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GPR40 receptor activation leads to CREB phosphorylation and improves cognitive performance in an Alzheimer's disease mouse model

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ABSTRACT

Alzheimer's disease (AD) is a very complex neurodegenerative disorder as neuronal loss is a prominent and initial feature of AD. This loss correlates with cognitive deficits more closely than amyloid load. GPR40 receptor belongs to the class of G-protein coupled receptors, is expressed in wide parts of the brain including the hippocampus which is involved in spatial learning and memory. Till now, there are few studies investigating the functional role of GPR40 in brain. In this study, we evaluated the functional role of GPR40 receptor in the A-beta AD mice model. Administration of $A\beta_{1-42}$ (410 pmol intracerebroventricularly (i.c.v.) once at the beginning of experiment significantly impaired cognitive performance (in step-through passive test), the ability of spatial learning and memory in (Morris water maze test), working memory, attention, anxiety in (Novel object recognition test), and spatial working and reference-memory in (Hole board discrimination test) compared with the control group. The results revealed that GPR40 receptor treatment groups significantly ameliorated model mice cognitive performance. All GPR40 receptor agonist GW9508, treatment groups enhanced the learning and memory ability in Step-through passive test, Morris water maze test, Hole board discrimination test, Novel object recognition test. Furthermore, we have observed that activation of GPR40 receptor provoked the phosphorylation of the cAMP response element binding protein (CREB) and significant increase in neurotropic factors including (brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrohin-4 (NT-4) in mouse hippocampal neurons and contribute to neurogensis. These results suggest that GPR40 is a suitable therapeutic candidate for neurogensis and neuroprotection in the treatment and prevention of AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the loss of memory and cognitive abilities (Tapia-Rojas, Aranguiz, Varela-Nallar, & Inestrosa, 2016). In 2006, 0.40% of the global population (range 0.17–0.89%; absolute number 26.6 million, range 11.4–59.4 million) were affected by AD, and that the occurrence rate would triple and the absolute number would quadruple by the end of 2050 (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007). Currently there is no cure for AD, available treatments only offer small symptomatic relief and remain palliative. As of 2012 more than 1000 clinical trials have been conducted to test various compounds in AD (Clinical Trials). Scientist are more interested to discover modern and new therapeutic options for the treatment of AD.

Neurogenesis is recognized worldwide and is one of the most encouraging and exciting research fields in neuroscience. The efforts of many scientists are focusing on the understanding of neurogensis and development of new approaches for the treatment of various degenerative disorders including Alzheimer and Parkinson's disease. The useful impact of neurogenesis on learning and memory propose its contribution in AD, because poor memory function can predict AD up to 15 years before diagnosis (Kawas et al., 2003), which continues to decline during the course of the disease. The correlation between hippocampal neurogenesis and learning is reasonable. Hippocampal adult neurogenesis is important for learning and memory (Neves, Cooke, & Bliss, 2008).

GPR40, also known as free fatty acid receptor 1 (FFAR1) is a member of G protein–coupled receptors (GPCR), and it contains seven hydrophobic regions, consistent with the transmembrane spanning helices. GPR40 was identified downstream of cd22 on human chromosomal locus 19q13.1 (Sawzdargo et al., 1997). In humans, GPR40 mRNA is the most abundantly expressed in the brain and consequently in the pancreas (Briscoe et al., 2003). GPR40 is present throughout the primate brain (Ma et al., 2007).

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GPR40 expression in the new-born neurons is in the dendrites, cytoplasm, and synaptic buttons, while its presence in the mature neurons are in the nucleus and perinuclear regions (Yamashima, 2008; Ma et al., 2008). GW9508 is a Potent and selective agonist for the free fatty acid receptor FFA1 (GPR40) (Briscoe et al., 2006) and GW1100 is selective antagonist of GPR40 receptor (Nakamoto et al., 2013).

CREB (cAMP response element-binding, protein) belongs to the family of leucine zipper transcription factors. It binds to certain DNA sequences called cAMP response elements (CRE) and regulate the transcription of various genes including BDNF, c-fos, neuropeptides (such as somatostatin, enkephalin, and corticotropin-releasing hormone) and tyrosine hydroxylase (Purves et al., 2008). CREB regulates cell proliferation, differentiation, and survival in the developing brain, and mediates such responses as neuronal plasticity, learning, and memory in the adult brain (Lonze & Ginty, 2002). Currently there is no consensus regarding the exact upstream regulator of CREB-signaling in adult-born neurons, however it is interesting to note that GPR40, is one of the GPCR that is extensively expressed in the hippocampal neurogenic niche of adult monkeys is capable of activating CREB-signaling and contribute to the neurogensis (Yamashima, 2012). Recently, Zamarbide et al., reported that GPR40 activation can leads to CREB and ERK Phosphorylation in primary cultures of neurons from the mouse CNS and in human neuroblastoma cells (Zamarbide et al., 2014). Neurotrophins are chemicals that help to stimulate and control neurogenesis, BDNF being one of the most active and most extensively distributed neurotrophin in the central nervous system (Pencea, Bingaman, Wiegand, & Luskin, 2001). CREB regulate the transcripation of BDNF gene (Purves et al., 2008). Various studies have reported altered levels of BDNF in the circulation, i.e. serum or plasma, of patients with Alzheimer's disease (AD), and low BDNF levels in the CSF as predictor of future cognitive decline in healthy older subjects. In the late onset of AD low level of plasma BDNF was detected and lowest level of BDNF was detected in the demented patients affected by diabetes (Passaro et al., 2014).

The aim of this study was to evaluate the effect of GPR40 activation on mice cognitive performance. A second aim was to confirm whether or not there is a direct link between GPR40 activation and CREB Phosphorylation using AD mice model. The following tests (Morris water maze, Passive-avoidance test (PA), Novel object recognition test NOR, and hole-board) of learning and memory were administered to measure cognitive functions known to be affected in AD followed by neurotropic factors determination through ELISA and western blot analysis for determination of CREB Phosphorylation in mice hippocampus. The results indicate significant improvement in the cognitive performance in GPR40 receptor agonist, GW9508 treated groups (4 μg/mouse, 10 μg/mouse, 25 μg/mouse). Furthermore, GPR40 receptor activation lead to CREB Phosphorylation and increase hippocampal neurotropic factors (BDNF, NGF, NT3, NT4) in AD mouse model.

2. Methods and materials

2.1. Animals and drug administration

Male ICR mice (18–22 g) were purchased from Animal Experimental Center of Yangzhou University (Yangzhou, China), approval number: SCXK (su) 2012-0004. Pellet feed was purchased from Animal Breeding Grounds of Nanjing Jiangning Qinglong Mountain (Nanjing, China). Animals were housed in a room that was automatically maintained at 21–25 °C and relative humidity (45–65%) with a controlled light–dark cycle. The mice were kept under free access to food and water. All procedures were conducted in accordance with the Guidelines on the Care and Use of Laboratory Animals (Chinese Council on Animal Research and the Guidelines of Animal Care). The study was approved by the Ethical Committee of China Pharmaceutical University.

After 2 weeks of acclimatization, mice were randomly divided into seven groups (each consisting of 12 mice): control group, model group $A\beta_{1-42}$, donepezil group ($A\beta_{1-42}$ + donepezil), low dosage, middle dosage and high dosage of GPR40 agonist GW9508 groups and GPR40 antagonist group (GW1100 + GW9508). Except for the control group, mice were administered with $A\beta_{1-42}$ at the dose of 410 pmol once at the beginning of experiment by intracerebroventricular (i.c.v.) injection, while those of control group were treated with the same volume normal saline. After 10 days, GW9508 was administrated by i.c.v injection with the following three doses, 4 μg/mouse, 10 μg/mouse, 25 μg/mouse once, to low-dosage, middle-dosage, and high-dosage GPR40 agonist groups respectively. GW1100 was administrated by (i.c.v) injection with the dose of 30 μg/mouse once followed by administration of GW9508 with the dose of 25 μg/mouse (i.c.v.) to the antagonist group. Donepezil was administrated intragastricaly with the dose of 1 mg/kg body weight once daily for 2 weeks to donepezil group. Same volume of normal saline was administrated to control group and model group.

2.2. Behavioral tests

The behavioral tests were performed in a silent, isolated room at the temperature of 22 ± 2 °C. The experimenter and the devices for data acquisition and analysis were located in an adjacent room. A video-camera viewing the experimental area was positioned on the vertical form at the center of the arena and connected to a personal computer. Mice movements were tracked and analyzed with dedicated software (Any-maze™, Stoelting Co., Chicago, USA).

2.3. Morris water maze

Spatial learning and memory ability was tested by Morris water maze. Methods for training of animals on the water maze task have previously been described (Foster, 2012; Foster, Defazio, & Bizon, 2012; Kumar & Foster, 2013; Kumar, Rani, Tchigranova, Lee, & Foster, 2012).The apparatus consisted of a black round pool (about 6 feet in diameter and 3 feet deep) filled with tap water, which maintained at 25 ± 1 °C. The pool was divided into four quadrants of equal area: I, II, III, and IV. An escape platform (10 cm in diameter) was placed 1 cm below the water surface at the midpoint of the IV quadrant (the target quadrant). Several visual cues helping mice to learn the location of the hidden platform were arrayed surrounding the maze. The experiment included two phases e.g. acquisition training session and the probe trail. The acquisition training session was consist of five days. On first and second day mice were trained in the presence platform (Visible platform training) while on day third, fourth and fifth mice were trained in the absence of platform (Hidden platform training). In the acquisition training session, each mouse was submitted to four trials successively per day. In each trial, the time a mouse spent from being put into the water to finding and climbing onto the hidden platform was recorded as escape latency. Mice were given a maximum of 90 s for finding the platform. If a mouse failed to locate the platform within 90 s, it was placed on the platform and stayed there for 10 s, and its escape latency was recorded as 90 s. On the sixth day, the platform was removed from the pool for the probe trail. The mice were released into the water on the opposite side of the target quadrant and allowed to swim freely for 90 s. The total number of time each mouse crossed the position where the escape platform was once placed (crossing number) and time it spent in the III quadrant (time in target quadrant %) was recorded.

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