

Novel dual GLP-1/GIP receptor agonists show neuroprotective effects in Alzheimer's and Parkinson's disease models

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Short title: Novel *GLP-1 / GIP analogues as treatments for AD and PD*

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abstract

Type 2 diabetes is a risk factor for several chronic neurodegenerative disorders such as Alzheimer's or Parkinson's disease. The link appears to be insulin de-sensitisation in the brain. Insulin is an important neuroprotective growth factor. GLP-1 and GIP are growth factors that re-sensitise insulin and GLP-1 mimetics are used in the clinic to treat diabetes. GLP-1 and GIP mimetics initially designed to treat diabetes show good protective effects in animal models of Alzheimer's and Parkinson's disease. Based on these results, several clinical trials have shown first encouraging effects in patients with Alzheimer's or Parkinson' disease. Novel dual GLP-1/GIP receptor agonists have been developed to treat diabetes, and they also show good neuroprotective effects that are superior to single GLP-1 analogues. Several newer dual analogues have been tested that have been engineered to cross the blood –brain barrier. They show clear neuroprotective effects by reducing inflammation and oxidative stress and apoptotic signalling and protecting memory formation, synaptic numbers and synaptic activity, motor activity, dopaminergic neurons, cortical activity and energy utilisation in the brain. These results demonstrate the potential of developing disease-modifying treatments for Alzheimer's and Parkinson's disease that are superior to current single GLP-1 mimetics.

Highlights

- analogues of the incretin hormones GLP-1 and GIP show good neuroprotective effects
- first clinical trials testing insulin or GLP-1 mimetics show good effects in patients
- novel dual GLP-1/GIP receptor agonists have been developed
- in animal models of neurodegenerative disorders, they show superior neuroprotective effects

Key words: insulin; neurodegeneration; inflammation; growth factors; GIP; GLP-1;

1. A new dawn – novel drug targets for treating Alzheimer’s disease

Our understanding what causes progressive neurodegenerative disorders such as Alzheimer’s or Parkinson’s disease (PD) is still sketchy. The findings of Alois Alzheimer when staining the brain of a patient showed distinct protein aggregates which appeared to define the disease (Alzheimer, 1907; Alzheimer et al., 1995). Brain Derived Neurotrophic Factor (gene delivery systems are under development to circumvent this barrier. The injection of BDNF directly into the brain is not a suitable treatment for the clinic. No such clinical trial has been successful so far (Beck et al., 2005; Gao et al., 2016; Lopes et al., 2017; Schulte-Herbruggen et al., 2007; Zuccato and Cattaneo, 2009) NGF was found to protect memory formation, synapse numbers and LTP in AD mouse models or in nonprimate monkeys (Clarris et al., 1994; Covaceuszach et al., 2009; Kordower et al., 1997). Gene delivery systems have been developed to be able to use NGF as a drug to treat AD. Clinical trials testing this technique have not been successful so far (Bradbury, 2005; Covaceuszach et al., 2009; Heese et al., 2006; Mandel, 2010; Rafii et al., 2014; Schulte-Herbruggen et al., 2007). In PD, glial-cell line derived neurotrophic factor (GDNF) has attracted considerable interest, as it protects dopaminergic neurons from stress and degeneration, and has shown considerable neuroprotective effects in preclinical tests. However, as it does not cross the BBB either, and the same obstacles of enhancing GDNF levels in the brain exist (Blits and Petry, 2016; Tenenbaum and Humbert-Claude, 2017). Ideally, a growth factor that can cross the BBB and has similar neuroprotective effects should be used.

2.1 New opportunities: growth factors that can cross the BBB

Fortunately, there are growth factors that can cross the BBB. Insulin is an important growth factor that is essential for the control of energy metabolism, cell growth and cell repair in neurons (Freiherr et al., 2013; Holscher, 2014b)(see also the reviews on this topic in this special issue). Insulin can cross the BBB (Banks, 2004; Banks et al., 1997). Type 2 diabetes is a risk factor for developing AD, and a potential driver for the progressive neurodegeneration in AD and PD is the loss of insulin signalling in the brain (Arvanitakis et al., 2004; Baker et al., 2011; Biessels et al., 2006; Schrijvers et al., 2010; Talbot et al., 2012). Cell growth, repair and energy utilisation gradually decays and may be a key mechanism that underlies the progressive degenerative process. A biochemical analysis of brain tissue of AD patients showed a clear profile of insulin desensitisation, even in people that were not diabetic (Lester-Coll et al., 2006; Moloney et al., 2010; Steen et al., 2005; Talbot et al., 2012). It was found that insulin receptor subunits and

IRS1/IRS2 was found to be hyper-phosphorylated and inactivated, a biochemical profile also seen in diabetics in the periphery (Moloney et al., 2010; Talbot et al., 2012). In PD, insulin desensitisation was also observed in central brain areas such as the basal ganglia and substantia nigra (Moroo et al., 1994; Morris et al., 2011; Morris et al., 2008; Pellecchia et al., 2014). Energy utilisation, mitochondrial function, insulin signalling and dopamine transmission was found to be impaired (Morris et al., 2011; Morris et al., 2008; Numao et al., 2014). These effects were also found in non-diabetic subjects and are therefore unlikely to be caused exclusively by diabetes. However, clinical tests showed that a higher percentage of PD patients are diabetic or glucose intolerant compared with age-matched controls (Aviles-Olmos et al., 2013b).

2.2 Treating AD patients with insulin – proof of concept trials

Just as insulin improves diabetes, treating AD patients with insulin shows improvements in cognition, attention, reducing levels of biomarkers for AD, and normalising cortical activity and brain energy utilisation (Okereke et al., 2008; Reger et al., 2008a; Watson and Craft, 2004; Zhao et al., 2004). Insulin cannot be given to people who are not diabetic. Delivering insulin by nasal application where it enters the brain more directly with causing only little increases in blood levels can circumvent the problem of inducing hypoglycaemia. Nasal application of insulin improved attention and memory formation even in non-diabetic people (Craft, 2007; Reger et al., 2008a; Reger et al., 2008b). A phase II clinical trial in AD patients showed improved cognition in patients with mild cognitive impairments (MCI). It furthermore improved the amyloid1-40/1-42 ratio in the cerebrospinal fluid and increased brain activation as seen in ¹⁸FDG-PET scans which measure brain activity and energy utilisation, and furthermore showed improvement in mental tasks (Claxton et al., 2015; Craft, 2010; Craft et al., 2012). However, as in patients with diabetes, insulin appears to enhance brain insulin desensitisation and worsen cognitive decline in some patients (Claxton et al., 2015). For a review, see (Freiherr et al., 2013; Holscher, 2014a).

2.3 Mimetics of Glucagon-like peptide 1 (GLP-1) have neuroprotective properties

GLP-1 is a growth factor of the glucagon family type and has similar properties that insulin has (Baggio and Drucker, 2007; Doyle and Egan, 2007; Holscher, 2014b), see Fig. 1. GLP-1 enhances insulin release and increases insulin signalling, making it an attractive treatment for type II diabetes mellitus (Baggio and Drucker, 2007; Doyle and Egan, 2007; Long-Smith et al., 2013). GLP-1 mimetics that are protease-resistant and have a much enhanced biological half-life in the blood stream are currently on the market to treat diabetes (Baggio and Drucker, 2007; Campbell and Drucker, 2013). These drugs do not affect glycaemia levels directly and therefore are safe to

take by non-diabetics (Lean et al., 2014). Importantly, GLP-1 mimetics can cross the BBB (Athauda et al., 2017; Christensen et al., 2015; Hunter and Holscher, 2012; Kastin and Akerstrom, 2003; Kastin et al., 2002; McClean et al., 2011b). GLP-1 receptors are expressed in the brains of rodents, primates and humans (Cork et al., 2015; Farr et al., 2016; Heppner et al., 2015; Merchenthaler et al., 1999).

2.3.1 GLP-1 mimetics show neuroprotective effects in animal models of AD

In several transgenic mouse models of AD, GLP-1 mimetics were neuroprotective. The GLP-1 mimetic exendin-4 (Byetta®, Bydureon®) showed good neuroprotective effects in a triple transgenic mouse model of AD (Li et al., 2010). The drug is on the market as a treatment for diabetes. Exendin-4 showed neuroprotective effects in other animal models of neurodegeneration as well (Eakin et al., 2013; Perry and Greig, 2005; Perry et al., 2007; Rachmany et al., 2013). Liraglutide (Victoza®), another drug on the market to treat diabetes (Courreges et al., 2008), reduced AD hallmarks such as memory loss, synapse loss, impaired synaptic transmission (LTP), chronic inflammation in the brain, and amyloid load in the brain after 8 weeks of once-daily treatment (McClean et al., 2011b). The same drug treatment regime in aged APP/PS1 mice 14-16 months old still had protective effects, suggesting that treatment at more progressed AD stages may still show benefits (McClean and Holscher, 2014a). When treating APP/PS1 mice from 2 months onward for 8 months, the drug did reduce disease development and therefore has the potential to be used prophylactically (McClean et al., 2015). The GLP-1 mimetic lixisenatide (Lyxumia®) also had similar neuroprotective effects compared to liraglutide in this APP/PS1 mouse model (McClean and Holscher, 2014b). A study has been published that showed no effects of liraglutide on amyloid plaque load. One reason for this may be that one of the transgenic mouse models chosen which expresses the London APP mutation actually hardly develops extracellular plaques at all, which makes it impossible for a drug to reduce the levels (Hansen et al., 2016a). Furthermore, liraglutide showed protective effects in the human P301L mutated tau-expressing mouse, a model of fronto-temporal lobe dementia. Liraglutide treatment reduced the amount of tangles and hyperphosphorylated tau (Hansen et al., 2015a). In the accelerated senescence SAMP8 mouse model, liraglutide had beneficial effects on memory formation and reduced neuronal loss (Hansen et al., 2015b). Liraglutide furthermore improved insulin desensitisation and inflammation induced by the injection of amyloid oligomers into the cortex of cynomolgous monkeys (Lourenco et al., 2013). GLP-1 mimetics normalise neuronal progenitor cell proliferation and neurogenesis in mouse models of AD and of diabetes, GLP-1 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., 2011b; Parthsarathy and Holscher, 2013; Porter et al., 2010a; Porter et al., 2010b) and protect against ER stress and autophagy impairments (Panagaki et al., 2017; Sharma et al., 2013), see Fig. 2.

2.3.2 GLP-1 mimetics show effects in animal models of PD

Exendin-4 has shown good neuroprotective effects in several animal models of PD. In the 6-hydroxydopamine (6-OHDA) rat lesion model, animals were treated for 3 weeks and showed functional recovery. In the substantia nigra, dopaminergic neurons were partly protected from the toxic effects of 6-OHDA (Bertilsson et al., 2008; Harkavyi et al., 2008). Exendin-4 protected dopaminergic neurons and rescued motor function in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion mouse model of PD (Li et al., 2009). When comparing the next generation GLP-1 mimetics liraglutide and lixisenatide with the first generation drug exendin-4, we found that liraglutide and lixisenatide had good protective effects while exendin-4 showed only minor protection under the conditions chosen in this MPTP mouse model experiment. Motor coordination was partly rescued, and dopaminergic neurons were protected in the substantia nigra. Pro-apoptotic mitochondrial cell signalling was reduced, while growth factor kinase activity was enhanced (Liu et al., 2015). One study did not find neuroprotective effects testing liraglutide in the 6-OHDA rat model, but the lack of effect is most likely due to the design of the study. In one study, liraglutide was only given to the rats six weeks after the 6-OHDA lesion, a time point when neurons will have long died (Hansen et al., 2016b).

3. Clinical trials testing GLP-1 mimetics in AD or PD patients

The results obtained in the preclinical studies show an impressive range of neuroprotective effects of GLP-1 and GIP mimetics. As several GLP-1 mimetics are already on the market as treatments for T2DM with a good safety profile, clinical trials have started that investigate the neuroprotective effects of exendin-4 or liraglutide in PD or AD patients.

3.1 Parkinson's disease

A clinical pilot trial of exendin-4 in PD patients showed good effects. This study tested the effects of exendin-4 in a randomised, open label trial in 45 patients. The patients had already progressed in the disease as the average time since diagnosis was 10 years. None of the patients had diabetes. The drug was administered over 12 months, followed by a 2 month wash-out period. In a single-

blinded rating of motor activity, clear improvements were found, and cognitive measures were improved in the drug group compared to controls in the Mattis DRS-2 cognitive test. Exendin-4 treated patients had a mean improvement at 12 months on the MDS-UPDRS of 2.7 points, compared to a mean decline of 2.2 points in control patients. Importantly, the drug group showed a clear improvement in the cognitive test, suggesting that exendin-4 has beneficial effects on cognition and memory (Aviles-Olmos et al., 2013a). The group was re-tested 12 months after the trial had finished, and the clear differences between groups in motor performance and cognitive scores had not changed (Aviles-Olmos et al., 2014).

A phase II double-blind, placebo controlled trial testing the once-weekly formulation of exendin-4, Bydureon®, had been conducted as a follow-up. The patients had a shorter average time since diagnosis of 5 years and were not diabetic. The trial confirmed the protective effects of exendin-4 in motor skills as measured in the MDS-UPDRS part 3 test. After 48 weeks, the drug group was 4.3 points superior to the placebo group. After a wash-out period of 12 weeks when no drug was given, the difference between groups was still 3.5 points, demonstrating that the drug treatment has disease-modifying properties (as defined as improvements still present even when the drug is no longer present in the body). CSF analysis confirmed that the drug crossed the BBB, and that there was no drug present after wash-out (Athauda et al., 2017) (see also the review by Athauda and Foltynie in this special issue). This result confirms the results of the pilot study, and is a proof of concept that GLP-1 mimetics can halt or slow down PD in the human brain.

3.2 Alzheimer's disease

A pilot double blind, randomized pilot trial tested the effects of liraglutide vs. placebo in AD patients, using ¹⁸FDG-PET imaging to estimate cortical activity and PIB-PET imaging to measure plaque load (Egefjord et al., 2012). The drug treatment only lasted for 6 months, which is very short for an AD trial, and the control group did not deteriorate significantly in that short period, making it impossible to test a drug effect. However, there was a clear effect in the brain ¹⁸FDG-PET scans. Deoxyglucose (DG) is a modified glucose molecule, and the uptake of labelled DG in the brain correlates well with cortical activity, synaptic activity, and disease progression (Femminella and Edison, 2014). While the placebo group showed the typical reduction in the ¹⁸FDG-PET scans of up to 20%, the drug group showed no reduction at all and even showed improved activity in some brain areas (Gejl et al., 2016) (see also the review by Rungby in this special issue). This result confirms a previous study, testing the effects of liraglutide in a transgenic mouse model of AD. In this study, PET scans showed clear protection by the drug of the deterioration of the ¹⁸FDG-PET signal seen in control tg mice (McClean et al., 2011a). It

demonstrates that the results from animal models translate into the clinic, which has not been seen in previous drug studies. In a separate pilot study, Liraglutide showed good protective effects in a pilot study in people with mood disorder. In behavioural analysis and in MRI brain scans, improvements were observed (Mansur et al., 2017a; Mansur et al., 2017b).

A randomised, placebo controlled double blind phase II clinical trial testing liraglutide in AD patients is currently ongoing. It will analyse ^{18}FDG -PET brain activity, PET inflammation markers (microglia activation), MRI brain scan changes, CSF samples to test levels of pro-inflammatory cytokines and amyloid /tau levels, and several cognitive tests such as the ADAS Exec score (Femminella and Edison, 2014). Patient recruitment is currently ongoing (NCT01843075).

4. GIP analogues show good neuroprotective effects in animal models of AD and PD

Glucose-dependent insulinotropic polypeptide (GIP) is a 42-amino acid incretin growth factor which activates pancreatic islets to enhance insulin secretion and to help reduce hyperglycaemia, similar to GLP-1 (Finan et al., 2016; Gault et al., 2003). GIP receptors are also expressed in the brain and are found on larger neurons such as the pyramidal cortical neurons (Nyberg et al., 2005), which is very similar to the pattern of expression of GLP-1 receptors (Hamilton and Holscher, 2009; Nyberg et al., 2007). Stable analogues such as D-ala²-GIP or N-glyc-GIP facilitate synaptic plasticity in the hippocampus, while the antagonist Pro³-GIP impairs LTP (Gault and Holscher, 2008). The GIP analogue D-Ala²-GIP showed neuroprotective effects in the APP/PS1 mouse model of AD. In 12 months old tg mice, synaptic plasticity the hippocampus and spatial memory formation was protected by D-Ala²-GIP. Synapse loss was prevented by the drug. The amyloid plaque load was reduced, as was the chronic inflammation response. Neuronal progenitor cell levels in the dentate gyrus were also increased (Duffy and Holscher, 2013; Faivre and Holscher, 2013b). The drug also had protective effects on synaptic transmission in 19 month old tg mice, increased synaptic numbers and reduced the overall plaque load (Faivre and Holscher, 2013a). GIP injection ip. had protective effects on spatial learning in memory tasks and also reduced plaque formation and amyloid load in a different AD mouse model (Figueiredo et al., 2010). See (Ji et al., 2016a) for a review.

When testing GIP in the MPTP mouse model of PD, it was found that the long-lasting protease resistant analogue D-Ala2-GIP-glu-PAL showed good protective effects. Motor activity was partly rescued, and the number of dopaminergic neurons in the substantia nigra was increased. Synapse

numbers were increased, and the cAMP/PKA/CREB growth factor second messenger pathway was shown to be activated by the drug (Li et al., 2016). In a chronic model of MPTP lesion, the same drug protected motor activity, dopaminergic neurons, and inhibited the increased levels of expression of alpha-synuclein in the brain induced by MPTP. Furthermore, drug treatment reduced chronic neuroinflammation, oxidative stress and lipid peroxidation, and increased the expression of BDNF (Li et al., 2017). Others found very similar effects with D-Ala2-GIP in the MPTP mouse model (Verma et al., 2017). GIP treatment also protected rat brains in a model of mild traumatic brain injury (Yu et al., 2016).

5. Novel dual GLP-1/GIP receptor agonists

New dual GLP-1 and GIP receptor agonists have been developed to treat diabetes. Some have already been tested in clinical trials and show superior performance when compared with the single GLP-1 analogue liraglutide (Finan et al., 2013; Frias et al., 2017). There are currently 5 different dual agonists that we have named DA1-DA5 (see table 1 for details). DA1-DA3 have been developed as treatments for diabetes (Finan et al., 2013). We also have developed two novel dual agonists that have been modified to enhance BBB penetration (DA-JC4 and DA-CH5, see table 1).

When testing DA-JC1 in the MPTP mouse model of PD, we found that it rescued motor activity, synapse numbers, numbers of dopaminergic neurons in the substantia nigra, and reduced chronic inflammation. Interestingly, the expression of BDNF was enhanced, which can explain some of the neuroprotective effects observed (Cao et al., 2016; Ji et al., 2016b). DA-JC1 was furthermore tested in the 6-OHDA lesion rat model of PD. When treating rats for 6 weeks with DA-JC1, motor activity as tested in the Rotarod and in the open field was much improved. In the amphetamine and apomorphine circling behaviour tests, the 6-OHDA induced impairments were much reduced by the DA-JC1 treatment. Dopaminergic neuron numbers in the substantia nigra were decreased by 6-OHDA lesion and normalised by DA-JC1 treatment. Dopamine levels in the basal ganglia were reduced by 6-OHDA lesion and increased by DA-JC1. The levels of the growth factor GDNF and pAkt/CREB growth factor cell signaling was enhanced by DA-JC1. Interestingly, the autophagy marker Beclin1 was also activated by the drug. (Jalewa et al., 2017). We furthermore tested DA1-JC in a mouse model of stroke. The drug protected neuronal survival and prevented secondary neuronal degeneration (Han et al., 2015). The DA-JC1 peptide was also tested in a rat model of mild traumatic brain injury (Tamargo et al., 2017). In a cell culture study testing a range of

analogues of GLP-1, GIP, oxyntomodulin and DA-JC1, it was found that DA-JC1 was the most potent drug in the SH-SY5Y rotenone stress model (Jalewa et al., 2016).

When testing the novel DA-CH3 peptide, it was found that it is superior to liraglutide in the MPTP mouse model of PD at a dose of 25nmol/kg ip once-daily for 7 days. In the Rotarod and grip strength assessment, DA-CH3 was superior to liraglutide in reversing the MPTP-induced motor impairment. Dopamine synthesis as indicated by levels of tyrosine hydroxylase was much reduced by MPTP in the substantia nigra and striatum, and DA-CH3 reversed this while liraglutide only partially reversed this. The chronic inflammation response as shown in increased levels of activated microglia and astrocytes was reduced by both drugs. Importantly, expression levels of the neuroprotective growth factor Glial Derived Neurotrophic Factor (GDNF) was much enhanced by both DA3-CH and liraglutide. The results demonstrate that the combination of GLP-1 and GIP receptor activation is superior to single GLP-1 receptor activation alone (Yuan et al., 2017). The novel DA-JC4 peptide was tested in the icv. STZ rat model of insulin de-sensitisation. Treatment with DA-JC4 (10 nmol/kg ip.) once-daily for 14 days after STZ icv. administration significantly prevented spatial learning deficits in a Y- maze test and Morris water maze tests, and decreased phosphorylated tau levels in the rat cerebral cortex and hippocampus. DA-JC4 also alleviated the chronic inflammation response in the brain (activated astrocytes and microglia). Apoptosis was reduced as shown in the reduced ratio of pro-apoptotic BAX to anti- apoptotic Bcl-2 levels. Importantly, insulin signaling was re-sensitized as demonstrated by a reduction of phospho-IRS1^{Ser1101} levels and phospho-Akt^{Ser473} up-regulation (Shi et al., 2017).

When comparing the 3 novel dual agonists DA-JC1, DA-JC4 and DA-CH5 with the GLP-1 analogue liraglutide (all drugs at 25nmol/kg ip once-daily for 6 days) in the MPTP mouse model of PD, DA-JC4 and DA-CH5 were most effective. In the Rotarod and grip strength assessment, DA-CH5 performed best in reversing the MPTP-induced motor impairment. Dopamine synthesis as indicated by levels of tyrosine hydroxylase was much reduced by MPTP in the substantia nigra and striatum, and DA-CH5 was most effective in reversing this. Pro-inflammatory cytokines were reduced the most by DA-CH5, while expression levels of the neuroprotective growth factor GDNF was most increased by DA-JC4. Synapses were protected best by DA-JC4 and DA-CH5. Both DA-JC1 and liraglutide showed inferior effects (Feng et al., 2018). Furthermore, novel triple receptor agonists that activate GLP-1, GIP and glucagon receptors have been developed and first preclinical studies show encouraging effects (Tai et al., 2018).

In conclusion, the novel dual GLP-1/GIP receptor agonists show improved neuroprotective effects and protect motor activity, dopaminergic neurons, dopamine synthesis, protect energy utilisation

and insulin sensitivity, reduce chronic inflammation and oxidative stress. The peptides DA-CH3, DA-JC4 and DA-CH5 showed superior effects to liraglutide in a direct comparison. However, more pharmacokinetic experiments and dose-response tests need to be conducted to establish the best peptide and the most effective dose. As some of these dual agonists are already in clinical trial in diabetes and show good effects with few side effects, it is feasible that the best neuroprotective dual agonist can be brought into the clinic fairly quickly.

Conflict of interest declaration

Dr. Holscher 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.

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Peptide sequences of the GLP-1/GIP dual agonists

DA-JC1 (Finan et al., 2013)

YXEGTFTSDYSIYLDKQAA^XEFVNWLLAGGPSSGAPPSK-NH₂

X = aminoisobutyric acid; **K** = Lys-C₁₆ acyl

DA2 – (Finan et al., 2013)

YXEGTFTSDYSIYLDKQAA^XEFVCWLLAGGPSSGAPPSK-NH₂

X = aminoisobutyric acid; **C** = Cys-40kDa PEG

DA-CH3 – (Finan et al., 2013)

YXEGTFTSDYSIYLDKQAA^XEFVNWLLAGGPSSGAPPSK-NH₂

X = aminoisobutyric acid

DA-JC4 (Shi et al., 2017)

YXEGTFTSDYSIYLDKQAA^XEFVNWLLAGGPSSGAPPSKKKKKK-NH₂

X = aminoisobutyric acid

DA-CH5

YXEGTFTSDYSIYLDKQAA^XEFVNWLLAGGPSSGAPPSKRRQRRKRGY-NH₂

X = aminoisobutyric acid

Table 1: Peptide sequence of the GLP-1/GIP dual agonists discussed in this review. For details see (Finan et al., 2013; Shi et al., 2017; Yuan et al., 2017).

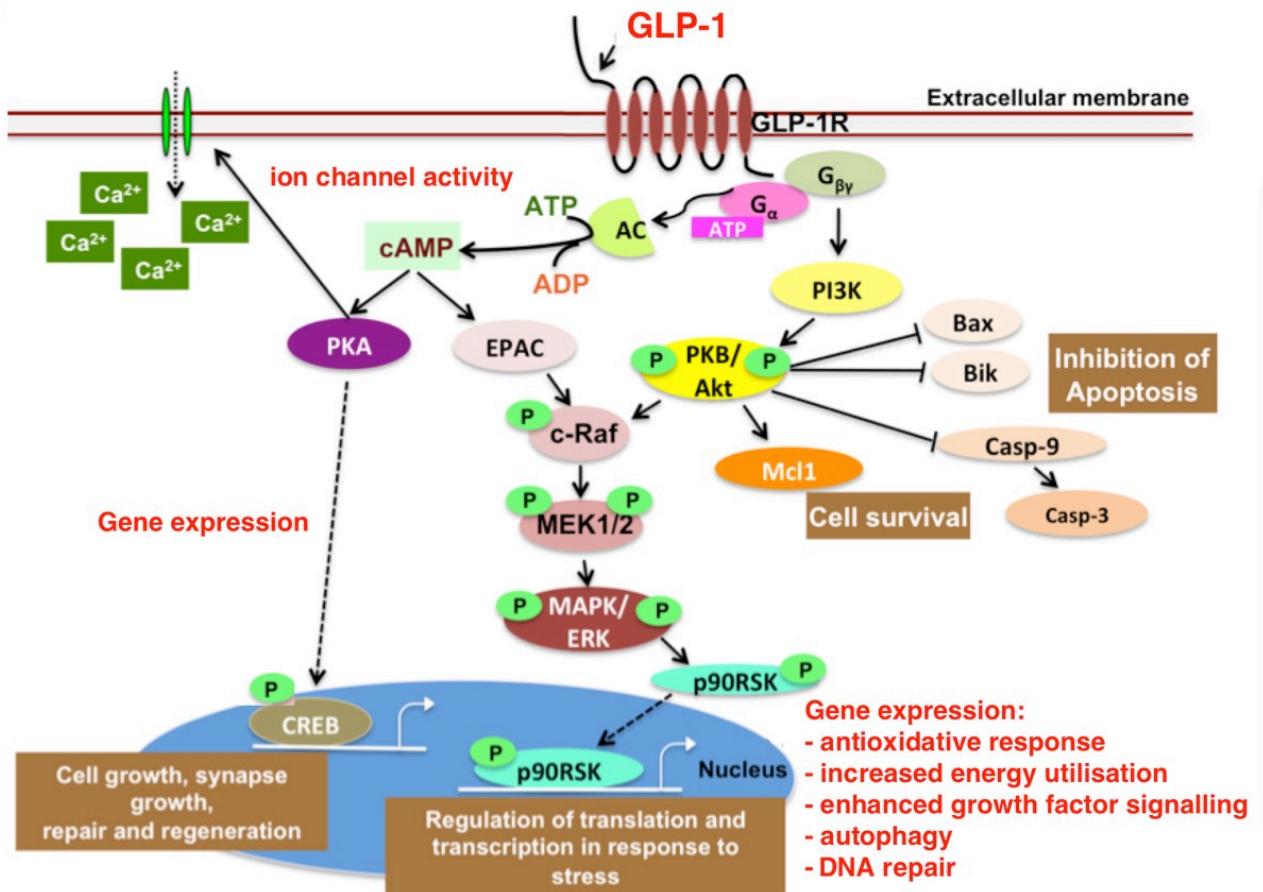


Fig. 1: Schematic representation of the cell signalling pathways that are activated by GLP-1 receptor activation and that produce the neuroprotective effects. Activation of the GLP-1R results in an increase of cAMP levels further leading to intracellular events such as cell survival, inhibition of apoptosis, activation of Ca²⁺ channels, cell growth, repair and regeneration and regulation of translation/transcription in response to stress. Modified from (Sharma et al., 2013). Abbreviations: GLP-1R, GLP-1 receptor; PKA, protein kinase A; PI3K, phosphoinositide 3 kinase; PKB, protein kinase B; AC, adenylate cyclase; EPAC, exchange proteins directly activated by cAMP; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; CREB, cyclic AMP response element binding protein; P90RSK, ribosomal S6 kinase; MEK1/2, MAPK or Erk kinases; c-Raf, cellular Raf gene (Rapidly accelerated fibrosarcoma); Mcl1, myeloid cell leukemia protein-1; Casp-9, caspase 9; Casp-3, caspase 3; Bax, Bcl2 associated X protein; Bik, Bcl2-interacting killer; Ca²⁺, calcium ions.

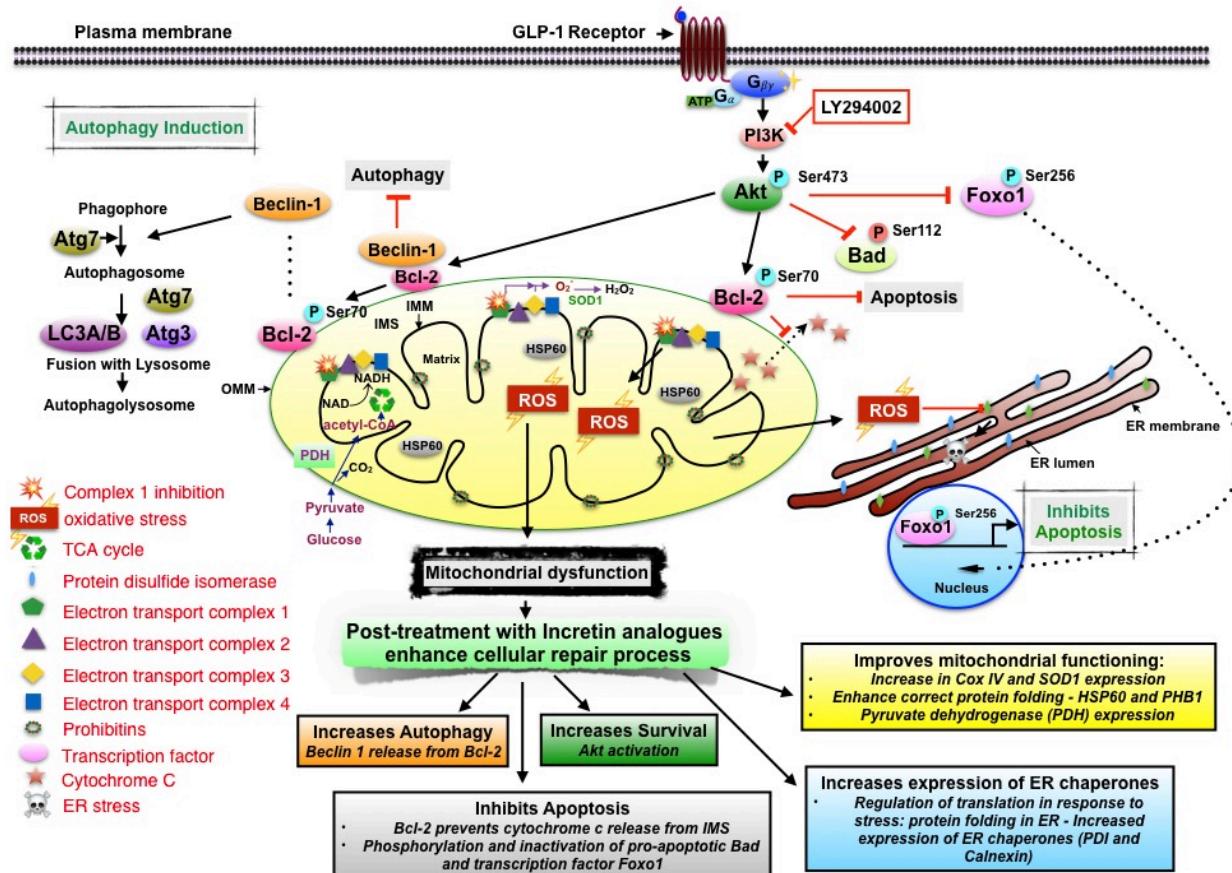


Fig. 2: Schematic representation of the protective cell signalling pathways protecting from ER stress and modulating autophagy activated by the GLP-1R. The diagrammatic representation of the effect of post-treatment with Incretin analogues against Rotenone stress summarises different events involved in the cellular signalling. When GLP-1 binds to the GLP-1 receptor, it activates PI3K resulting in phosphorylation of Akt at site Ser473. Akt activation results in 1. Increase in autophagy by releasing Beclin-1 from Bcl-2, 2. Increase in survival by inhibition of apoptosis - phosphorylation of Bcl-2 that prevents cytochrome c release from inner mitochondrial space (IMS), 3. Phosphorylation and inactivation of pro-apoptotic Bad and transcription factor Foxo1. Post-treatment with incretin analogues ameliorate mitochondrial dysfunctioning by 1. an increased expression of (cytochrome c oxidase) Cox IV and (superoxide dismutase) SOD1, 2. Increase in (heat shock protein 60) HSP60 and Prohibitins (PHB1), 3. Increased Pyruvate dehydrogenase (PDH) expression. Abbrev.: GLP-1R = glucose-dependent insulinotropic peptide receptor; GIPR = glucose-dependent insulinotropic peptide receptor; PI3K = phosphoinositide 3 kinase; OMM = outer mitochondrial membrane; IMM = inner mitochondrial membrane; IMS = inter membrane space; Bcl-2 = B-cell lymphoma 2; Bad = Bcl-2-associated death promoter; SOD1 = superoxide dismutase; PDH = pyruvate dehydrogenase; Atg7 = Autophagy-related protein 7; Atg3 = Autophagy-related protein 3. Modified from (Jalewa et al., 2016).