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Exendin-4, a glucagon-like peptide 1 receptor agonist, protects against amyloid-β peptide-induced impairment of spatial learning and memory in rats

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HIGHLIGHTS

- Aβ1–42 resulted in a significant decline of spatial learning and memory of rats.
- Exendin-4 protected against the Aβ1–42-induced impairment of spatial memory.
- Exendin-4 decreased the level of Bax and caspase-3 in Aβ1–42-induced rats.
- Exendin-4 increased the level of Bcl2 in Aβ1–42-induced Alzheimer-like symptoms rat.

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ABSTRACT

Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) share specific molecular mechanisms, and agents with proven efficacy in one may be useful against the other. The glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 has similar properties to GLP-1 and is currently in clinical use for T2DM treatment. Thus, this study was designed to characterize the effects of exendin-4 on the impairment of learning and memory induced by amyloid protein (Aβ) and its probable molecular underlying mechanisms. The results showed that (1) intracerebroventricular (*i.c.v.*) injection of Aβ1–42 resulted in a significant decline of spatial learning and memory of rats in water maze tests; (2) pretreatment with exendin-4 effectively and dose-dependently protected against the Aβ1–42-induced impairment of spatial learning and memory; (3) exendin-4 treatment significantly decreased the expression of Bax and cleaved caspase-3 and increased the expression of Bcl2 in Aβ1–42-induced Alzheimer's rats. The vision and swimming speed of the rats among all groups in the visible platform tests did not show any difference. These findings indicate that systemic pretreatment with exendin-4 can effectively prevent the behavioral impairment induced by neurotoxic Aβ1–42, and the underlying protective mechanism of exendin-4 may be involved in the Bcl2, Bax and caspase-3 pathways. Thus, the application of exendin-4 or the activation of its signaling pathways may be a promising strategy to ameliorate the degenerative processes observed in AD.

1. Introduction

Alzheimer's disease (AD), which affects more than 40 million people worldwide, is a chronic, age-related and irreversible neurodegenerative disorder characterized by progressive cognitive dysfunction and the inability to perform complex daily activities [3]. One of the hallmarks of AD is the presence of a high density of senile plaques composed of amyloid protein (Aβ) in the hippocampus and cerebral cortex [67]. Aβ is a proteolytic cleavage product of the sequential cleavages of amyloid precursor proteins (APPs) by the β-site of the APP-cleaving enzyme (BACE), followed by γ-secretase, to generate a family of Aβ peptides of 40 and 42 amino acids that aggregate to form oligomers capable of causing synaptic dysfunction and neuronal degeneration [25,54]. The neurotoxicity of Aβ peptides, including different Aβ fragments such as Aβ1–42, Aβ1–40, Aβ25–35 and Aβ31–35, have been widely reported *in vivo* and *in vitro* [26,46,60]. Consequently, it is important to clear the aggregated Aβ or block the Aβ toxicity in the brain for the prevention and clinical treatment of AD.

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Interestingly, it has recently been reported that AD and type 2 diabetes mellitus (T2DM), another age-related degenerative disorder in the elderly, share several common clinical, pathological and biochemical characteristics [21,32,45]. For example, some risk factors implicated in the development of T2DM, such as genetic predisposition, oxidative stress, age, diet, obesity, and physical inactivity, appear to be involved in AD, implicating T2DM as a risk factor for the development of AD [21,31]. Moreover, AD is associated with the peripheral and central insulin abnormalities, including the desensitization of insulin receptors in the brain [11,32,41]. Therefore, an effective treatment strategy against T2DM could also have potential value in AD.

Emerging evidence indicates that glucagon-like peptide 1 (GLP-1), an incretin hormone, can cross the blood–brain barrier (BBB) on its receptor (GLP-1R) and facilitate insulin signaling [19,44]. GLP-1R is a member of the secretin receptor family of the G-protein-coupled receptor and is expressed in the brain. It has been reported that GLP-1 binds to its receptor and triggers cellular signaling pathways, which has shown a protective effect on neuronal cells against apoptosis [49,51]. However, due to the shorter half-life of GLP-1 by the rapid inactivation of the enzyme dipeptidyl peptidase-4 [45], its potential therapeutic application is greatly limited. Exendin-4 is a glucagon-like peptide-1 (GLP-1) receptor agonist with a longer plasma half-life than GLP-1 and has been developed as a first-in-class diabetes therapy. It has been reported that exendin-4 could readily cross the blood-brain barrier [34]. In the brain, GLP-1R is expressed widely [22], and its activation produces multiple biological responses [43,48]. Some studies showed that exendin-4 could reduce endogenous levels of Aβ in AD transgenic mice [42] and enhance neuronal progenitor proliferation in the brains of diabetic mice [23]. Therefore, it is of interest to further investigate whether the Aβ-induced impairment in the central nervous system can be effectively alleviated by exendin-4. In this study, we first observed the effects of intracerebroventricular (*i.c.v.*) injection of exendin-4 on Aβ1–42-induced impairment of spatial learning and memory of rats in the Morris water maze (MWM) test. We also revealed the possible molecular mechanism of exendin-4 against the Aβ1–42-induced impairments in the spatial cognition of rats.

2. Materials and methods

2.1. Animal and surgical procedure

With approval of the Shaanxi Animal Research Ethics Committee, adult male Sprague–Dawley (SD) rats (230–250 g) supplied by the Research Animal Center of Xi'an Jiaotong University were divided randomly into six groups: control, Aβ1–42, exendin-4 0.02 nmol, 0.2 nmol, 2 nmol and exendin-4 plus Aβ1–42. The surgical preparation procedure was similar to our previous report (Zhang et al., 2006). Briefly, SD rats were anesthetized (chloral hydrate, 0.3 g/kg, i.p.) and placed in a stereotaxic apparatus (Narishige, Tokyo, Japan), and a hole was drilled in the right side of the skull (0.8 mm posterior to bregma and 1.3 mm lateral to the midline) to introduce a guide cannula for *i.c.v.* injection of drugs/vehicle.

2.2. Chemicals and drug application

Both exendin-4 and Aβ1–42 were purchased from Sigma (St. Louis, MO, USA) and dissolved in normal saline, and Aβ1–42 was "aged" before surgery by incubation at 37 °C for 4 days [60,64]. Aβ1–42 (5 nmol per rat), exendin-4 (0.02, 0.2, 2 nmol per rat) and vehicle solutions were injected *i.c.v.* using a Hamilton microsyringe over 10 min in a total volume of 5 μl. All rats in the co-application group were pretreated with exendin-4 15 min before the Aβ1–42 injection. Two weeks later, the Morris water maze (MWM) test was performed.

2.3. Morris water maze (MWM) test

Rats' spatial learning and memory were evaluated using a classic MWM, a device commonly used for studying cognitive deficits in APP transgenic mice [50]. The Morris water maze consisted of a large circular pool (diameter, 150 cm; height, 50 cm) and a small circular platform (diameter, 14 cm; height, 29 cm). The inner surface of the pool and the platform were painted black, and the platform was placed at the midpoint of one quadrant and submerged approximately 1.0 cm below the surface of the water at a temperature of 23 ± 2 °C. Prominent visual cues were provided in the testing area of the room. The swimming path of the rats during each trial was monitored *via* a camera mounted overhead above the center of the pool and connected to a video tracking system (EthoVision, VA, USA). Tracking was achieved using a white rat on a black background. The hidden platform test was performed 2 weeks after drug injection. The tests were performed four times per day for 5 consecutive days. In each trial, the rats were released into the water facing the pool wall in one of the four quadrants (Zones 1, 2, 3, and 4) designated by computer software and were allowed to swim freely to the escape platform. After climbing onto the platform, the rats were allowed to rest on it for 20 s. In probe trials on the sixth day, the platform was removed from the pool, and rats were given 120 s to swim in the pool to measure the spatial bias. The percentages of total time and distance spent in the different quadrants were measured. After the probe trials, motor skill ability and visual acuity were also tested using a visible platform located opposite the original position within the pool. The test comprised four trials with the visible platform elevated above the water level (approximately 2 cm), and the latency and swimming speed at which the animal arrived at the platform were recorded. These data were recorded and analyzed by a behavioral software system (EthoVision 3.0, Noldus Information Technology, Wageningen, the Netherlands).

2.4. Real-time PCR

After finishing the MWM test, the rats were rapidly decapitated, and the bilateral hippocampi were collected. Total RNA from the hippocampus was isolated using Trizol reagent (Life Technologies, Grand Island, NY, USA), and the concentration of RNA was determined by NanoDrop 2000 Spectrophotometer (THERMO, USA). Prior to RT-PCR, RNA was initially treated with DNase I (Ambion Inc., Austin, TX) to degrade genomic DNA. Nearly 2 μg of total RNA was reverse transcribed with the RevertAid First Strand cDNA Synthesis Kit (THERMO, USA). Quantitative real time qPCR was processed using the DNA Engine Opticon 2 system (Bio-Rad, USA) with the protocol of denaturation at 94 °C for 5 min, followed by 34 cycles of denaturation at 94 °C for 1 min and annealing/extension at 52 °C or 30 s. Data analysis was performed by Mx3005P QPCR Systems (STRATAGENE, USA). The mRNA content was determined as a relative value normalized to GAPDH mRNA level. The sequences of the primers used were as follows: For GAPDH, forward: 5′-AAGAAGGTGGTGAAGCAGGC-3′ and reverse: 5′-TCCACCACCCTGTTGCTGTA-3′; for Bcl2, forward: 5′-ACTGAGTACCTGAACCGGCATC-3′ and reverse: 5′-GGAGAAATCAAACAGAGGTCGC-3′; for Bax, forward: 5′-AGTGTCTCAGGCGAATTGGC-3′ and reverse: 5′-CACGGAAGAAGACCTCTCGG-3′. All experiments were repeated three times.

2.5. Western blot analysis

Proteins were extracted from the collected whole hippocampus of the rats (n = 6 per group). The protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, USA). Proteins (50 μg) were separated by SDS-PAGE and transferred to membranes. The protocol for western blotting was described previously [8]. The primary antibodies used were rabbit anti-cleaved caspase-3 (#9661, Cell Signal Tech) and mouse anti-actin (A5441, Sigma). The films were