Neuroscience 344 (2017) 39–47

RESVERATROL AMELIORATES SPATIAL LEARNING MEMORY IMPAIRMENT INDUCED BY $A\beta_{1-42}$ IN RATS

RUI WANG, YU ZHANG, JIANGUO LI AND CE ZHANG *

National Key Disciplines, Key Laboratory for Cellular Physiology of Ministry of Education, Department of Neurobiology, Shanxi Medical University, #56 Xin Jian South Road, Taiyuan 030001, Shanxi Province, PR China

Abstract—β-amyloid (Aβ) deposition is considered partially responsible for cognitive dysfunction in Alzheimer's disease (AD). Recently, resveratrol has been reported to play a potential role as a neuroprotective biofactor by modulating Aβ pathomechanisms, including through anti-neuronal apoptotic, anti-oxidative stress, and anti-neuroinflammatory effects. In addition, SIRT1 has been demonstrated to modulate learning and memory function by regulating the expression of cAMP response binding protein (CREB), which involves in modulating the expression of SIRT1. However, whether resveratrol can alleviate Aβ-induced cognitive dysfunction, whether SIRT1 expression and CREB phosphorylation in the hippocampus are affected by Aβ, and whether resveratrol influences these effects remain unknown. In the present study, we used a hippocampal injection model in rats to investigate the effects of resveratrol on $A\beta_{1-42}$-induced impairment of spatial learning, memory and synaptic plasticity as well as on alterations of SIRT1 expression and CREB phosphorylation. We found that resveratrol significantly reversed the water maze behavioral impairment and the attenuation of long-term potentiation (LTP) in area CA1 that were induced by hippocampal injection of $A\beta_{1-42}$. Interestingly, resveratrol also prevented the $A\beta_{1-42}$-induced reductions in SIRT1 expression and CREB phosphorylation in rat hippocampus. In conclusion, in rats, resveratrol protects neurons against $A\beta_{1-42}$-induced disruption of spatial learning, memory and hippocampal LTP. The mechanisms underlying the neuroprotective effects may involve rescue of SIRT1 expression and CREB phosphorylation. © 2016 Published by Elsevier Ltd on behalf of IBRO.

Key words: amyloid beta peptide, learning and memory, LTP, resveratrol, SIRT1, CREB.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia (Querfurth and LaFerla, 2010) and is characterized by extracellular senile plaques containing a β-amyloid peptide (Aβ) core, intracellular neurofibrillary tangles (NFTs), and the selective loss of neurons (Selkoe, 2001; Tanzi and Bertram, 2005). Aβ deposition is thought to play a pivotal role in a cascade of harmful events, including inflammatory responses, oxidative stress, synaptotoxins, neuronal injury and death (Klein et al., 2004; Lesne et al., 2006; Nunomura et al., 2006; Haass and Selkoe, 2007; Walsh and Selkoe, 2007). To date, treatments for AD remain at the level of epidemiological correlates for which the therapeutic mechanisms are unknown. Although a number of therapeutic interventions, including vitamin E (Farina et al., 2012; Dysken et al., 2014), statins (Feldman et al., 2010; Sano et al., 2011) and omega-3 fatty acids (Chew et al., 2015), have been applied, none proved effective when tested in the clinic.

Resveratrol (trans-3,4,5′-trihydroxystilbene), a potential activator of SIRT1, has been shown to have a broad range of beneficial effects in mammals, including anti-oxidative, anti-inflammatory, chemopreventive, and cardioprotective effects as well as neuroprotective effects against neurodegenerative diseases (Baur and Sinclair, 2006). Early studies showed that moderate consumption of red wine containing polyphenols could ameliorate clinical dementia in AD patients (Orgogozo et al., 1997) and attenuate amyloid neuropathology and AD-like cognitive impairment in animal models (Luo et al., 2002; Luchsinger et al., 2004; Wang et al., 2006, 2008).

In addition, studies have been reported that sirtuin 1 (SIRT1) is essential for synaptic plasticity and normal cognitive functions (Michan et al., 2010), in which SIRT1 modulates memory function through the miR-134-mediated post-transcriptional regulation of cAMP response binding protein (CREB) (Gao et al., 2010). Interestingly, levels of SIRT1 and p-CREB are significantly reduced in the brains of AD patients, and this reduction is closely associated with Aβ deposition in the cerebral cortex of AD patients (Yamamoto-Sasaki et al., 1999; Julien et al., 2009). Furthermore, a recent study demonstrated that resveratrol could increase SIRT1 mRNA expression in nucleus pulposus cells of degenerated human intervertebral disks (Wu et al., 2015). Although an increasing number of studies have focused on the beneficial effects of resveratrol against diverse Aβ neuropathological processes, no study has demonstrated

*Corresponding author.
E-mail addresses: wr_00866@sina.com (R. Wang), zhyucnm@163.com (Y. Zhang), lijg71@163.com (J. Li), cezh2002@yahoo.com (C. Zhang).

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; Aβ, β-amyloid peptide; BDNF, brain-derived neurotrophic factor; CREB, cAMP response binding protein; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LTP, long-term potentiation; MWM, Morris water maze; PPF, paired-pulse facilitation; Resv, resveratrol; SIRT1, silence mating type information regulation 2 homolog 1; TQ, target quadrant.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.051
0306-4522/

whether resveratrol can alleviate $A\beta_{1-42}$-induced cognitive and synaptic plasticity dysfunction in a rat model.

Accordingly, the present study was designed to test the following hypothesis: $A\beta_{1-42}$ injection impairs spatial learning and memory and synaptic plasticity by downregulating SIRT1 expression and CREB phosphorylation, while resveratrol can prevent these effects. To test this hypothesis, we used a rat hippocampal injection model and investigated spatial memory with the Morris water maze (MWM), synaptic plasticity with hippocampal long-term potentiation (LTP) *in vivo*, and hippocampal expression of SIRT1 and phosphorylated CREB with western blot.

EXPERIMENTAL PROCEDURES

Animals and drugs

The adult male Sprague–Dawley (SD) rats (weight range 220 to 240 g, $N = 138$) used in this study were purchased from the Research Animal Center of Shanxi Medical University. The animals were housed 3–4 per cage and maintained on a 12-h light:12-h dark cycle (lights on from 7:00 A.M.). They had free access to water and food. The protocols for animal handling and care were approved by the Animal Care and Use Committee of Shanxi Medical University.

$A\beta_{1-42}$ (A9810, Sigma-Aldrich, Beijing, China) was dissolved in saline (0.22 nmol/μl) (in 0.1% DMSO). Resveratrol (R5010, Sigma-Aldrich, Beijing, China) was dissolved in DMSO (0.22 nmol/μl) to a final concentration of 0.1%. To obtain aggregated $A\beta_{1-42}$, the peptide solution was incubated in a 37 °C water bath for 96 h. The resveratrol solution (0.1% in DMSO/saline) was freshly prepared immediately before each injection.

Resveratrol was injected directly into the hippocampus, and the spatial extent of diffusion was confirmed through the use of Pontamine sky blue dye (data not shown). At the injection point (−2.80 mm from bregma), dark blue was observed in the section, and in subsequent sections (−3.60 mm from bregma to −4.10 mm from bregma), the color of dye gradually faded. At −4.30 mm from bregma, the color was completely faded. Assuming the spatial field of drug diffusion is spherical, it appears to have a radius of approximately 1.3 mm (−2.80 mm from bregma to −4.10 mm from bregma). According to the formula for the volume of a sphere, the volume of the diffusion sphere may thus be close to 10 mm^3 for an injection volume of 10 μl. Consequently, 0.22 nmol resveratrol in this volume results in a concentration of 22 μM. Thus, the doses of 0.005 nmol, 0.0125 nmol, 0.025 nmol, 0.05 nmol, 0.22 nmol, and 0.44 nmol used in the hippocampal injection might exhibit effects equal to those of concentrations of 0.5 μM, 1.25 μM, 5 μM, 22 μM, and 44 μM, respectively.

MWM test

SD rats ($N = 114$) were randomly assigned into 11 groups ($n = 8$–18): vehicle ($n = 18$), $A\beta_{1-42}$ ($n = 17$), resveratrol (0.005 nmol, 0.0125 nmol, 0.025 nmol, 0.05 nmol, 0.22 nmol, or 0.44 nmol) + $A\beta_{1-42}$ ($n = 8$–9), and resveratrol (0.05 nmol, 0.22 nmol, or 0.44 nmol) + vehicle ($n = 9$).

After the administration of chloral hydrate (0.3 g/kg, i.p.) as an anesthetic, the animals were placed in stereotaxic apparatus (Narishige, Japan) for drug injection. Small burr holes were drilled to implant a stainless steel guide cannula. $A\beta_{1-42}$ (1 μl) or an identical volume of vehicle (1 μl) was injected into the hippocampus (0.2 μl/min) bilaterally [anterior/posterior (AP), −3.0 mm from the bregma; medial/lateral (ML), ±2.2 mm from the bregma; dorsal/ventral (DV), −3.1 mm from the dura]. After the injection, the syringe was held in place for another 2 min to ensure complete diffusion of the drugs. Using the same procedure, resveratrol was administered 15 min before the $A\beta_{1-42}$ hippocampal injection. The resveratrol solution (0.1% in DMSO/saline) was freshly prepared immediately before each injection. After surgery, the animals were allowed to recover for 8 days.

The DMS-2 MORRIS system (Institute of Materia, Chinese Academy of Medical Sciences, China) was used. A tank (60 cm in height; 150 cm in diameter) was filled with water (23 ± 2 °C) to a depth of 30 cm. A black platform (10 × 10 cm) was placed at the center of the target quadrant (TQ) and submerged in the water to a depth of approxiamtely1 cm. Animal movement was tracked with a video camera that was mounted overhead. The MWM test was performed in three phases. (1) The hidden platform test was performed for 5 days. The animals were placed in the pool facing the pool wall at water level (not dropped) and allowed to freely swim. If they did not reach the platform within 120 s, the animals were gently guided to it and placed in their home cage for 30 s before the next test. All animals were trained 4 times per day. (2) A probe trial was performed the day after the last hidden platform test (day six A.M.) to evaluate reference memory. After removing the platform, the animals were placed in a novel start position and given 120 s to swim freely while the percentage of time spent in the different quadrants was measured (Morris, 1984; Vorhees and Williams, 2006). (3) The visual platform test was conducted 6 hours after the probe trials. The platform was attached to a highly visible cover and elevated to approximately 1 cm above the water surface. The time needed to reach the platform and the swimming speed were recorded (Puzzo et al., 2009; Wang et al., 2010).

In vivo hippocampal LTP recording

To evaluate the electrophysiological effects of $A\beta_{1-42}$ and resveratrol *in vivo*, rats ($N = 24$) were randomly assigned to four groups ($n = 6$) and administered different treatments: vehicle, $A\beta_{1-42}$, resveratrol (0.22 nmol) and resveratrol (0.22 nmol) + $A\beta_{1-42}$.

After administration of an anesthetic (urethane 1.5 g/kg, i.p.), the animals were placed into a stereotaxic apparatus (Narishige, Japan). A small hole was drilled to implant a stainless-steel guide cannula for hippocampal drug injection (AP, −2.8 mm; ML, 2.0 mm; DV, −3.0 mm). Additionally, another hole was drilled