Physiology & Behavior 138 (2015) 297–304

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Resveratrol exerts anti-inflammatory and neuroprotective effects to prevent memory deficits in rats exposed to chronic unpredictable mild stress

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HIGHLIGHTS

- Resveratrol attenuates the deficits in cognition seen in stressed rats.
- Resveratrol decreased proinflammatory cytokine concentrations in plasma.
- Resveratrol improved neurothrophic factor expression in hippocampus and amygdala.
- Resveratrol have a role in reversing the deleterious effects of stress on cognition.

ARTICLE INFO

Article history:
Received 25 April 2014
Accepted 10 October 2014
Available online 22 October 2014

Keywords:
Resveratrol
Chronic mild stress learning
Memory
BDNF
c-Fos
TNF-α
IL-1β

ABSTRACT

A number of studies have recently focused on the neuroprotective and anti-inflammatory effects of resveratrol. In prior studies, we described its beneficial effects on scopolamine-induced learning deficits in rats. The aim of this study was to investigate the effects of resveratrol on emotional and spatial cognitive functions, neurotropic factor expression, and plasma levels of proinflammatory cytokines in rats exposed to chronic unpredictable mild stress (CUMS), which is known to induce cognitive deficits. Resveratrol (5 or 20 mg/kg) was administered intraperitoneally for 35 days. Rats in the CUMS group and in the 5 mg/kg resveratrol + CUMS group performed poorly in tasks designed to assess emotional and spatial learning and memory. The 20 mg/kg resveratrol + CUMS group showed improved performance compared to the CUMS group. In addition, the CUMS procedure induced lower expression of brain-derived neurotrophic factor and c-Fos in hippocampal CA1 and CA3 and in the amygdala of stressed rats. These effects were reversed by chronic administration of resveratrol (20 mg/kg). In addition, plasma levels of tumor necrosis factor-alpha and interleukin-1 beta were increased by CUMS, but were restored to normal by resveratrol. These results indicate that resveratrol significantly attenuates the deficits in emotional learning and spatial memory seen in chronically stressed rats. These effects may be related to resveratrol-mediated changes in neurotrophin factor expression in hippocampus and in levels of proinflammatory cytokines in circulation.

1. Introduction

Chronic stress is an unavoidable life experience that can induce depression [1], impair spatial cognition [2], and cause abnormalities in neuroendocrine function [3] and plasticity [4]. Based on these observations, an animal model of chronic unpredictable mild stress (CUMS) has been developed to mimic the development and progress of stress-associated clinical depression [5] and cognitive deficits [6].

It has been proposed that the learning and memory deficits associated with chronic stress may be alleviated using novel therapeutics such as dietary and medicinal phyto-antioxidants. One such nutraceutical is resveratrol. It is a dietary polyphenol found in a wide variety of foods such as berries, nuts, grape skins, and red wine. An increasing research effort is aimed at identifying potential therapeutic roles of resveratrol in human health given its various, and potentially beneficial, antioxidant, anti-inflammatory, and neuroprotective activities [7,8]. Recent studies focusing on the neuroprotective effects of resveratrol have shown that it attenuates amyloid beta peptide- [9,10] and kainic acid-induced toxicities

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http://dx.doi.org/10.1016/j.physbeh.2014.10.010
0031-9384/

[11], and protects against cerebral ischemic injury [12]. The neuroprotective properties of resveratrol have been attributed to its potent antioxidant activity [13].

Cognitive improvement was observed in a mouse model of Alzheimer's disease after treatment with resveratrol [14]. Similar findings have been reported in animals after consumption of red wine in moderate quantities [15] and on administration of a polyphenolic extract from grape seed [16]. Data from preclinical studies support the theory that resveratrol could be a useful therapeutic agent for Alzheimer's disease [14,17]. Similarly, a previous study in our laboratory showed that resveratrol improved cognitive deficits induced by scopolamine [18]. In addition, Schmatz and co-workers [19] reported that resveratrol prevents the increase in acetylcholinesterase activity and consequent memory impairment typically observed in diabetic rats. Despite numerous studies of the beneficial effects of resveratrol on cognition, its therapeutic potential in ameliorating memory related to chronic stress is not fully understood. Therefore, the present study was designed to investigate the effects of resveratrol on CUMS-induced spatial and emotional cognitive deficits using the water maze and passive avoidance tasks, respectively. To investigate the possible molecular mechanisms underlying the therapeutic effects of resveratrol, we also assessed hippocampal expression of brain-derived neurotrophic factor (BDNF) and c-Fos protein and plasma levels of TNF-α and IL-1β.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Kocaeli University, Experimental Medical Research and Application Center, Kocaeli, Turkey) weighing 250–300 g were kept in an animal colony, at a density of ~5 to 6 per cage, for 2 weeks prior to beginning these experiments. All behavioral tests were conducted between 9:00 A.M. and 12:00 P.M. under standard laboratory conditions (22 ± 2 °C room temperature; 12-h light/dark cycle with lights on at 7:00 A.M.). Tap water and food pellets were provided ad libitum. All animals used in this study were naive to the experimental tests, and different rats were used in each experiment.

The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Project number: HADYEK 28, Kocaeli, Turkey).

Animals were divided into five groups (n = 10 per group). One control group received physiological saline and a second control group received dimethylsulfoxide (DMSO) for 5 weeks. The CUMS group was given physiological saline during 5 weeks of CUMS. The groups receiving resveratrol treatment were administered resveratrol during CUMS at a dose of 5 mg/kg/day or 20 mg/kg/day.

2.2. Unpredictable chronic mild stress procedure

CUMS was applied as described previously by Yazir and co-workers [20]. Briefly, the CUMS groups with or without treatment were subjected to nine different types of stressors, as listed: restraint for 4 h, cage tilting for 24 h, wet bedding for 24 h, swimming in 4 °C cold water for 5 min, swimming in 45 °C hot water for 5 min, pairing with another stressed animal for 48 h, level shaking for 10 min, nip tail for 1 min, and inversion of the light/dark cycle for 24 h. These nine stressors were randomly applied for 35 days, and each stressor was applied 4–5 times during this period. A rat received only one of these stressors per day, and the same stressor was not applied on 2 consecutive days to prevent animals from predicting the occurrence of stimulation. The stress procedure did not involve any food or water deprivation. The control groups receiving no stress had free access to food and water.

2.3. Locomotor activity test

Locomotor activity was measured with a computerized system (May Commat, Ankara, Turkey; in a 40 cm × 40 cm × 35 cm box). Total locomotor activity was measured before the cognitive behavioral tests and was expressed as the sum of the stereotypic, ambulatory, and vertical activities of the animals. Locomotor activity was evaluated over a 5-min period.

2.4. Passive avoidance test

A one-trial, light–dark passive avoidance apparatus (Ugo Basile model 7551, Italy) was used for the evaluation of emotional memory based on contextual fear conditioning [21]. In this test, the animal learns to avoid a specific place associated with an aversive event. The reduction of latency to avoid was used as a learning index. The apparatus consisted of two compartments, each measuring 22 × 21 × 22 cm. An illuminated white box was connected to a dark box equipped with an electrifiable grid floor. An inescapable electrical shock could thus be delivered to the animal's feet via a shock generator. The two boxes were separated by a flat-box partition, including an automatically operated sliding door at floor level.

A training trial was carried out as described by Monleon and co-workers [22]. A preacquisition trial was performed on the first day of training in which the rats were placed individually into the light compartment and allowed to explore the boxes. The door between the two boxes was opened after 30 s and the animal was able to move freely into the dark compartment. Fifteen minutes after the preacquisition trial, an acquisition (training) trial was performed. Rats were again placed in the light compartment of the passive avoidance apparatus. After 30 s of familiarization with the apparatus, the door between the compartments was opened. When the animal entered the dark compartment completely, the sliding door between the chambers was closed automatically and an electric foot-shock (0.5 mA) of 3-s duration was delivered through the grid floor. The time taken to enter the dark compartment was recorded as the training latency. If the animal failed to cross over from the illuminated to the dark compartment within 300 s, it was excluded from the experiment. The animals were then removed from the dark box and put back in their home cages. Both compartments of the box were cleaned thoroughly between each training session to remove any confounding olfactory cues.

Twenty-four hours after the acquisition trial, a retention trial was performed. Recall of the shock stimulus was evaluated by returning the animals to the light compartment and recording their latency to enter the dark compartment (four paws in). No foot shock was applied in this trial. If the animal did not enter the dark compartment within 300 s, it was returned to its home cage and a latency of 300 s was recorded. This latency served as a measure of the retention performance of the passive avoidance response.

2.5. Morris water maze test

Performance in the Morris task was assessed in a water tank (150 cm in diameter) as previously described [23]. The rats underwent three trials during five daily sessions. During the first four days, the platform, which was situated in the center of the southwest quadrant, was submerged 1.5 cm below the surface of water, and small black pieces of plastic were placed on the water surface. The plastic was invisible to the rats due to its placement, and it was used to monitor spatial learning. The platform position remained unchanged over 4 days, and latency to find the platform was assessed. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of three starting