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LIXISENATIDE RESCUES SPATIAL MEMORY AND SYNAPTIC PLASTICITY FROM AMYLOID β PROTEIN-INDUCED IMPAIRMENTS IN RATS

H.-Y. CAI,[a] C. HÖLSCHER,[b] X.-H. YUE,[a] S.-X. ZHANG,[c] X.-H. WANG,[d] F. QIAO,[a] W. YANG[a] AND J.-S. QI[a]*

[a] *Department of Physiology, Shanxi Medical University, Taiyuan 030001, China*

[b] *Division of Biomed and Life Sciences, Faculty of Health and Medicine Lancaster University, Lancaster LA1 4YQ, UK*

[c] *Second Affiliated Hospital of Shanxi Medical University, Taiyuan 030001, China*

[d] *Department of Pathology, Shanxi Medical University, Taiyuan 030001, China*

Abstract—Alzheimer's disease (AD) is a progressive and degenerative disorder accompanied by cognitive impairment, but effective strategies against AD are currently not available. Interestingly, glucagon-like peptide-1 (GLP-1) used in type 2 diabetes mellitus (T2DM) has shown neuroprotective effects in preclinical studies of AD. Lixisenatide, an effective GLP-1 receptor (GLP-1R) agonist with much longer half life than GLP-1, has been licensed in the EU as a treatment for T2DM. However, the neuroprotective effects of lixisenatide in the brain remain to be clarified. In the present study, we report for the first time the effects of lixisenatide on the amyloid β (Aβ) protein-induced impairments in spatial learning and memory of rats, and investigated its electrophysiological and molecular mechanisms. We found that: (1) bilateral intrahippocampal injection of Aβ25–35 resulted in a significant decline in spatial learning and memory of rats, as well as a suppression of *in vivo* hippocampal long-term potentiation (LTP); (2) lixisenatide treatment effectively prevented the Aβ25–35-induced impairments; (3) lixisenatide inhibited the Aβ25–35 injection-induced activation of glycogen synthase kinase 3β (GSK3β), with a significant increase in the phosphorylation of ser9 and a significant decrease in the phosphorylation of Y216. These results indicate that lixisenatide, by affecting the PI3K-Akt-GSK3β pathway, can prevent Aβ-related impairments in synaptic plasticity and spatial memory of rats, suggesting that lixisenatide may be a novel and effective treatment for AD.

Key words: lixisenatide, amyloid β-protein, Morris water maze, long-term potentiation, glycogen synthase kinase 3β, Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is an age-related and neurodegenerative disorder characterized by progressive memory loss and cognitive decline (Selkoe, 2001). One of the hallmarks of AD is the accumulation of amyloid β (Aβ) plaques in the hippocampus and cerebral cortex (Arnold et al., 1991; Thal et al., 2002; Holscher et al., 2007; Zetterberg et al., 2010). The neurotoxicity of Aβ has been widely reported (Chen et al., 2000; Deshpande et al., 2006). For example, infusion of synthetic Aβ peptides into the brain caused learning and memory deficits in rats (Nitta et al., 1997); Aβ oligomers acutely impaired synaptic functions when added to hippocampal slices or slice cultures (Shankar et al., 2007; Li et al., 2009); and Aβ induced dysfunction of synapses in neural networks (Palop and Mucke, 2010). In our previous experiments, we found that not only full length of Aβ peptides such as Aβ1–42 and Aβ1–40 but also Aβ fragments including Aβ25–35 and Aβ31–35 significantly impaired the spatial memory (Pan et al., 2010) and hippocampal synaptic plasticity (Wang et al., 2010; Li et al., 2011) in rats. However, no effective neuroprotective strategies against Aβ neurotoxicity are currently available.

Interestingly, it has been reported that AD and type 2 diabetes mellitus (T2DM), share several common clinical and pathological characteristics. T2DM has been viewed as a risk factor for developing AD (Stewart and Liolitsa, 1999; Hoyer, 2004; Luchsinger et al., 2004; Holscher, 2005; Craft, 2007; Perry et al., 2007). AD is also associated with the desensitization of insulin signaling in the brain (Hoyer, 2004; Craft, 2007; Li and Holscher, 2007; Talbot et al., 2012). It seems that the impairment of insulin signaling in the brain may be closely related to the development of neurodegenerative disorders (Hoyer, 2004; Holscher, 2005; Craft, 2007; Talbot et al., 2012). Therefore, one of the promising strategies for developing novel AD treatments is to normalize insulin

*Corresponding author. Address: Department of Physiology, Shanxi Medical University, Taiyuan, Shanxi 030001, China. Tel: +86-351-413-5091.
E-mail address: jinshunqi2006@yahoo.com (J.-S. Qi).

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; ANOVA, analysis of variance; BBB, blood–brain barrier; DPPIV, DPPIV dipeptidyl peptidase IV; fEPSPs, Field excitatory postsynaptic potentials; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GSK3β, glycogen synthase kinase 3β; LTP, long-term potentiation; MWM, Morris water maze; PPF, paired-pulse facilitation; SD, Sprague Dawley; SEM, means ± standard errors; TBST, Tris-buffered saline containing 0.05% Tween-20; T2DM, type 2 diabetes mellitus.

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signaling in the brain. GLP-1 receptor (GLP-1R) agonists including lixisenatide can cross the blood–brain barrier (BBB) and show protective effects (Kastin et al., 2002; McClean et al., 2011; Gengler et al., 2012; Hunter and Hölscher, 2012); glucagon-like peptide-1 (GLP-1) and GLP-1R are also expressed in some critical memory-related brain areas, such as the hippocampus (During et al., 2003; Hamilton and Holscher, 2009). Importantly, GLP-1 possesses neurotrophic properties; GLP-1R stimulation decreased endogenous Aβ accumulation in cellular and animal models of AD (Li et al., 2010; McClean et al., 2011) and protects neurons against Aβ-induced cytotoxicity and apoptosis (Perry et al., 2002; Sharma et al., 2013); over expressing of the GLP-1R in the hippocampus improved learning and memory (During et al., 2003), while knocking out the GLP-1R impaired hippocampal long-term potentiation (LTP) and spatial learning and memory in mice (Abbas et al., 2009). Unfortunately, the natural GLP-1 peptide is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPPIV). The half-life of GLP-1 in blood plasma is only several minutes (Deacon et al., 1995), which seriously limits the application of GLP-1 in clinical practice.

It is reported that a new GLP-1 analog, Lixisenatide, has been developed recently, which is resistant to DPP-IV cleavage and is effective for the treatment of T2DM (Werner et al., 2010; Horowitz et al., 2013). However, the neuroprotective effects and the mechanisms of lixisenatide in the brain remain to be clarified. Therefore, it is of interest to investigate whether the Aβ-induced dysfunction in the brain can be effectively alleviated by lixisenatide. In this study, we tested the effects of lixisenatide on the Aβ25–35-induced deficits in spatial learning and memory of rats using classical Morris water maze (MWM), and investigated its probable electrophysiological and molecular mechanisms by recording *in vivo* hippocampal LTP and measuring LTP induction-related glycogen synthase kinase 3β (GSK3β) expression and activation in the brain.

EXPERIMENTAL PROCEDURE

Animals and drugs

Adult male Sprague–Dawley (SD) rats (200–230 g) used in the present study were provided by the Animal Research Center of the Shanxi Medical University. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive (86/609/EEC) and the Guidelines laid down by the NIH in the US. 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. The rats in the control group received only saline.

Intrahippocampal injection

All rats were randomly divided into four groups: vehicle + saline, vehicle + Aβ25–35, lixisenatide + saline and lixisenatide + Aβ25–35. Intrahippocampal injection was performed as previously described (Ryu and McLarnon, 2006, 2008). 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 twice injected into the bilateral 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). Lixisenatide (2 μl) or vehicle (2 μl) was firstly injected into the bilateral hippocampi. 15 min later, Aβ25–35 (2 μl) or saline (2 μl) was applied. To make sure the drug/vehicle was fully dispersed into the hippocampus, a 5 min-retention of the injection syringe in the brain was given after every injection. Two weeks later, MWM test, *in vivo* hippocampal LTP recording, and western blot assay were performed.

The MWM task

Rats were tested in a spatial reference memory version of the water maze as described previously (Morris, 1984; Prediger et al., 2007; Terry, 2009). The water maze was mainly composed of a pool for swimming and a platform for escape. The pool, 150 cm in diameter and 50 cm in height, was filled with tap water at 23 ± 2 °C. The platform, 14 cm in diameter and 29 cm in height, was placed in the center of a fixed quadrant and was hidden 1 cm under the water surface. Some cues were arranged on the walls of the maze. A video camera located above the pool was used to track the movement of rats.

Hidden platform acquisition phase. The acquisition phase consisted of 5 training days (1–5d), with four trials each rat per day. A rat was placed in the water facing the pool wall and was allowed to swim freely to the escape platform. If the rat could not reach the platform within 120 s, it was guided to the platform and permitted to stay there for 30 s before being returned to its home cage. Rats were kept dry in a plastic holding cage filled with paper towels between trials. The escape latency of rats was analyzed by a behavior software system (Ethovision 3.0, Noldus Information Technology, Wageningen, the Netherlands).

Probe trial. On the second day after finishing the acquisition task, a probe trial was performed to assess the spatial memory of rats. In the experiment, the platform was removed from the pool, and rats were allowed to swim freely for 120 s. Spatial acuity was calculated by the time spent in the exact area where the escape platform was located in training days 1–5. The swimming distances of rats were also recorded.

In vivo hippocampal LTP recording

In view of the close relationship between hippocampal LTP and MWM performance (Bliss and Collingridge, 1993), an *in vivo* electrophysiological recording of LTP in hippocampal CA1 region of rats was performed after

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behavioral study. Rats were anesthetized with urethane (ethyl carbamate, Sigma–Aldrich, UK, 1.5 g/kg, i.p.) for surgery. The body temperature of rats was monitored throughout the experiment, and a heating pad was used to control the body temperature at 37 ± 0.5 °C. A small hole was drilled on the right side of the skull for inserting stimulating and recording electrodes. A pair of parallel stimulating/recording electrodes was inserted in the hippocampus under the guidance of a stereotaxic apparatus (Narishige, Japan). The tip of stimulating electrode (Sequim, WA, USA) was located at the Schaffer collateral/commissural pathway (AP: −4.2 mm; ML: +3.8 mm) and the tip of the recording electrode was positioned at the stratum radiatum in the CA1 region (AP: −3.4 mm; ML: +2.5 mm). Field excitatory postsynaptic potentials (fEPSPs) were recorded of the right hippocampal hemisphere in response to baseline fEPSPs and elicited from the stratum radiatum in the CA1 region by stimulating the Schaffer collateral/commissural pathway at an interval of 30 s. LTP was induced by one set of high-frequency stimulation (HFS), which included three trains of 20 pulses (200 Hz) and 30 s of intertrain intervals. The fEPSPs were recorded for at least one hour after HFS. The averaged value of fEPSPs amplitude during 30 min of baseline recording was taken as 100%, and all recorded fEPSPs were normalized to the baseline value. A ⩾30% increase of fEPSP was considered as a successful LTP. All evoked fEPSPs were recorded by a signal processing system (Chengdu Instruments Ltd., PR China), which triggered an electronic stimulator (SEN-3301, Japan) to generate constant current pulses through a stimulus isolation unit (ss-102J, Japan). All events were stored on a computer for further off-line analysis. The paired-pulse facilitation (PPF) in the CA1 region was also examined, in which two paired test stimuli with an interval of 50 ms were given to induce two paired fEPSPs. The change in PPF was expressed as the ratio of the second fEPSP amplitude over the first one, which is associated with the change in neurotransmitter release from presynaptic terminals.

Western blot assay

After the LTP experiment, the hippocampi of rats were dissected. Protein of these tissues was extracted (Tissue Protein Extraction Reagent by Boster, Inc.) and supplemented with complete protease inhibitor (Boster, Inc). The protein concentration was measured using a bicinchoninic acid protein assay kit after removing debris by low-speed centrifugation. 30-μg sample protein was separated on 12% SDS–polyacrylamide gels. After electrophoresis, protein was transferred onto PVDF membrane and nonspecific binding was blocked with 5% BSA in Tris-buffered saline containing 0.05% Tween-20 (TBST). The membrane was incubated with the primary antibody overnight at 4 °C, followed by the secondary antibody for 2 h. The following primary antibodies (all from Abcam, Inc.) were used: anti-GSK3β antibody (dilution 1:1000), anti-pGSK3β (S9) antibody (dilution 1:700) and anti-pGSK3β (Y216) antibody (dilution 1:700). The secondary antibody was anti-rabbit IgGHRP (dilution 1:100,000, ZSGB-BIO, Inc.). The membrane was rinsed with TBST and the immunocomplex was visualized by using an enhanced chemiluminescence detection kit (Beyotime, Inc.). The signals of the membrane were scanned with the FluorChem Scanner and quantified with the Alpha View SA software.

Data analysis

All values in the experiments were expressed as means ± standard errors (SEM). The SPSS 13.0 and SigmaPlot 11.0 statistical packages were used for statistical analyses. The data from LTP recording and western blotting were examined by a two-way repeated measures analysis of variance (ANOVA). For the data from MWM, the escape latency was analyzed using a three-way ANOVA and other data were analyzed using a two-way ANOVA. The statistical significance level was defined as $p < 0.05$.

RESULTS

Lixisenatide treatment prevented Aβ25–35 induced impairments in spatial learning and memory

As shown in the Fig. 1A, the escape latency in the hidden platform acquisition phase of the MWM test decreased with the increase of the training day ($F_{(4,144)} = 82.954$; $p < 0.001$). There was a significant main effect of intrahippocampal injection (Aβ25–35 vs. saline) ($F_{(1,36)} = 35.052$; $p < 0.001$) and drug treatment (lixisenatide vs. vehicle) ($F_{(1,36)} = 13.832$; $p < 0.001$) on the escape latency and a significant interaction between intrahippocampal Aβ25–35 injection and lixisenatide treatment ($F_{(1,36)} = 36.066$; $p < 0.001$). The increased escape latency ($p < 0.001$) on the 2, 3, 4 and 5 training days in vehicle + Aβ25–35 group ($n = 10$) indicated that Aβ25–35-injected rats learned more slowly in finding the hidden platform than the saline-injected rats. Compared to vehicle + Aβ25–35 group, the rats in lixisenatide + Aβ25–35 group ($n = 10$) had a decreased escape latency ($p < 0.001$), indicating that lixisenatide treatment could prevent Aβ25–35-induced impairment in spatial learning, although lixisenatide alone (lixisenatide + saline, $n = 10$) did not affect the learning ability of rats ($p > 0.05$).

In order to assess the spatial memory of rats, the probe trial was performed on the sixth day in the MWM task. As shown in the Fig. 1B, a two-way ANOVA showed that Aβ25–35 injection and lixisenatide treatment had significant main effects on the swimming time of rats in the target quadrant (Aβ25–35 injection: $F_{(1,36)} = 64.955$, $p < 0.001$; lixisenatide treatment: $F_{(1,36)} = 26.818$, $p < 0.001$; Aβ25–35 injection by lixisenatide treatment interaction: $F_{(1,36)} = 38.739$, $p < 0.001$). Tukey's *post hoc* test showed that Aβ25–35 injection significantly decreased the swimming time of rats in the target quadrant ($n = 10$, $p < 0.001$), while the decrease was effectively reversed by lixisenatide treatment ($n = 10$, $p < 0.001$). The representative

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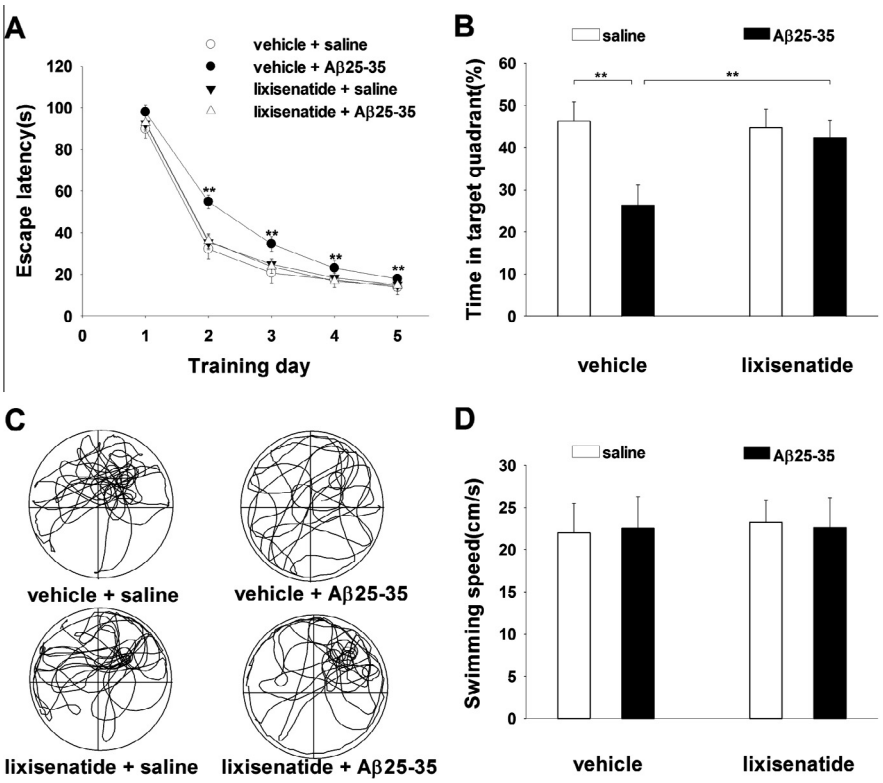

Fig. 1. Lixisenatide treatment reversed Aβ25–35-induced impairments in spatial learning and memory of rats. (A) Plots demonstrating the effects of Aβ25–35 and lixisenatide on the escape latency of rats during 5 consecutive days of the hidden platform test. A significant increase in the escape latency can be found on the 2–5 training days in vehicle + Aβ25–35 group compared to the control ($n = 10$, $p < 0.001$), while lixisenatide treatment reversed the increase in lixisenatide + Aβ25–35 group ($n = 10$, $p < 0.001$). Error bars indicate SEM. $^{**}p < 0.001$. (B) Histograms showing the average swimming time of rats in the target quadrant, with a significant decrease in the time in vehicle + Aβ25–35 rats compared to the control group ($n = 10$, $p < 0.001$), and a significant reversal in lixisenatide + Aβ25–35 group compared to the Aβ25–35 alone group ($n = 10$, $p < 0.001$). Error bars indicate SEM. $^{**}p < 0.001$. (C) Representative swimming traces of rats during the probe test. (D) Histograms showing the average swimming speeds of rats in all groups ($n = 10$, $p > 0.05$).

swimming traces of rats during the probe trial were demonstrated in the Fig. 1C.

To exclude the possibility that the change in learning and memory of rats may be caused by the impairment of its motor ability, we compared the average swimming speeds of rats in all groups (see Fig. 1D) and found no significant difference between groups ($n = 10$, $p > 0.05$).

These results from the MWM test above indicated that the Aβ25–35-induced impairments in spatial learning and memory could be effectively prevented by the lixisenatide treatment.

Lixisenatide protected against Aβ25–35-induced suppression of hippocampal LTP

In view of the close relationship between spatial cognition and hippocampal synaptic plasticity, we further observed the effects of Aβ25–35 and lixisenatide on the hippocampal LTP. Firstly, the PPF in the hippocampal CA1 region was examined to clarify whether the presynaptic mechanism was involved in the effects of Aβ25–35 and lixisenatide on the hippocampal synaptic plasticity. After paired pulses were applied to the Schaffer collaterals, the PPF in CA1 stratum radiatum always appeared with larger amplitude of second fEPSP (Fig. 2A). Two-way ANOVA showed that Aβ25–35 injection and lixisenatide treatment had no effects on the PPF ($n = 6$, $P > 0.05$), suggesting that neither Aβ25–35 nor lixisenatide affected presynaptic neurotransmitter release.

Next, HFS (20 pulses at 200 Hz repeated three times at an interval of 30 s) was applied to induce hippocampal LTP in the CA1 region (Fig. 2B, C). Two-way ANOVA showed that Aβ25–35 injection and lixisenatide treatment had significant main effects on the magnitude of LTP (Aβ25–35 injection: $F_{(1,20)} = 22.303$, $p < 0.001$; lixisenatide treatment: $F_{(1,20)} = 25.586$, $p < 0.001$; Aβ25–35 injection by lixisenatide treatment interaction: $F_{(1,20)} = 30.966$, $p < 0.001$). Tukey's *post hoc* test showed that Aβ25–35 injection produced a significant decrease in the magnitude of LTP in vehicle-treated rats ($n = 6$, $p < 0.001$), and this decrease was reversed by lixisenatide treatment ($n = 6$, $p < 0.001$). These results suggested that lixisenatide treatment could protect against Aβ25–35-induced impairment in hippocampal LTP.

Lixisenatide treatment suppressed Aβ25–35-induced activation of GSK3β in the hippocampus

The neuroprotection of lixisenatide might be involved in the PI3K-Akt-GSK3β pathway (Gao et al., 2012). In order to clarify the possible mechanism by which lixisenatide prevented Aβ25–35-induced deficits in

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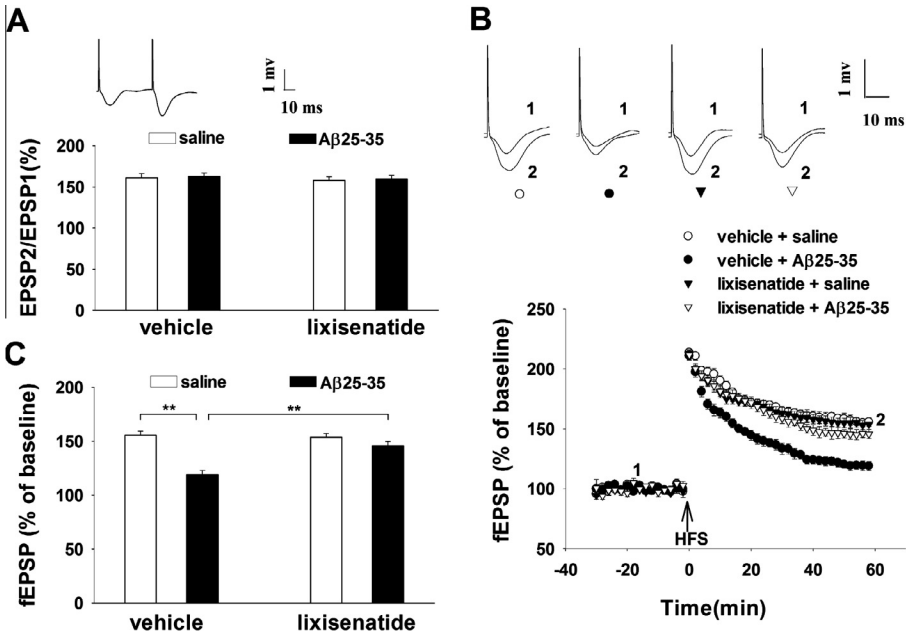

Fig. 2. Lixisenatide treatment prevented Aβ25–35-induced suppression of hippocampal LTP. (A) Neither Aβ25–35 nor lixisenatide affected the PPF (EPSP2/EPSP1) in the hippocampal CA1 region ($n = 6$, $p > 0.05$). Inset, a representative paired pulse-induced fEPSP trace. (B) Time course of fEPSPs recorded in the four groups, and typical fEPSP traces from the four groups at the time points. Bars = 1 mV and 10 ms. (C) Histograms showing the average fEPSPs in the four groups at the last 10 min. Aβ25–35 injection produced a significant decrease in the magnitude of LTP in vehicle-treated rats ($n = 6$, $p < 0.001$), but this decrease was prevented by lixisenatide treatment ($n = 6$, $p < 0.001$). Error bars indicate SEM. $^{**}p < 0.001$.

spatial cognition and LTP, we examined the levels of GSK3β and its two phosphorylation forms: pGSK3β (S9) and pGSK3β (Y216) in the hippocampus. As shown in the figure 3A and 3B, Aβ25–35 injection and lixisenatide treatment had no effects on the level of GSK3β ($n = 6$, $p > 0.05$). But, a two-way ANOVA showed that Aβ25–35 injection and lixisenatide treatment had significant main effects on the level of pGSK3β (S9) (Aβ25–35 injection: $F_{(1,20)} = 6.809$, $p < 0.05$; lixisenatide treatment: $F_{(1,20)} = 5.544$, $p < 0.05$; Aβ25–35 injection by lixisenatide treatment interaction: $F_{(1,20)} = 5.076$, $p < 0.05$). Tukey's *post hoc* test showed that Aβ25–35 injection produced a significant decrease in the level of pGSK3β (S9) in vehicle-treated rats ($n = 6$, $p < 0.05$), and this decrease was reversed by lixisenatide treatment ($n = 6$, $p < 0.05$, see Fig. 3A, C). Two-way ANOVA showed that Aβ25–35 injection and lixisenatide treatment had significant main effects on the level of pGSK3β (Y216) (Aβ25–35 injection: $F_{(1,20)} = 14.585$, $p < 0.05$; lixisenatide treatment: $F_{(1,20)} = 7.692$, $p < 0.05$; Aβ25–35 injection by lixisenatide treatment interaction: $F_{(1,20)} = 8.387$, $p < 0.05$). Aβ25–35 injection significantly increased the level of pGSK3β (Y216) in vehicle-treated rats ($n = 6$, $p < 0.05$), while the increase was also reversed by lixisenatide treatment ($n = 6$, $p < 0.05$, see Fig. 3A, D). These results indicated that Aβ25–35-induced impairments in spatial memory and synaptic plasticity may be involved in the activation of GSK3β in the hippocampus, while the lixisenatide treatment-induced inhibition of GSK3β might contribute to the neuroprotection of lixisenatide.

DISCUSSION

Aβ production and aggregation in the brain is thought to be one of the factors responsible for the cognitive deficits in AD. In our previous experiments, we have reported that different Aβ fragments, including Aβ1–40, Aβ25–35 and even a shorter fragment Aβ31–35, could induce apoptosis in cultured cortical neurons (Yan et al., 1999), enhance intracellular Ca^{2+} loading by forming new cation selective channels (Qi and Qiao, 2001a), suppress potassium (Ik) channels and large conductance Ca^{2+}-activated potassium (BK) channels in isolated hippocampal neurons (Qi and Qiao, 2001b; Qi et al., 2004) and also impair *in vivo* hippocampal synaptic plasticity and spatial memory in rats (Ye et al., 2004a,b; Zhang et al., 2006, 2009; Wang et al., 2010; Li et al., 2011). The present study focused on the neuroprotective properties of the GLP-1 analog, lixisenatide.

GLP-1 is an endogenous incretin hormone (Lovshin and Drucker, 2009), released by L cells of the distal intestinal mucosa (Impey et al., 1996). Because GLP-1 does not affect blood glucose levels in normoglycemic people (Vella et al., 2002; Gallwitz, 2006), and both GLP-1 and GLP-1R are expressed in the brain (During et al., 2003; Hamilton and Holscher, 2009), and GLP-1 possesses neurotrophic properties (Perry et al., 2002), GLP-1 could be beneficially applied to the patients with neurodegenerative disease, such as AD, whether they have diabetes or not. Indeed, it has been reported that exenatide, a GLP-1 analog with a 60–90 min half-life

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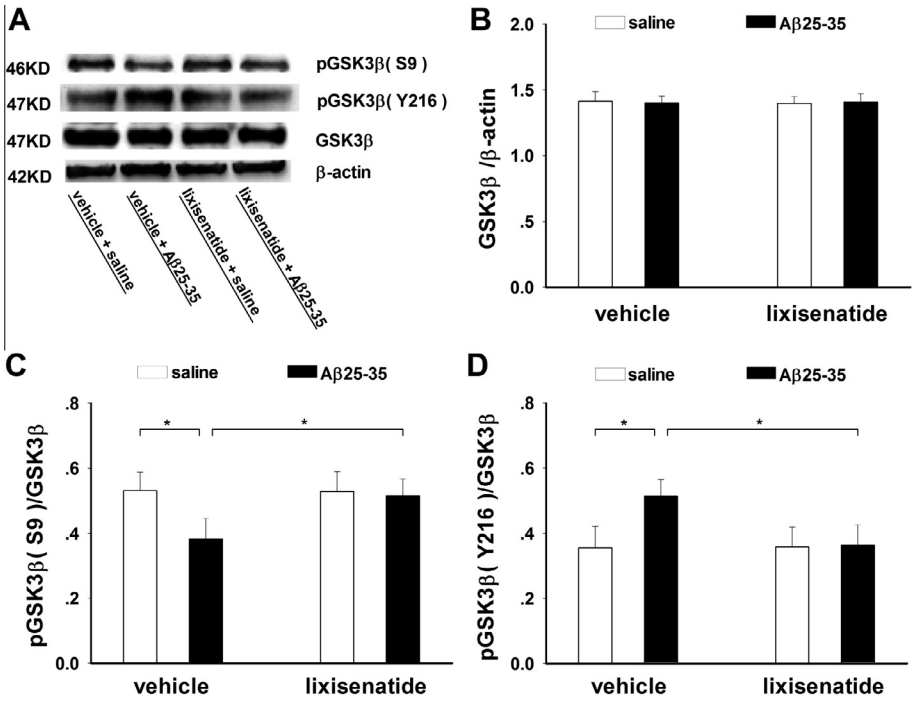

Fig. 3. Lixisenatide treatment inhibited GSK3β activation induced by Aβ25–35. (A) The expression of pGSK3β (S9), pGSK3β (Y216) and GSK3β in the hippocampus. (B) Aβ25–35 injection and lixisenatide treatment had no effects on the level of GSK3β ($n = 6$, $p > 0.05$). (C) Aβ25–35 injection produced a significant decrease in the level of pGSK3β (S9) in vehicle-treated rats ($n = 6$, $p < 0.05$), this decrease was reversed by lixisenatide treatment ($n = 6$, $p < 0.05$). (D) Aβ25–35 injection produced a significant increase in the level of pGSK3β (Y216) in vehicle-treated rats ($n = 6$, $p < 0.05$), which was inhibited by lixisenatide treatment ($n = 6$, $p < 0.05$). Error bars indicate SEM. $^{*}p < 0.05$.

(Timmers et al., 2009), protected cultured hippocampal neurons against death induced by Aβ (Perry et al., 2003), reduced endogenous levels of Aβ in transgenic AD mice (Li et al., 2010) and enhanced neuronal progenitor proliferation in the brain of diabetic mice (Hamilton et al., 2011). Most recently, our study also showed that liraglutide and Val^{8}-GLP-1(7–36), two long-lasting GLP-1 analogs, effectively and dose-dependently protected against Aβ25–35- or Aβ1–40-induced impairments in spatial memory and LTP (Wang et al., 2010). In APP/PS1 mice, liraglutide prevented memory impairments in object recognition and water maze tasks, and prevented synapse loss and deterioration of synaptic plasticity in the hippocampus; after treatment of liraglutide, overall β-amyloid plaque count in the cortex and dense-core plaque numbers were reduced by 40–50%, while the numbers of young neurons in the dentate gyrus were increased (McClean et al., 2011).

Another novel long-lasting GLP-1 analog, lixisenatide, with profound resistance to DPPIV, higher permeability across BBB and greater biological activity than liraglutide (Hunter and Hölscher, 2012) was used in the present study. We first confirmed that bilateral intrahippocampal injection of Aβ25–35 resulted in a significant impairment of spatial learning and memory in rats, while treatment with lixisenatide effectively prevented the Aβ-induced cognitive behavior impairment. Then, the electrophysiological effects of lixisenatide on synaptic transmission and plasticity were investigated. We found that lixisenatide alone did not affect the basal synaptic transmission in the hippocampal CA1 region, but significantly prevented Aβ25–35-induced suppression of LTP, which may explain the behavioral improvement seen in the MWM test. Considering the long-lasting beneficial effects of lixisenatide in neuroprotection and anti-diabetes, we suggest that lixisenatide might be of great significance in preventing or reducing the development of AD.

One of the possible mechanisms by which lixisenatide prevents Aβ-induced deficits in spatial cognition and LTP may be the activation of the PI3K-Akt pathway (Chang et al., 2013), which results in the inhibition of GSK3β activity (Gao et al., 2012). GSK3 is a multifunctional serine/threonine (ser/thr) kinase that is originally identified as a regulator of glycogen metabolism (Embi et al., 1980). There are two highly conserved isoforms of GSK3: GSK3α and GSK3β. Only GSK3β, however, is highly enriched in the brain (Woodgett, 1990), where it has been implicated in several CNS dysfunctions, such as AD (Anderton, 1999; Alvarez et al., 2002; Eldar-Finkelman, 2002; Bhat et al., 2004). GSK3β is an unusual enzyme in that it has high basal activity, which is primarily determined by the phosphorylation status of ser9 and Y216. pGSK3β (S9) induces an inhibition of GSK3β activity, while pGSK3β (Y216) results in the activation of GSK3β (Zhou et al., 2009). In the present study, Aβ25–35 injection induced a decrease in the pGSK3β (S9) level and an increase in the pGSK3β (Y216) level, indicating the activation of GSK3β. It has been reported that the overexpression or overactivation of GSK3β can induce a series of pathological changes, most of which are hallmarks of AD and T2DM. For example, activation of GSK3β promoted the aggregation of Aβ (Gao et al., 2012); overexpression of GSK3β

impaired spatial learning in transgenic mice (Hernandez et al., 2002). On the contrary, specific inhibition of GSK3β, but not GSK3α, markedly reduced Aβ deposition and neuritic plaque formation, and rescued memory deficits in double transgenic AD mouse models (Ly et al., 2013); following LTP induction, GSK3β activity was also inhibited in rat hippocampal slices (Peineau et al., 2007). In the present study, we found that lixisenatide injection not only reversed the Aβ-induced activation of GSK3β in the brain, but also rescued spatial learning and memory deficits and hippocampal LTP suppression of rats. These findings strongly suggest that the activation of PI3K-Akt pathway and inhibition of GSK-3β level might be important for the neuroprotective function of GLP-1 analogs, and lixisenatide may be a novel and promising candidate to protect learning and memory in neurodegenerative diseases such as AD.

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