Peptides 61 (2014) 38–47

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Lixisenatide improves recognition memory and exerts neuroprotective actions in high-fat fed mice

Rachael Lennox, Peter R. Flatt, Victor A. Gault*

The SAAD Centre for Pharmacy and Diabetes, School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine BT52 1SA, County Londonderry, Northern Ireland, UK

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 26 August 2014
Accepted 26 August 2014
Available online 3 September 2014

Keywords:
Cognitive function
Glucagon-like peptide-1 (GLP-1)
Hippocampus
Lixisenatide

ABSTRACT

The metabolic benefits of lixisenatide as an anti-diabetic agent are recognized but potential extra-pancreatic effects of this glucagon-like peptide-1 (GLP-1) mimetic in the brain are less well known. This study examines actions within the hippocampus following chronic 40-day peripheral administration of lixisenatide to high-fat fed mice with established obesity, insulin resistance and impaired cognition. Lixisenatide (50 nmol/kg bw, twice-daily) resulted in marked improvements in glycemic status, insulin secretion and insulin sensitivity. Examination of pancreatic tissue revealed decreased islet area, increased islet number, and increased insulin content, with no evidence of pancreatic inflammation. Lixisenatide improved recognition memory during a novel object recognition task and this was associated with up-regulation of hippocampal expression of neurotrophic tyrosine kinase receptor type 2 (NTRK2) and mammalian target of rapamycin (mTOR) genes involved in modulating synaptic plasticity and long-term potentiation. Lixisenatide also enhanced progenitor cell proliferation and increased the number of immature neurons in the hippocampal dentate gyrus. These data indicate that lixisenatide is not only a new efficacious drug for treatment of diabetes but it also exerts favorable neuroprotective effects, reversing memory impairment in obesity-diabetes. Further clinical studies are necessary to fully assess potential beneficial actions of lixisenatide in the hippocampus and cognition in man.

Introduction

Recent studies have highlighted a growing realization that obesity and diabetes are associated with cognitive impairment and an increased risk of developing neurodegenerative disorders [9]. While several hypotheses have been proposed to explain the underlying mechanisms for the cognitive dysfunction observed in obesity-diabetes [33] studies in both animal models and humans suggest that cognitive decline is associated with worsening glycemic status [6,37]. Recent evidence suggests that therapeutic agents such as the glucagon-like peptide-1 (GLP-1) receptor agonists may improve cognitive function in animal models of obesity-diabetes and other neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease [11]. Furthermore, the two lead GLP-1 mimetics, exenatide and liraglutide, are currently undergoing clinical trials to examine their therapeutic efficacy in patients with mild cognitive impairment (MCI), early phase AD and PD [21].

Lixisenatide is one of the latest GLP-1 receptor agonists to reach the market and is currently prescribed as a once-daily treatment for type 2 diabetes [40]. Lixisenatide comprises 44 amino acids and is similar in structure to exendin-4, differing by absence of a C-terminal proline at position 38 and addition of six C-terminal lysine residues at position 39. These structural modifications result in a significantly higher affinity for the GLP-1 receptor which together with inherent DPP-IV stability and less frequent adverse events including hypoglycemia, make it a useful GLP-1 therapeutic [7,27,45,49]. Lixisenatide restores insulin secretion, accelerates glucose disposal, reduces postprandial glucose and slows gastric emptying [3,32,44]. Indeed, during a four-week, randomized, open-label, repeated-dose, phase II study in type 2 diabetes patients insufficiently controlled on metformin, lixisenatide elicited a markedly greater lowering of postpandrial glucose compared with liraglutide [27]. These data together with recent observations during the GetGoal trials indicate that lixisenatide is a good option for type 2 diabetes patients [40].

Although the metabolic actions of lixisenatide have been reported, recent evidence indicates that lixisenatide can readily diffuse through the blood–brain-barrier [24]. However the precise actions of lixisenatide within the brain have not been studied

* Corresponding author. Tel.: +44 28 7012 3322; fax: +44 02870324965.
E-mail address: va.gault@ulster.ac.uk (V.A. Gault).

http://dx.doi.org/10.1016/j.peptides.2014.08.014
0196-9781/

in detail. It is now recognized that GLP-1 receptors are widely expressed in various brain regions and structures including the hypothalamus, cortex, hippocampus and sub-ventricular zone [11]. Furthermore, GLP-1 receptor agonists have been shown to improve hippocampal synaptic plasticity and enhance learning and memory in animal models of obesity-diabetes [14,23,42]. The importance of the GLP-1 signaling pathway is further corroborated by studies demonstrating that rats over-expressing the GLP-1 receptor in the hippocampus exhibit improved learning and memory [12] whereas mice lacking the GLP-1 receptor have memory and learning impairments [1]. Therefore, we hypothesized that peripheral administration of lixisenatide could reverse learning and memory impairment in high fat fed mice, a model which has previously been shown to demonstrate cognitive decline [17]. In addition to assessing metabolic parameters and indices of cognition, we examined actions of lixisenatide on hippocampal cell proliferation, and expression of key hippocampal genes involved in the regulation of learning and memory processes. The results show that lixisenatide improves learning and memory function in obesity-diabetes and opens up further opportunities for using this drug for the potential treatment of neurodegenerative disorders.

Materials and methods

Peptide synthesis, purification, characterization and DPP-IV degradation

Native GLP-1, lixisenatide and exenatide were purchased from GL Biochem Ltd. (Shanghai, China; >95% purity). Peptide purity and identity were confirmed by HPLC and MALDI-TOF MS as described previously [15]. Peptide characteristics are shown in Table 1. For DPP-IV degradation studies, synthetic peptides were incubated in the presence of TEA-HCl and DPP-IV for 0, 4 and 8 h and reaction products separated as described previously [16]. HPLC peak area data was used to calculate percentage of intact peptide remaining at each time point.

Animals

Acute in vivo studies were carried out in 22–24-week old male lean mice (derived from a colony maintained at Aston University, Birmingham) maintained on a standard rodent diet (10% fat, 30% protein and 60% carbohydrate; % total energy 12.99 kJ/g; Trouw Nutrition). Longer-term experiments were performed on 6–8-week old male Swiss NIH (National Institutes of Health) mice (Harlan, UK) fed a high-fat diet (45% fat, 20% protein and 35% carbohydrate (% total energy 26.15 kJ/g; Special Diets Service, Essex, UK) for 4 months. These mice displayed increased body weight (61.1 ± 0.8 vs 49.8 ± 1.4 g; $P < 0.05$) and hyperglycemia (12.9 ± 1.2 vs 6.0 ± 0.2 mmol/l; $P < 0.05$) compared with age-matched mice maintained on normal laboratory chow. All mice were housed individually in an air conditioned room at 22 ± 2 °C with a 12 h light (08.00–20.00 h) and 12 h dark (20.00–08.00 h) cycle with free access to drinking water. Animal experiments were performed in accordance with the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised) as well as UK Animals (Scientific Procedures) Act 1986. A time line for the methods is provided in Fig. 1.

Acute and persistent in vivo actions of synthetic peptides

Four hour-fasted lean mice received glucose alone (18 mmol/kg body weight; i.p. injection) or in combination with lixisenatide (1, 10, 50 and 100 nmol/kg body weight; i.p. injection) or exenatide (25 nmol/kg body weight; i.p. injection; positive control). In other experiments, glucose (18 mmol/kg body weight; i.p. injection) was administered 4 h following injection of saline vehicle (0.9% v/w NaCl; i.p. injection), lixisenatide (at 50 or 100 nmol/kg body weight; i.p. injection) or exenatide (25 nmol/kg body weight; i.p. injection.

Table 1
Characteristics of synthetic peptides.

Peptide	Lot number	Purity (%)	HPLC retention time (min)	Experimental M_r (Da)	Theoretical M_r (Da)	DPP-IV stability (h)
GLP-1	P111010-XZ182634	>95	28.8	3296.0	3297.7	3.3
Lixisenatide	P111010-XZ171587	>95	30.8	4858.9	4858.6	>8
Exenatide	P090616-KC52143	>95	26.4	4185.5	4186.6	>8

Peptide purity was assessed by HPLC using a Phenomenex C-18 analytical column and retention times recorded. Purified peptides were spotted onto a stainless steel sample plate (in combination 10 mg/ml cyano-4-hydroxycinnamic acid in acetonitrile/ethanol) and applied to a Voyager-DE BioSpectrometry Workstation and M_r values recorded. Peptides were incubated with DPP-IV for 0, 2, 4 and 8 h and reaction products examined by HPLC with degradation calculated as percentage intact peptide present relative to major degradation peak, GLP-1(9-36).

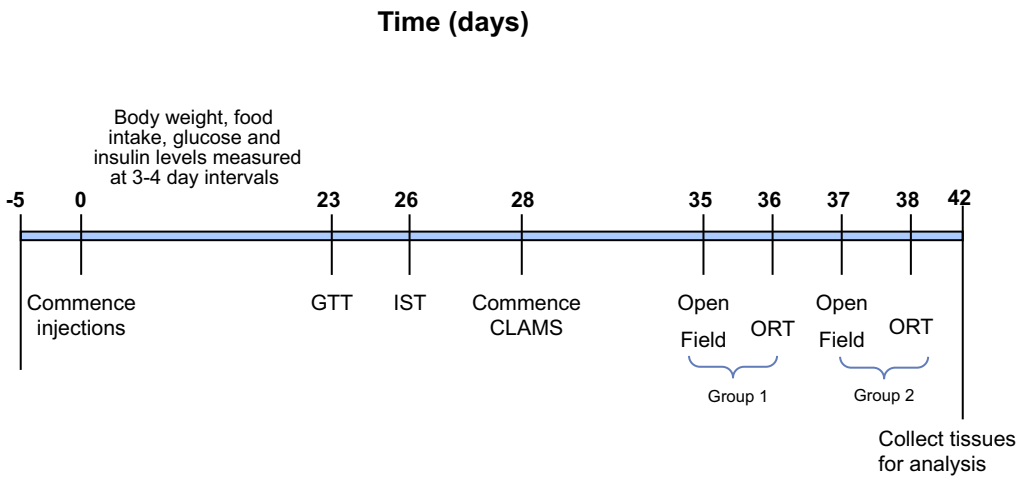

Fig. 1. Experimental timeline of the study.