Endocrine Pharmacology

Glucagon-like peptide-1 analogues enhance synaptic plasticity in the brain: A link between diabetes and Alzheimer's disease

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A B S T R A C T

Type 2 diabetes has been identified as a risk factor for patients with Alzheimer’s disease. Insulin signalling is often impaired in Alzheimer’s disease, contributing to the neurodegenerative process. One potential strategy to help prevent this is the normalisation of insulin signalling in the brain. Therefore, the present study was designed to test the effects of novel enzyme-resistant analogues of the insulin-releasing incretin hormone, glucagon-like peptide 1 (GLP-1). The effects of Liraglutide (Victoza) and other novel GLP-1 analogues were tested on synaptic plasticity (LTP) in area CA1 of the hippocampus. At a dose of 15 nmol in 5 µl i.c.v., Liraglutide (P<0.005), Asp7GLP-1 (P<0.001), N-glyc-GLP-1 (P<0.01), and Pro3GLP-1 (P<0.001). In contrast, the GLP-1 receptor antagonist exendin(9-39)amide impaired LTP (P<0.001). Co-injection of exendin(9-39) and Liraglutide showed no effect on LTP. These results clearly demonstrate that Liraglutide and other GLP-1 analogues elicit effects on neurotransmission in the brain. Furthermore, GLP-1 peptides are not only effective in modulating insulin-release and achieving glycaemic control in type 2 diabetes, but are also effective in modulating synaptic plasticity. These findings are consistent with our previous observations that the novel analogue (Val8)GLP-1 enhances LTP and reverses the impairments of LTP induced by beta-amyloid fragments. Therefore, the drug effects seen here could potentially ameliorate the impairments in neuronal communication and cognitive processes observed in Alzheimer’s disease.

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1. Introduction

Epidemiological studies have identified type 2 diabetes mellitus as a risk factor for Alzheimer’s disease (Li and Hölscher, 2007; Luchsinger et al., 2004; Ristow, 2004). A contributing factor is the desensitisation of insulin receptors in the brains of patients with Alzheimer’s disease (Biessels et al., 2006; Carro and Torres-Aleman, 2004a; 2004b; Hoyer, 2004; Steen et al., 2005). Building on this, new strategies are being developed to normalise insulin signalling in the brain, such as the use of the incretin hormone glucagon-like peptide-1 (GLP-1) as a new treatment for Alzheimer’s disease (Hölscher and Li, 2008; Perry and Greig, 2004). GLP-1 is an incretin hormone which regulates postprandial glucose levels through glucose-dependent insulin secretion (Gault et al., 2003). Currently, the GLP-1 receptor agonists exendin-4 (Exenatide, Byetta) and Liraglutide (Victoza) are approved for treatment of Type 2 diabetes, and others are in late stage clinical trials (Lovshin and Drucker, 2009). Interestingly, GLP-1 also plays an important role in the brain, it is expressed in neurons and acts as a neurotransmitter (Sarkar et al., 2003). GLP-1 receptors are also expressed on neurons (Hamilton and Holscher, 2009). GLP-1 has growth factor-like properties and protects neurons from neurotoxic influences (Biswa et al., 2008; During et al., 2003; Perry et al., 2003). GLP-1 also reduces the induction of apoptosis of hippocampal neurons (During et al., 2003; Qin et al., 2008) and improves spatial and associative learning (During et al., 2003).

Several stable GLP-1 analogues have been developed by amino acid substitutions in the native GLP-1 peptide at positions 7-9, where the endogenous protease dipeptidyl-peptidase IV (DPP-IV) degrades native GLP-1, eg. (Pro3)GLP-1 or (Asp7)GLP-1. Some of these substitutions have been shown to prevent protease degradation whilst retaining long-lasting GLP-1 agonist effects and improve the symptoms of type 2 diabetes (Green and Flatt, 2007; Green et al., 2005). We have previously shown that GLP-1 and the novel agonist analogue (Val8)GLP-1 have prominent effects on synaptic plasticity in vivo when injected icv (Gault and Hölscher, 2008a). In addition, (Val8)GLP-1 crosses the blood brain barrier and chronic administration prevents the impairment of synaptic plasticity that is found in a mouse model of Alzheimer’s disease (Gengler et al., 2008; Radde et al., 2006).

Liraglutide (NN2211) is a modified form of human GLP-1 which has been released on the market in Europe as a treatment for type 2 diabetes mellitus (trade name Victoza). The Liraglutide molecule has a fatty acid (C-16 palmitoyl) conjugated to the side-chain of Lys26 and...
an Arg for Ser amino acid substitution at position 34. Together, these modifications result in a significantly prolonged circulating biological half-life in vivo primarily due to binding to serum albumin and resistance to DPP-IV (Lovshin and Drucker, 2009). Another analogue that we tested is N-glyc-GLP-1 which has two polyethylene glycol attached, and this modification also prolongs the biological half life (Green et al., 2005). In this study, we characterise the role of GLP-1 analogues on long term potentiation of synaptic transmission (LTP) in the hippocampus (Collingridge and Bliss, 1987; Hölscher, 2001) to analyse whether these drugs modulate neuronal transmission and potentially alleviate impairments induced by the accumulation of beta-amyloid in the brain (Gautl and Holscher, 2008a,b).

2.2. Peptides

Peptides used in this study were synthesised on an Applied Biosystems automated peptides synthesizer (Model 432A) using standard solid-phase Fmoc protocols. Peptides were judged pure by reversed-phase HPLC on a waters Millennium 2010 chromatography system and peptides characterised using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry as described previously (Gengler et al., 2007; Hölscher et al., 2007). Peptides were stored in dry form and dissolved in double distilled water before the experiments. 5 µl of peptides solution were injected icv. Injection sites were verified by injection of ink after the experiments.

2.3. Statistics

Data were analysed using two-way ANOVA to discriminate between groups (PRISM, GraphPad software Inc, USA). Error bars in the figures depict SEMs.

3. Results

3.1. Effects of Liraglutide on LTP

When injecting 15 nmol Liraglutide in 5 µl i.c.v., a significant enhancement of LTP induced by a weak stimulation protocol was found (Fig. 2). A two-way ANOVA showed a difference between the drug group and control (DF1,10; F = 16.3; P < 0.005) and over time DF1,119; F = 1.5; P < 0.001). Interaction between factors was not significant (see Fig. 1).

3.2. Effects of N-glyc-GLP-1 on LTP

When injecting 15 nmol N-glyc-GLP-1 in 5 µl i.c.v., a significant enhancement of LTP induced by a weak stimulation protocol was found. A two-way ANOVA showed a difference between the drug group and control (DF1,10; F = 9.2; P < 0.01) and over time (DF1,119; F = 1.41; P < 0.005). Interaction between factors was not significant (see Fig. 2).

3.3. Effects of (Pro9)GLP-1 on LTP

When injecting 15 nmol (Pro9)GLP-1 in 5 µl i.c.v., a significant enhancement of LTP induced by a weak stimulation protocol was found. A two-way ANOVA showed a difference between the (Pro9) GLP-1 group and controls (DF1,10; F = 12.1; P < 0.01) and over time (DF1,119; F = 1.7; P < 0.001). Interaction between factors was not significant (see Fig. 3).

![Fig. 1. Male Wistar rats were injected icv with either vehicle (Control, □) or 15 nmol Liraglutide (♦). LTP was induced 30 min post-injection by HFS (weak protocol), and the change in EPSP assessed and graphed to represent the change in LTP. A two-way repeated measures ANOVA showed a difference between the drug group and control (P < 0.005). All groups n = 6. Averaged EPSPs are shown recorded 5 min pre-tetanus and 1 h post-tetanus. Calibration bars are 10 ms horizontal, 1 mV vertical.](image)
3.4. Effects of (Asp7)GLP-1 on LTP

When injecting 15 nmol Asp7GLP-1 in 5 µl i.c.v., a significant enhancement of LTP induced by a weak stimulation protocol was found. A two-way ANOVA showed a difference between the Asp7GLP-1 group and control (DF1,10; F = 21.4; P < 0.001) and over time DF1,110; F = 1.6; P < 0.001). Interaction between factors was not significant (see Fig. 4).

3.5. Effects of the GLP-1 receptor antagonist exendin(9-39)amide on LTP

When injecting 15 nmol exendin(9-39)amide in 5 µl i.c.v., a significant impairment of LTP induced by a strong stimulation protocol was found. A two-way ANOVA showed a difference between the exendin(9-39) group and control (DF1,110; F = 21.2; p = 0.001) and over time DF1,110; F = 1.8; P < 0.01). Interaction between factors was not significant (see Fig. 5).

3.6. Effects of the GLP-1 antagonist exendin(9-39)amide in combination with Liraglutide on LTP

When injecting 15 nmol exendin(9-39)amide and 15 nmol Liraglutide in 5 µl i.c.v., no impairment of LTP induced by a strong stimulation protocol was found. A two-way ANOVA showed no difference between groups or over time (see Fig. 6).

4. Discussion

The results from this study confirm our previous work that GLP-1 and novel protease-resistant analogues facilitate LTP (Gault and Holscher, 2008b). In this study, we show for the first time that the long-acting novel GLP-1 peptide Liraglutide (NN2211, Victozza) has a fast acting facilitatory effect on LTP. The other novel protease resistant GLP-1 agonists N-glyc-GLP-1, (Asp7)GLP-1 and (Pro9)GLP-1 also facilitate LTP, clearly demonstrating that GLP-1 receptor activation on neurons has modulatory effects on the processes that underlie synaptic plasticity. Importantly, the GLP-1 antagonist exendin(9-39)amide impaired LTP, and a combination of the agonist Liraglutide and the antagonist exendin(9-39)amide had no significant effect on LTP, showing that the effects of the peptides tested are linked to the activation of GLP-1 receptors. Since GLP-1 analogues such as exendin-4 (Exendide, Byetta, by Amylin) (Flatt et al., 2009) and Liraglutide (Victoza, by Novo Nordisk) are already on the market (Tomillero and Moral, 2009; Vilsboll, 2009; Zinman et al., 2009) (see also the Novo Nordisk company webpage for an update), it is of vital importance that more information on the effects of these drugs on neuronal activity in the brain is collected. Liraglutide is much longer lasting than exendin-4 (Buse et al., 2009) or other GLP-1 analogues (Green and Flatt, 2007), which means that diabetic patients only need one injection per day independent of meals. Several studies have shown that GLP-1 and analogues such as exendin-4 (Kastin and Akerstrom, 2003; Kastin et al., 2002), (Val8)GLP-1 and Liraglutide (McClean et al., 2009) can cross the blood brain barrier. Therefore, patients that are
prescribed such drugs to treat type 2 diabetes and inject these subcutaneously will also experience drug effects in the brain. The fact that these novel protease-resistant GLP-1 analogues can cross the blood brain barrier is also of central importance if these compounds are to be considered as potential therapies for neurodegenerative diseases such as Alzheimer’s disease. The results presented here show that all GLP-1 receptor agonists tested have pronounced effects on synaptic activity and support the concept that GLP-1 is a neurotransmitter and plays an important role in modulating neuronal activity. This is in agreement with previous studies that demonstrated potent and fast modulating actions of neuronal activity employing such peptides. One study found that injection of GLP-1 icv greatly increased the spontaneous firing rate of neurons in the hippocampus (Oka et al., 1999). Furthermore, in a patch clamp experiment recording from hypothalamic neurons, GLP-1 enhanced the frequency of miniature and spontaneous excitatory post-synaptic currents, which is a measure of synaptic vesicle release, illustrating that part of the GLP-1 activity observed is due to a presynaptic mode of action. Postsynaptic processes such as sodium channel and voltage-dependent calcium channel (VDCC) activation were also observed (Acuna-Goycolea and van den Pol, 2004). Furthermore, injection of GLP-1 icv increased the release of glutamate transmitter by neurons in the basal ganglia (Mora et al., 1992), highlighting that the effect of GLP-1 is in part pre-synaptic. These effects can only be explained by a rapid and direct effect of GLP-1 on neurotransmission, eg. through activation of GLP-1 receptors that are located on the pre-synapse and controlling the release of neurotransmitter. These results also demonstrate that the GLP-1 receptors in the brain affect neuronal activity directly, most likely through modulation of cAMP levels (Drucker et al., 1987; Green et al., 2004), calcium channels activity (Acuna-Goycolea and van den Pol, 2004; Gilman et al., 2003). There are clear parallels to the effects that GLP-1 has in the pancreas. GLP-1 receptors on pancreatic beta-cells modulate insulin release via a mechanism that involves closure of $K^+$ channels and depolarisation of the cell membrane which activates VDCCs. GLP-1 receptors are also linked to a adenylate cyclase that increases CAMP levels which in turn activates the mechanisms of vesicle exocytosis to release insulin into the extra-cellular space (Green et al., 2004; Leech and Habener, 1997; Suzuki et al., 1997). The same biochemical mechanisms that control the release of insulin are also found in neurons and control neurotransmitter release into the synaptic cleft (Okamoto et al., 1994; Wheeler et al., 1994; Winder and Conn, 1993). Indeed, it has been shown that in neuronal cell cultures, GLP-1 modulates glutamate-induced $Ca^{2+}$ influx. This effect was due to enhanced VDCC activity. GLP-1 furthermore induced cAMP formation, activated PKA, MAP kinases and other second messenger systems that are involved in transmitter vesicle release (Gilman et al., 2003).

In conclusion, GLP-1 and protease resistant GLP-1 analogues have neuroprotective properties which make them potentially useful to treat or prevent neurodegenerative diseases such as Alzheimer’s disease (Hölscher and Li, 2008). Several in vitro studies of cultured neurons showed that GLP-1 agonists protect cells from oxidative stress and reduce the induction of apoptosis (Perry et al., 2002a; Qin et al., 2008). GLP-1 also has growth-factor like properties in neurons (Perry et al., 2002b) and increases stem cell proliferation and beta-cell numbers in the pancreas (Irwin et al., 2006). Importantly, GLP-1 crosses the blood brain barrier (Kastin et al., 2002), and we have recently shown that GLP-1 analogues such as (Val$^8$)GLP-1 and now Liraglutide also cross the blood brain barrier (McClelland et al., 2009), and that these drugs have physiological effects in the brain (Gault and Holscher, 2008). Chronic injection of (Val$^8$)GLP-1 ip has protective effects and prevents the deterioration of LTP in a mouse model of Alzheimer’s disease that overexpresses human mutated APP and PS-1 (Gengler et al., 2008; Radde et al., 2006). These exciting data open up the possibility that GLP-1 and novel protease-resistant analogues can have beneficial effects in neurodegenerative diseases of the brain such as Alzheimer’s disease. The use of novel GLP-1 analogues that are protease resistant and can cross the blood brain barrier could be a promising new strategy of treating neurodegenerative diseases. Since some of these analogues have successfully passed drug trials and showed only minor side effects (Courreges et al., 2008; Monami et al., 2009), they appear to be a safe option for chronic use. In addition, they show little effect on insulin levels when blood glucose levels are normal. Only during hyperglycaemic periods, GLP-1 analogues increase insulin release and reduce blood glucose (Green and Platt, 2007). There are few side effects such as nausea or reduced appetite, which appear to be acceptable for a treatment of a devastating disease such as Alzheimer’s disease. However, further studies on the efficacy of GLP-1 agonists on neurodegenerative processes are needed to further develop this new treatment strategy.

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