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Lixisenatide, a drug developed to treat type 2 diabetes, shows neuroprotective effects in a mouse model of Alzheimer's disease

Paula L. McClean [a], Christian Hölscher [b, *]

[a] Clinical Translational Research and Innovation Centre, University of Ulster, Derry/Londonderry, BT47 6SB, Northern Ireland, UK
[b] Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, LA1 4YQ, UK

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ABSTRACT

Type 2 diabetes is a risk factor for developing Alzheimer's disease (AD). In the brains of AD patients, insulin signalling is desensitised. The incretin hormone Glucagon-like peptide-1 (GLP-1) facilitates insulin signalling, and analogues such as liraglutide are on the market as treatments for type 2 diabetes. We have previously shown that liraglutide showed neuroprotective effects in the APP*swe*/PS1ΔE9 mouse model of AD. Here, we test the GLP-1 receptor agonist lixisenatide in the same mouse model and compare the effects to liraglutide. After ten weeks of daily i.p. injections with liraglutide (2.5 or 25 nmol/kg) or lixisenatide (1 or 10 nmol/kg) or saline of APP/PS1 mice at an age when amyloid plaques had already formed, performance in an object recognition task was improved in APP/PS1 mice by both drugs at all doses tested. When analysing synaptic plasticity in the hippocampus, LTP was strongly increased in APP/PS1 mice by either drug. Lixisenatide (1 nmol/kg) was most effective. The reduction of synapse numbers seen in APP/PS1 mice was prevented by the drugs. The amyloid plaque load and dense-core Congo red positive plaque load in the cortex was reduced by both drugs at all doses. The chronic inflammation response (microglial activation) was also reduced by all treatments.

The results demonstrate that the GLP-1 receptor agonists liraglutide and lixisenatide which are on the market as treatments for type 2 diabetes show promise as potential drug treatments of AD. Lixisenatide was equally effective at a lower dose compared to liraglutide in some of the parameters measured.

1. Introduction

Type 2 diabetes (T2DM) has been identified as a risk factor for Alzheimer disease (AD) (Craft, 2007; Hoyer, 2004). Impaired insulin signalling has been linked to cerebral degenerative processes in aged T2DM patients (Zhao et al., 2004) and AD patients (Ohara et al., 2011) and insulin receptor desensitisation is observed in the Alzheimer's disease brain (Craft, 2007; Talbot et al., 2012). The desensitisation could play a role in the development of neurodegenerative disorders, as insulin is a growth factor with neuroprotective properties (Freiherr et al., 2013; Holscher, 2011; Hoyer, 2004).

GLP-1 is an incretin hormone (Lovshin and Drucker, 2009). Currently, the GLP-1 receptor agonists exendin-4 (Exenatide, *Byetta*®), liraglutide (*Victoza*®) and lixisenatide (Lyxumia®) are approved for treatment of T2DM (Elkinson and Keating, 2013; Lovshin and Drucker, 2009). These analogues are injected subcutaneously and are well tolerated. As they do not directly affect blood glucose levels, they can be given to non-diabetic people as well (George et al., 2014; van Bloemendaal et al., 2014). The GLP-1 receptor is expressed on large neurons in the hippocampus and the neocortex (Darsalia et al., 2013; Hamilton and Holscher, 2009; Lee et al., 2011).

GLP-1 also acts as a growth factor in the brain, and has been shown to induce neurite outgrowth and to protect against oxidative stress and reduces apoptosis (Perry et al., 2007; Sharma et al., 2013). Furthermore, mice that overexpress GLP-1 receptors in the hippocampus exhibited increased neurite growth and improved learning (During et al., 2003). The deletion of the GLP-1 receptor impairs learning and long-term potentiation of synaptic transmission (LTP) in the hippocampus (Abbas et al., 2009). Moreover, liraglutide and exendin-4 have been shown to reduce levels of beta-amyloid in the brain (McClean et al., 2011; McClean and Holscher, 2014; Perry et al., 2003). GLP-1 analogues induce the proliferation

* Corresponding author. Lancaster University, Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Furness college, B65, Lancaster, LA1 4YQ, UK. Tel.: +44 (0)1524 594870.
E-mail addresses: c.holscher@lancaster.ac.uk, christian_holscher@mac.com (C. Hölscher).

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of neuronal progenitor cells in the brains of mice (During et al., 2003; Hamilton et al., 2011; Hunter and Holscher, 2012). This induction of cell proliferation has the potential to facilitate the repair of neuronal networks in cortical tissue and could have beneficial effects in patients with AD (Greenberg and Jin, 2006). We have previously shown that injection of Val(8)GLP-1 *ip.* for 3 weeks rescued LTP in the hippocampus of a APP/PS1 mouse model of AD (Gengler et al., 2012). Importantly, GLP-1 and GLP-1 analogues such as liraglutide and lixisenatide cross the blood brain barrier (BBB) in mice (Gengler et al., 2012; Hunter and Holscher, 2012; Kastin et al., 2002; McClean et al., 2011; Perry and Greig, 2002; Tweedie et al., 2013). We tested liraglutide in 7–9 month old APP/PS1 mice and showed that at an intermediate stage of disease progression, when mice are beginning to display behavioural deficits, 8 weeks treatment with liraglutide ip. improved learning and memory, enhanced LTP, reduced beta amyloid plaque load and chronic inflammation, reduced the total levels of APP and amyloid oligomers. Importantly, body weight and blood glucose levels were not affected (McClean et al., 2011). A subsequent study investigated whether liraglutide would have restorative effects in late-stage Alzheimer's disease in the same transgenic mice. Accordingly, 14-Month-old APP/PS1 and littermate control mice were injected with liraglutide (25 nmol/kg bw) ip. for 2 months. Spatial memory was improved by liraglutide-treatment in APP/PS1 mice compared with APP/PS1 saline-treated mice, and the overall plaque load and inflammation response was reduced by 33%, while neuronal progenitor cell count in the dentate gyrus was increased by 50%. LTP was significantly enhanced in liraglutide-treated APP/PS1 mice, and the numbers of synapses in the hippocampus and cortex were much increased (McClean and Holscher, 2014). We have shown that lixisenatide crosses the BBB and enhanced neuroprogenitor proliferation and neurogenesis in the dentate gyrus of mice (Hunter and Holscher, 2012). In addition, lixisenatide has neuroprotective effects on memory formation and synaptic plasticity in rats injected intrahippocampally with beta-amyloid and drug (Cai et al., 2014).

The current study was designed to compare the effects of lixisenatide and liraglutide in the APP/PS1 mouse model and analyse the effects on cognition, synaptic plasticity in the hippocampus, amyloid plaque and inflammation levels in the cortex. Liraglutide is given to diabetic patients at a high dose (0.6mg-1.8 mg subcutaneously once daily) due to its high affinity to blood proteins and low bioavailability in the blood compared to exendin-4 and lixisenatide which require much lower doses (10–20 μg subcutaneously once daily for lixisenatide and twice daily for exendin-4)(Ahren, 2014; Schmidt et al., 2014; Tan and Bloom, 2013). Therefore we tested lixisenatide at a lower dose than liraglutide to account for this difference.

2. Materials and methods

2.1. Animals

$APP_{swe}/PS1_{\Delta E9}$ mice with a C57Bl/6 background were bred at the animal unit of The University of Ulster. Heterozygous males were bred with wild-type C57/Bl6 females bought locally (Harlan, UK). Offspring were ear punched and genotyped using PCR with primers specific for the APP-sequence (Forward "GAATTCCGACATGACTCAGG", Reverse: "GTTCTGCTGCATCTTGGACA"). For details see (Gengler et al., 2010). Mice not expressing the transgene were used as wild-type controls. Male animals were used in all studies. Animals were caged individually and maintained on a 12/12 light–dark cycle (lights on at 08h00, off at 20h00), in temperature-controlled room (T:21.5 °C ± 1). Food and water were available *ad libitum.* Animals were handled daily for two weeks prior to commencement of the study.

APP/PS1 and wild-type animals were 7 months of age when treatment began. They were randomized and were administered their designated treatment intraperitoneally (i.p.) once daily (at 15:00 h).

Mice were injected with lixisenatide (10 nmol/kg) or lixisenatide (1 nmol/kg) or liraglutide (25 nm/kg bw) or liraglutide (2.5 nmol/kg) or saline (0.9% w/v). Approximate doses for a typical 30 g mouse were; 0.075 nmol or 0.28 μg per mouse per day for the 2.5 nmol/kg liraglutide group; 0.75 nmol or 2.81 μg per mouse per day for the 10 nmol/kg liraglutide group; 0.03 nmol or 0.146 μg per mouse per day for the 1 nmol/kg lixisenatide group and 0.3 nmol or 1.46 μg per mouse per day for the 10 nmol/kg lixisenatide group.

Treatments were staggered so as to ensure 10 weeks of chronic administration to each animal. Treatment groups comprised n = 10–12. All experiments were licensed by the UK home office in accordance with the Animal (scientific procedures) Act of 1986.

Table 1
Amyloid plaque load analysis.

Treatment group	Mean ± S.E.M. percentage of stained area per photomicrograph	n=	Change vs control	p value (vs control)
Saline	1575 ± 0.155	11	–	–
Lixisenatide, 1 nmol/kg	0.808 ± 0.102	12	−49%	$p < 0.001$
Liraglutide, 2.5 nmol/kg	0.847 ± 0.087	12	−46%	$p < 0.001$
Lixisenatide, 10 nmol/kg	0.901 ± 0.113	11	−43%	$p < 0.01$
Liraglutide, 25 nmol/kg	0.865 ± 0.105	12	−45%	$p < 0.001$

2.2. Peptides

Lixisenatide was supplied by Sanofi in powdered form (Batch No: AVE0010, Ch-B:B004, 30.3.12). Liraglutide was purchased from GL Biochem Ltd. (Shanghai). The purity of the peptide was analysed by reversed-phase HPLC and characterised using matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry, with a purity > 99%.

Peptides were reconstituted in ultrapure® water to a concentration of 1 mg/ml in polypropylene tubes and frozen in aliquots to permit fresh preparation of doses required for injection.

2.3. Object recognition task

The object recognition task was conducted in a grey-coloured aluminium open-field arena (58 cm in diameter; 31 cm high wall) as previously described (Abbas et al., 2009). Objects for exploration were red cubes (1.8 cm diameter) and white balls (2.6 cm diameter). The arena and objects were cleaned with 70% ethanol between trials to prevent the build-up of olfactory cues.

Mice received a session of 5min in the empty open-field, 24 h prior to exposure to objects, in order to habituate them to the apparatus and test room. Motor activity was recorded by total path, number of lines crossed, and speed. The number of rearing events (forepaws elevated from the floor) was considered to be an index of exploratory behaviour. The number of grooming sessions and the number of fecal were recorded as measures of anxiety in mice.

Twenty-four hours after habituation, each mouse was subject to a 10min acquisition trial, during which they were placed in the open-field in presence of two identical objects (cube or ball) situated at 15 cm from the arena wall. After a 3 h retention interval, the mice were placed back into the arena and exposed to the familiar object and to a novel object for a further 10min. Object location and novel and familiar objects were randomized throughout the trial.

Total time spent exploring each of the two objects (when the animal's snout was directly toward the object at a distance ≤2 cm), was recorded. Recognition index was defined as the amount of time exploring the novel object over the total time spent exploring both objects multiplied by 100, and was used to measure recognition memory (TB/(TA + TB))*100 where A represents familiar object and B, novel object.

Table 2
Dense-core plaque analysis.

Treatment group	Mean ± S.E.M. percent staining area per photomicrograph	n=	Change vs control	p value (vs control)
Saline	0.267 ± 0.047	11	–	–
Lixisenatide, 1 nmol/kg	0.109 ± 0.014	12	−59%	$p < 0.001$
Liraglutide, 2.5 nmol/kg	0.143 ± 0.016	12	−47%	$p < 0.05$
Lixisenatide, 10 nmol/kg	0.141 ± 0.019	11	−47%	–
Liraglutide, 25 nmol/kg	0.136 ± 0.012	11	−49%	$p < 0.05$