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Research report

Liraglutide prevents cognitive decline in a rat model of streptozotocin-induced diabetes independently from its peripheral metabolic effects

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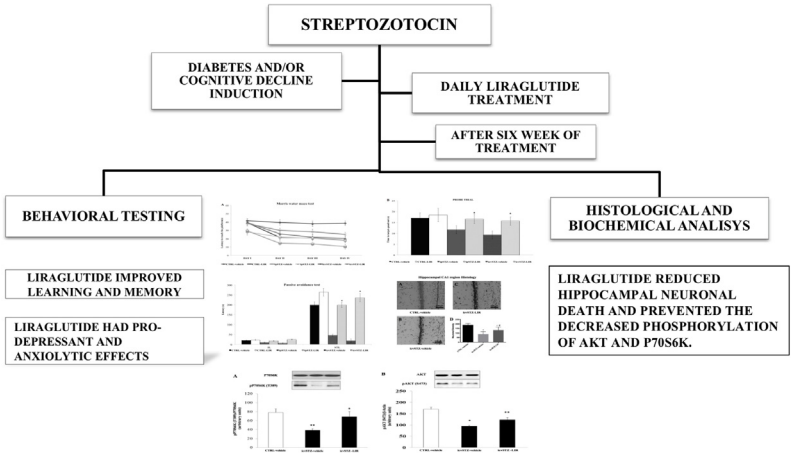
HIGHLIGHTS

- Liraglutide might have anxiolytic and pro-depressant effects.
- Liraglutide demonstrated neuroprotective effects and improved learning and memory.
- Liraglutide protected from hippocampal neurodegeneration likely through mTOR pathway.
- Liraglutide reduced hippocampal neuronal death.
- Liraglutide treatment might be a relevant option for cognitive decline.

GRAPHICAL ABSTRACT



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ABSTRACT

Diabetes has been identified as a risk factor for cognitive dysfunctions. Glucagone like peptide 1 (GLP-1) receptor agonists have neuroprotective effects in preclinical animal models. We evaluated the effects of GLP-1 receptor agonist, liraglutide (LIR), on cognitive decline associated with diabetes. Furthermore, we studied LIR effects against hippocampal neurodegeneration induced by streptozotocin (STZ), a well-validated animal model of diabetes and neurodegeneration associated with cognitive

Abbreviations: AD, Alzheimer's disease; APP/PS1, amyloid precursor protein/presenilin 1; Aβ, β-amyloid; CNS, central nervous system; CTRL, control; EPM, elevated plus maze; FST, forced swimming test; GLP-1R, glucagon-like peptide-1 receptor; i.c.v., intracerebroventricular; i.p., intraperitoneally; IL, initial latency; IT, immobility time; LIR, liraglutide; mTOR, mammalian target of rapamycin; MWM, Morris water maze; NOS, nitric oxide synthase; OF, open field arena; p70S6 K,p70, ribosomal protein S6 kinase; PD, Parkinson's disease; s.c., subcutaneously; STL, step-through latency; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-alpha.

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decline. Diabetes and/or cognitive decline were induced in Wistar rats by intraperitoneal or intracerebroventricular injection of STZ and then rats were treated with LIR (300 μg/kg daily subcutaneously) for 6 weeks. Rats underwent behavioral tests: Morris water maze, passive avoidance, forced swimming (FST), open field, elevated plus maze, rotarod tests. Furthermore, LIR effects on hippocampal neurodegeneration and mTOR pathway (AKT, AMPK, ERK and p70S6K) were assessed. LIR improved learning and memory only in STZ-treated animals. Anxiolytic effects were observed in all LIR-treated groups but *pro*-depressant effects in CTRL rats were observed. At a cellular/molecular level, intracerebroventricular STZ induced hippocampal neurodegeneration accompanied by decreased phosphorylation of AMPK, AKT, ERK and p70S6K. LIR reduced hippocampal neuronal death and prevented the decreased phosphorylation of AKT and p70S6K; AMPK was *hyper*-phosphorylated in comparison to CTRL group, while LIR had no effects on ERK. LIR reduced animal endurance in the rotarod test and this effect might be also linked to a reduction in locomotor activity during only the last two minutes of the FST. LIR had protective effects on cognitive functions in addition to its effects on blood glucose levels. LIR effects in the brain also comprised anxiolytic and *pro*-depressant actions (although influenced by reduced endurance). Finally, LIR protected from diabetes-dependent hippocampal neurodegeneration likely through an effect on mTOR pathway.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a public health problem currently increasing in prevalence [1]. T2DM is a complex endocrine and metabolic disorder also accompanied by a negative impact on the central nervous system (CNS), particularly in the elderly [2], leading to diabetic encephalopathy and concomitant increased incidence of cognitive problems; the latter are particularly associated with atrophy of the hippocampal formation that is known to be also involved in learning and memory processing [2]. Patients with T2DM show volumetric abnormalities in the hippocampus and amygdala, similar to those found in patients with major depression [3]. Furthermore, neurocognitive deficits in working memory, attention and executive function have been reported in both individuals with diabetes and mood disorders [2]. The precise pathophysiology of cognitive dysfunction and neuronal damage in T2DM is not completely understood, but it is likely that impaired glucose control (hyperglycemia, hypoglycemia), vascular damage, and insulin resistance play significant roles [3]. None of the known antidiabetic drugs currently used for T2DM has a proven effect on cognitive decline in patients, even if, there are some data on the efficacy of metformin and other antidiabetic drugs in animal models of diabetes and Alzheimer's disease (AD) [4–6] however, the glucagon-like peptide-1 receptor (GLP-1R) agonists, a new class of antidiabetic drugs now used therapeutically in diabetic patients, also exert powerful neuroprotective properties in animal models of neurodegenerative diseases, cerebral ischemia and traumatic brain injury [7,8], therefore might be valuable therapeutic tools for prevention of neurological comorbidity in T2DM. GLP-1 is an endogenous incretin (insulinotropic) peptide hormone secreted from the gastrointestinal tract, that plays a key physiological role in glucose homeostasis by enhancing pancreatic insulin secretion and by suppressing glucagon release and hepatic glucose output [9]. GLP-1 receptor (GLP-1R) agonists such as exenatide, liraglutide (LIR) and lixisenatide have been approved for treatment of T2DM [10]; in December 2014, liraglutide 3.0 mg was approved by the Food and Drug Administration and in March 2015 by the European Medicines Agency for obesity treatment. In addition to its peripheral metabolic effects, GLP-1 acts as a growth factor in the brain, inducing neurite growth and protecting against oxidative injury [11]. It is known that GLP-1Rs are expressed by pyramidal neurons in the hippocampus, hypothalamus, neocortex and Purkinje cells in the cerebellum and are involved in cell differentiation and neuroprotection as demonstrated both *in vitro* and *in vivo* [12]. GLP-1 and GLP-1 analogs also increase neuronal progenitor proliferation in the brain, as well as long-term potentiation and paired-pulse facilitation in the hippocampus [13,14]; accordingly, GLP-1R knock-out mice have an impairment of memory formation [14].

The neuroprotective effects of GLP-1 have been widely demonstrated in cultured neurons [15]. Human neuroblastoma cell lines over-expressing GLP-1Rs are protected from oxidative stress-induced cell death [16]. Excitotoxic L-glutamate-induced death of cultured rat hippocampal neurons is also decreased by GLP-1 [17]. The dual actions of GLP-1 in pancreatic β-cells and in neurons have recently generated therapeutic interest, considering that T2DM is a risk factor also for AD. Thus, GLP-1 has beneficial effects not only for the treatment of diabetes but also produces significant neuroprotection in animal models of cerebral ischemia and AD [18,19]. Indeed, GLP-1 reduces endogenous levels of β-amyloid (Aβ) in the rodent brain [20] and intracerebroventricular (i.c.v.) administration of GLP-1 enhances associative and spatial learning [13]. Furthermore, most GLP-1 agonists show remarkable neuroprotective effects in different neurodegenerative disorders such as AD, Parkinson's disease (PD) and stroke by reducing β-amyloid plaques, preventing loss of synapses and memory impairments, and reducing oxidative stress and chronic inflammatory responses in the brain. Recent studies have established that exenatide, a stable GLP-1R agonist, enhances neuronal progenitor proliferation in the brain of diabetic mice [21] and reduces endogenous levels of Aβ in transgenic AD mice [20]. Similarly, LIR has neuroprotective effects in rats [22]. It also has neuroprotective effects in an amyloid precursor protein/presenilin 1 (APP/PS1) mouse model of AD [23], increases neurogenesis, improves cognitive function and reduces amyloid plaque deposition in a mouse model of AD [19,24] and is now being tested in clinical trials for the treatment of AD patients [25]. LIR additionally attenuates the neuronal damage following cerebral ischemia in rats by preventing apoptosis and decreasing oxidative stress [26]. Recently, lixisenatide was tested in the APPswe/PS1DE9 mouse model of AD in comparison with LIR, showing that lixisenatide was equally effective but more potent [27]. Therefore, GLP-1 agonists apparently have a potential role to facilitate neuronal network repair in cortical tissue and consequently could have beneficial effects in patients with neurodegenerative disorders. Based on this background, we tested the effects of LIR on cognitive decline associated with diabetes mellitus in the established streptozotocin (STZ) rat model; furthermore, to exclude any influence of LIR effects on blood glucose levels, we studied the effects of LIR against the hippocampal neurodegeneration induced by i.c.v. injection of STZ, a well-validated model of neurodegeneration associated with cognitive impairment, not accompanied by diabetes [28]. Finally, in order to clarify the neuroprotective mechanism of action of LIR, we studied its effects on the mammalian