. Furthermore, since three GLP-1 mimetics are already on the market as treatments for T2DM with a good safety profile in chronic use, several clinical trials have started that investigate the neuroprotective effects of exendin-4 or liraglutide in PD or AD patients. GLP-1 analogues have low-to-absent potential of inducing hypoglycemia, as this propriety makes them potentially suitable as safe treatment of non-hyperglycemic conditions such as neurological disorders.

PD

Recently, a clinical trial of exendin-4 in PD patients has been completed. This proof of concept study tested the effects of exendin-4 in a randomised open label trial in 45 patients. The drug was given for 12 months followed by a 2 month wash-out period. The drug group was compared to a matched group that did not receive an injection. In was found that exendin-4 was well tolerated, although weight loss was common. In a single-blinded rating of the drug group, clinically relevant improvements in PD across motor and cognitive measures compared with the control group. Exenatide-treated patients had a mean improvement at 12 months on the MDS-UPDRS of 2.7 points, compared with mean decline of 2.2 points in control patients (P = 0.037). Most
interestingly, exendin-4 showed a clear improvement in the Mattis DRS-2 cognitive score, suggesting that exendin-4 has beneficial effects in the CNS on cognition and memory (Aviles-Olmos, et al. 2013).

**AD**

A randomized, double blind clinical trial to assess the safety and efficacy of Exendin-4 treatment in 230 MCI patients / early phase Alzheimer’s disease is currently ongoing at the NIH / NIA in the USA. This trial will take 3 years, with exendin-4 given. The outcomes are performance in the Clinical Dementia Rating (CDR) scale sum-of-boxes, Alzheimer’s Disease Assessment scale - cognitive sub-scale (ADAS-cog), Behavioral and cognitive performance measures, Changes on structural and functional MRI and MRS, Hormonal and metabolic changes and changes in cerebrospinal fluid and plasma AD biomarkers (http://clinicaltrials.gov/ct2/show/NCT01255163?term=exendin-4+AND+alzheimer&rank=1).

A small-scale trial with 34 patients has been completed in Denmark at the University of Aarhus, but the results have not been published yet. This double blind, randomized trial tests the effects of liraglutide vs. placebo on MCI patients, using FDG-PET imaging to estimate glucose uptake in neurons and PIB-PET imaging to measure plaque load, Cognitive tests were also scheduled, see (Egefjord, et al. 2012) for details of the study design. A caveat of this study is that it is underpowered to produce meaningful results. (ClinicalTrials.gov Identifier: NCT01469351).

A second larger scale phase II clinical trial with liraglutide in 206 MCI patients has started. This trial is conducted by Imperial College London. The trial has a randomised and placebo controlled double blind design and will analyse FDG-PET signal changes in neuronal metabolism and cortical activation, inflammation markers (microglia activation) in PET imaging, will take CSF samples for inflammation markers and amyloid /tau levels, and the change in z-scores for the ADAS Exec, and MRI changes. It will take 12 months, with a drug dose of 1.8mg subcut. per day in the drug group or placebo injection. (http://clinicaltrials.gov/ct2/show/NCT01843075?term=liraglutide+and+alzheimer&rank=1)

**5. Conclusions**

The preclinical experimental results demonstrate a wide range of important neuroprotective properties in engaging the therapeutic targets associated with neurodegenerative disease, such as impaired memory, synapse loss and impaired neuronal communication/ synaptic plasticity, beta-amyloid plaque formation (AD), motor function impairment, dopaminergic neuronal loss and dopaminergic loss in the basal ganglia (PD), chronic inflammation and reduced neuronal regeneration and neurogenesis (stroke MS, ALS). As GLP-1 mimetics are already on the market to treat T2DM are well tolerated (Nauck 2011) and show a range of additional benefits (Ussher and Drucker 2012), and clinical trials in patients with PD or AD testing the effects of exendin-4 or liraglutide have been started, GLP-1R activation shows great
promise to be helpful in treating a range of neurodegenerative disorders (Holscher 2010).

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Figure 1. Insulin signaling in neurons. The insulin receptor (IR) is expressed on neurons and activates growth-factor type cell signaling pathways. The IR plays an important role in neuronal growth, synaptic development, and control of neurotransmitter release at the synapse. Its role in glucose uptake is limited in neurons, as the insulin-dependent GLUT-4 glucose uptake transporter is only expressed in a sub-population of large excitatory neurons (Benomar, et al. 2006; Grillo, et al. 2009). Insulin binds to the α-subunit of the receptor. This activates the tyrosine kinase phosphorylation of the β-subunit. This activates second messenger pathways.

1. Activation of the insulin receptor MAP kinase pathway activates growth related gene expression required for the control of cell metabolism and energy homeostasis, cell growth, synapse growth, and for cell repair and maintenance (Biessels et al. 2006; Hoyer 1997).

2. Insulin also modulates synaptic neurotransmission and primes synapses for induction of long-term potentiation of synaptic transmission (LTP) (Biessels, et al. 2004). This pathway most likely involves binding of insulin receptor substrate-1 (IRS1) to activate the phosphatidylinositol 3-kinase (PI3K) (Zhao, et al. 2000). This may prime the synapse for increased neurotransmitter vesicle release (de la Monte and Wands 2006). Modulation of neurotransmission may be the basis for memory formation and information processing in the CNS (Hölscher 1999).

Figure 2: Overview of the main pathways induced by GLP-1 in neurons. The GLP-1 receptor is a member of a different class type of receptor compared to the IR. Activation of the GLP-1 R activates an adenylyl cyclase and increases cAMP levels. This activates PKA and other downstream kinases that are related to growth factor signaling. This may be the reason why GLP-1 mimetics can compensate for insulin desensitization in diabetics and in AD. For more details see (Holscher 2010; Holscher and Li 2010).
Pathways and functions of insulin receptor activation

Insulin

- activation of Ca\(^{2+}\) channels and neuronal receptors
- inhibition of apoptosis
- cell survival
- neuronal development, neuroprotection, memory formation
- cell growth, synapse growth, repair and regeneration

Glucose

IRS1,2

Pi3K

Akt/PKB

PDK

cPD3B

MAPK

Raf

Ras

Grb2/SOS

Shc

IR GLUT4

Increased release of neurotransmitter/facilitation of LTP

Gene transcription

Cell growth, synapse growth, repair and regeneration
Activity of GLP-1 in neurons

GLP-1 binding to GLP-1R activates Ca2+ channels, leading to neurotransmitter release. This in turn triggers the following signaling cascade:

- ADP activates PKA, leading to cAMP production.
- cAMP activates AC, further increasing cAMP levels.
- cAMP/GEF activates RAP1A, B-RAF, MEK, ERK, leading to gene transcription and inhibition of apoptosis.
- PKC and PI3K are involved in cell survival and growth processes, including Akt/PKB-mediated neuroprotection and neuronal development.

Increased release of neurotransmitter and facilitation of neurotransmission result in enhanced cell growth, synapse growth, repair, and regeneration.