Behavioural Brain Research 318 (2017) 28–35

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

# Lixisenatide attenuates the detrimental effects of amyloid β protein on spatial working memory and hippocampal neurons in rats

Hong-Yan Cai[a], Zhao-Jun Wang[b], Christian Hölscher[c], Li Yuan[b], Jun Zhang[b], Peng Sun[a], Jing Li[a], Wei Yang[b], Mei-Na Wu[b], Jin-Shun Qi (Ph. D & Prof.)[b,*]

[a] Department of Microbiology and Immunology, Shanxi Medical University, Taiyuan 030001, China
[b] Department of Physiology, Shanxi Medical University, Taiyuan 030001, China
[c] Division of Biomed and Life Sciences, Faculty of Health and Medicine Lancaster University, Lancaster, LA1 4YQ, UK

## HIGHLIGHTS

- Lixisenatide, a drug already on the market to treat diabetes, shows neuroprotective effects.
- In a rat model of Alzheimer's disease, lixisenatide prevented memory loss induced by amyloid ICV injection.
- Intracellular calcium levels were normalised by the drug.
- The protective effect was blocked by a MEK1/2 kinase inhibitor.

## ARTICLE INFO

*Article history:*
Received 19 August 2016
Received in revised form 17 October 2016
Accepted 20 October 2016
Available online 21 October 2016

*Keywords:*
Lixisenatide
Amyloid β protein
Working memory
Cell viability
Akt-MEK1/2 signal pathway
Intracellular calcium concentration

## ABSTRACT

Type 2 diabetes mellitus(T2DM) is a risk factor of Alzheimer's disease (AD), which is most likely linked to impairments of insulin signaling in the brain. Hence, drugs enhancing insulin signaling may have therapeutic potential for AD. Lixisenatide, a novel long-lasting glucagon-like peptide 1 (GLP-1) analogue, facilitates insulin signaling and has neuroprotective properties. We previously reported the protective effects of lixisenatide on memory formation and synaptic plasticity. Here, we describe additional key neuroprotective properties of lixisenatide and its possible molecular and cellular mechanisms against AD-related impairments in rats. The results show that lixisenatide effectively alleviated amyloid β protein (Aβ) 25-35-induced working memory impairment, reversed Aβ25-35-triggered cytotoxicity on hippocampal cell cultures, and prevented against Aβ25-35-induced suppression of the Akt-MEK1/2 signaling pathway. Lixisenatide also reduced the Aβ25-35 acute application induced intracellular calcium overload, which was abolished by U0126, a specific MEK1/2 inhibitor. These results further confirmed the neuroprotective and cytoprotective action of lixisenatide against Aβ-induced impairments, suggesting that the protective effects of lixisenatide may involve the activation of the Akt-MEK1/2 signaling pathway and the regulation of intracellular calcium homeostasis.



## 1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, afflicting more than 40 million people all around the world. It is characterized by memory loss and cognitive decline [55]. One of the prominent events in the pathogenesis of AD is the formation of abundant deposits of senile plaques composed of amyloid β protein (Aβ) [2,63]. The toxicity of Aβ has been widely reported. For example, prolonged infusion of synthetic Aβ into the brain impaired learning and memory in rats, including working memory and place learning in the eight-arm radial maze, Y-maze and water maze [44,60]; Aβ destabilized neuronal calcium homeostasis, altered cellular ionic activity, and rendered neurons more vulnerable to toxic stimuli in human cerebral cortical cell cultures [15,32]; Aβ acutely impaired synaptic functions when added to hippocampal slices or slice cultures [33]. Not only full length Aβ but also its fragments, such as Aβ25-35 and Aβ31-35, could induce apoptosis in cultured cortical neurons [70], in vivo LTP [18], and cognitive deficits in rats [1,71]. Unfortunately, effective neuroprotective strategies against Aβ neurotoxicity are still lacking.

* Corresponding author at: Department of Physiology, Shanxi Medical University, Taiyuan, Shanxi 030001, China.
*E-mail address:* jinshunqi2009@163.com (J.-S. Qi).

http://dx.doi.org/10.1016/j.bbr.2016.10.033
0166-4328/

Epidemiological studies found a clear correlation between type 2 diabetes mellitus (T2DM) and AD, in which T2DM has been identified as a risk factor for AD and the insulin resistance in the brain might initiate or accelerate the development of AD [37,46,62]. Based on these observations, it might be a promising strategy to normalize insulin signaling in the brain for the prevention and treatment of AD. Glucagon-like peptide 1 (GLP-1), an incretin hormone, and GLP-1 analogues that are protease resistant, have been reported to be able to cross the blood-brain barrier (BBB) and it facilitates insulin signaling [19,23,27]. Furthermore, it has been found that GLP-1 and the GLP-1 receptor (GLP-1R) are expressed in the rodent central nervous system [7,43], especially the memory-related brain regions, such as the hippocampus [7,12,21]. More importantly, GLP-1 possesses neurotrophic properties [24,50], and could protect neurons against glutamate-induced apoptosis and even decrease endogenous Aβ accumulation in cellular and animal models of AD [34,42]. However, endogenous GLP-1 is broken down quickly and lasts for only several minutes in blood plasma, which greatly limits its application in clinical practice [9]. Currently, several GLP-1 mimetics are on the market (e.g. exendin-4 (Byeatta), liraglutide (Victoza) and lixisenatide (Lyxumia)). They have been engineered and developed to resist protease degradation [6,26,38,65]. These GLP-1R agonists enhance cognition and reduce blood glucose levels in T2DM models [20], but do not affect blood glucose levels in non-diabetic animals or people [41]. It has been reported that exendin-4 enhanced nerve growth factor (NGF)-induced cell differentiation into neurons [49], and showed pronounced neuroprotective and anti-inflammatory effects in middle-aged diabetic animals [8], reversed T2DM-induced neuronal pathology in the piriform cortex of the rat [35], and antagonized Aβ1-42-induced suppression of hippocampal long-term potentiation in rats [66]. Liraglutide also enhanced synaptic plasticity in the hippocampus of the APP/PS1 AD mouse model [40], reduced the chronic inflammation response [39,48], and ameliorated working memory impairment in the Aβ-induced AD mouse model [51]. Lixisenatide, a novel long-lasting GLP-1 analogue, has a higher permeability across the BBB and greater biological activity than exendin-4 and liraglutide [28,36]. Our previous studies [4] showed that lixisenatide prevented Aβ25-35 -induced spatial memory deficits and hippocampal LTP suppression in rats. In the present study, we first observed the effects of lixisenatide on Aβ-induced deficits in spatial working memory of rats and cultured cell viability by using Y maze test and primary hippocampal neuronal cultures. Furthermore, the molecular and cellular mechanisms underlying the neuroprotective role of lixisenatide were investigated by using the Western blotting technique and intracellular calcium imaging.

## 2. Methods

### 2.1. Animals and drugs

Adult male Sprague Dawley (SD) rats (200–230 g) were provided by the Animal Research Center of Shanxi Medical University. All animal handling and procedures accorded with the guidelines of the Shanxi Animal Research Ethics Committee. During experiments, rats were kept at controlled room temperature (20–24 °C) and humidity (60%–80%). Aβ25–35 and lixisenatide (Sigma, St. Louis, MO, USA) were stored in dry form and dissolved in saline (5 nmol/μl) before experiments.

### 2.2. Intrahippocampal injection

Intrahippocampal injection was performed as previously described [53,54]. In brief, SD rats were anesthetized (chloral hydrate, 0.3 g/kg, i.p.) and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan). Lixisenatide (5 nmol/μl) or vehicle (0.9% NaCl) and Aβ25–35 (5 nmol/μl) or saline (0.9% NaCl) were bilaterally injected into the hippocampi (anterior-posterior: −3.0 mm; medial-lateral: ±2.2 mm; dorso-ventral: −3.0 mm, from bregma), with an injection rate of 0.2 μl/min under the control of a micropump (KD Scientific, Inc., KDS310 Plus, USA). In co-application group, lixisenatide was injected 15 min before Aβ25–35 application. To make sure the drugs were fully dispersed into the hippocampus, a 5 min-retention of the injection syringe in the brain was given after every injection.

### 2.3. Y maze test

Spontaneous alternation of Y maze was performed to examine the spatial working memory of rats. Rats were examined 10 days after drug administration. The Y maze has three redial arms (A–C) with the same angle (120°) between arms. Each arm is 45 cm long and 12 cm high. Rats were put into the junction of three arms and allowed to move freely for 8 min session. The entries of rats into each arm were recorded and every entry different from last two entries was considered as a successful alternation. The alternation percentage was calculated according to the following: [(number of alternations)/(total number of arm entries − 2)] × 100(%) [29].

### 2.4. Primary hippocampal neuronal cell culture

Primary hippocampal formation cultures were prepared from 24 h postnatal SD rats. Briefly, pups were anesthetized with ether, and sterilized in 75% ethanol. Rat brains were quickly removed into ice-cold dissection solution. The hippocampi were stripped and cut into small pieces (each cube <1 $mm^3$). The tissue pieces were incubated with 0.125% trypsin at 37 °C for 15–20 min, and then complete culture medium was added to stop enzymatic reaction. Single-cell suspensions were obtained by mechanical dissociation using a Pasteur pipette with a fire-polished tip in complete culture medium, and then filtered through a 200 mesh nylon screen, centrifuged for 5 min at 1000 rpm. The cells at the bottom were resuspended with fresh complete culture medium and plated on poly-D-lysine coated 96-well plates and 35 mm culture dishes (Corning Inc) at a density of $5 \times 10^5$ cells/ml. Cultures were maintained in 5% $CO_2$ at 37 °C in complete culture medium for 6 h. The culture media were then changed to serum-free B27/neurobasal medium. Afterward, half of the culture medium was replaced with fresh serum-free B27/neurobasal medium every 3 days. 7–10 days after plating, the mature cells were used for further experimental observation. The experiment was all carried out under sterile condition.

### 2.5. Cell viability assay

To assess the rate of cell viability, cell counting kit-8 (CCK-8) and lactate dehydrogenase (LDH) were used. Briefly, cells were inoculated in 96-well plates. After 7–10 days, cells were incubated with Aβ25–35 (10 μM) for 24 h. In co-application group, Lixisenatide (100 μM) were applied 24 h before Aβ25–35 treatment. Six wells were used for each sample, and each experiment was repeated three times. CCK-8 solution (10 μl) was added to each well, then cells were incubated at 37 °C for 2 h. The absorbance of samples was measured by a microplate reader (SpectraMAX 190, Molecular Devices, Sunnyvale, CA) at 450 nm. Supernatant was collected to measure the LDH (a stable cytosolic enzyme, which is released upon cell lysis) release according to the manufacturer's instructions.