Resveratrol Reverses the Synaptic Plasticity Deficits in a Chronic Cerebral Hypoperfusion Rat Model

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Background: Dementia is the most prevalent neurological disease in aged people. Chronic cerebral hypoperfusion (CCH) is one of the causes of vascular dementia (VaD) and is also an etiological factor for Alzheimer's disease (AD). However, effective therapy for those two diseases is still missing. Resveratrol is a polyphenol produced by plants that have multiple biological functions, such as increased life span and delay in the onset of diseases associated with aging. It is known supplement with resveratrol could exert neuroprotection against multiple injury factors induced neuronal death and degeneration, as well as the cognitive decline of CCH rat model. *Methods:* The morris water maze was used to evaluate the learning and memory, electrophysiological recording was used to detect the synaptic plasticity, the Golgi staining was used to examine the change of dendritic spines, the western blot was used to detect the proteins levels. *Results:* We reported that resveratrol pretreatment effectively restore the synaptic plasticity in CCH rats both functional and structural. We also found that the PKA-CREB activation may be a major player in resveratrol-mediated neuroprotection in CCH model. *Conclusions:* Our data provide the mechanistic evidence for the neuroprotective effects of resveratrol in vascular dementia. **Key Words:** Dementia—resveratrol—synaptic plasticity—dendritic spine—PKA—CREB.

Introduction

Vascular dementia (VaD) is another most common type of dementia after Alzheimer's disease in aged people.[1] It is reported that about 20% of dementia cases are diagnosed as VaD around the world.[2] Permanent occlusion of bilateral carotid arteries had been wildly used for the VaD model because of chronic cerebral hypoperfusion (CCH).[3] Previous studies had reported that bilateral carotid arteries caused not only neuronal loss and neuronal inflammation in the cortex and hippocampus, but also impairments in learning and memory in rats.[4,5] It is known that synaptic plasticity is the molecular biological basis of learning and memory.[6] Long-term potentiation (LTP) was recognized as a synaptic model of memory and was widely used as the parameter to evaluate the synaptic strength both in vivo and in vitro.[7] Plenty of studies suggested that impaired synaptic plasticity plays an important role in the learning/memory deficits of VaD animal models.[8] Thus, preservation of the ability of synaptic plasticity is a potential therapeutic strategy for the prevention of memory deficits in CCH.

Resveratrol (chemical name, 3,5,4′-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by some plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi.[9] The natural food sources of resveratrol include the skin of grapes, blueberries,

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raspberries, and mulberries.[10] It is reported that resveratrol has multiple beneficial activities similar to those associated with caloric restriction, such as increased life span and delay in the onset of diseases associated with aging.[11] In the nervous system, the protective effects of resveratrol had also been well studied. For example, resveratrol prevents axonal degeneration after injury by enhancing Sir2 activity,[12] attenuates Aβ peptide-induced neuronal loss by activation of PKC in vitro,[13] and rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons.[14] Meanwhile, resveratrol ameliorates ischemia-induced neuronal cell death in a gerbil model with global cerebral ischemic injury,[15] suggesting its protection from the brain injury induced by vascular factors. However, whether resveratrol could be protective to the synaptic plasticity of CCH rats model is not clear.

In this study, we found that preadministering resveratrol protects against impairments in spatial learning and memory in a CCH rats model. Resveratrol also prevents LTP inhibition and dendritic spine loss, as well as the synaptic proteins. The possible underlying mechanism for the prevention of resveratrol is through the activation of the protein kinase A (PKA) and cAMP-responsive element-binding protein (CREB) pathway.

Materials and Methods

Drugs and Antibodies

Resveratrol was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in dimethylsulfoxide. Polyclonal antibodies (pAbs) PKAα/β cαt against PKA catalytic α-subunit (1:1000) and PKA IIβ reg against PKA regulated β-subunit (1:1000), CREB against total CREB (1:1000), and phosphorylated CREB (p-CREB) against p-CREB at Ser133 site (1:1000) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The pAb PSD95 (1:1000) against PSD95 protein, pAb PSD93 (1:1000) against PSD93 protein, Syn-1 against synapsin 1 (1:1000), and pAb NR2B against N-methyl-D-aspartate receptor 2B were from Bioworld Technology, Inc. (St. Louis Park, MN). IRDye 800CW conjugated goat (polyclonal) antirabbit IgG (1:10000) and IRDye 800CW Conjugated Goat (polyclonal) antimouse IgG (1:10000) were from LI-COR Biosciences (Lincoln, NE).

Animals

Sixty male Wistar rats (200 ± 10 g) were purchased from Hubei Provincial Center for Disease Control and Prevention (Hubei, China). All animal experiments were performed according to the "Policies on the Use of Animals and Humans in Neuroscience Research," revised and approved by the Society for Neuroscience in 1995. The animals were acclimatized to our animal facility for 4 days after arrival. Then they were randomly divided into 4 groups: a sham group (sham, n = 15), a CCH group (n = 15), a CCH group with resveratrol (40 mg/kg, i.p.)[16,17] pretreatment for 4 weeks (CCH + resveratrol, n = 15), and a resveratrol administration group (resveratrol, n = 15). All rats were housed in polypropylene cages with a vivarium at a temperature of 22°C, under a 12:12-hour light/dark cycle (lights on at 8 am), and with access to food and water.

CCH

CCH was conducted by bilateral common carotid artery stenosis as previously described.[18] Briefly, rats were anesthetized with 10% chloral hydrate (350 mg/kg, i.p.) and the core body temperature was maintained at 37.0 ± .2°C using an automated heat blanket with temperature feedback. Then both common carotid arteries were exposed and freed from their sheaths via a midline cervical incision. For hypoperfusion, metal coils (180-μm inner diameter; Sawane Spring Company, Hamamatsu, Japan) were encircled onto the common carotid arteries, reducing blood flow to about 70%. All muscles and glands were guided back into place, the incision sutured, and local anesthetic applied to the wound before recovery. The procedures were accomplished within 15 minutes. Four weeks after surgery, the animals were carried for behavior test and electrophysiology recording.

Morris Water Maze Test

The Morris water maze test was used to assess the spatial memory of animals.[19] The maze was a circular pool with a diameter of 150 cm and a height of 50 cm. It was divided into 4 imaginary quadrants (NW, NE, SW, and SE) and filled with water with a temperature of 20°C-21°C. Briefly, the rats were trained over 5 days to find a platform in the SW quadrant that was submerged in 1-2 cm of water, by using a stationary array of cues outside the pool. In each trial, the rats were given a maximum of 60 seconds to find the hidden platform and were allowed to remain on it for 30 seconds. The trial ended when the rat climbed onto the platform. The rats that failed to locate the platform within 60 seconds were guided to the platform by the experimenter, and their data were discarded. Acquisition training consisted of a total of 20 trials, with 4 spaced trials per day for 5 consecutive days. After 1 day's rest, a test was conducted. The latency to reach the platform zone and the swimming pathways to find the hidden platform was recorded in all training trials and in the test.

Electrophysiology

The LTP record and assay methods were carried out according to previous studies.[20] Briefly, the animals were anesthetized with an intraperitoneal injection of ketamine and xylazine (85 and 15 mg/kg, respectively) and were placed on a stereotaxic instrument. The stimulating electrode was placed in the perforant path and the recording