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Journal of Nutritional Biochemistry 26 (2015) 190–199

Journal of Nutritional Biochemistry

Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring

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Received 11 April 2014; received in revised form 1 August 2014; accepted 7 October 2014

Abstract

Prenatal stress is known to induce emotional and cognitive dysfunction in the offspring of both humans and experimental animals. Hydroxytyrosol (HT), a major polyphenol in olive oil with reported ability modulating oxidative stress and mitochondrial function, was performed to investigate its preventive effect on prenatal stress-induced behavioral and molecular alterations in offspring. Rats were exposed to restraint stress on days 14–20 of pregnancy. HT was given at doses of 10 and 50 mg/kg/day. The spontaneous alternation performance and Morris water maze confirmed the impaired learning capacity and memory performance induced by prenatal stress in both male and female offspring, and these effects were markedly restored in the HT supplement groups. Through tissue analysis of the hippocampi of male offspring, we found that the stress-induced downregulation of neural proteins, including BDNF, GAP43, synaptophysin, NMDAR1, NMDANR2A and NMDANR2B, was prevented by HT. Prenatal stress-induced low expression of glucocorticoid receptor was also increased by HT, although basal fetal serum corticosterone levels were not different among the four groups. Oxidative stress and mitochondrial dysfunction in prenatally stressed rats were confirmed with changes in protein oxidation, SOD activity, the expression of mitochondrial complexes and mitochondrial DNA copy number. Meanwhile, HT significantly increased transcription factors FOXO1 and FOXO3, as well as phase II enzyme-related proteins, including Nrf2 and HO-1, which may contribute to the decreased oxidative stress and increased mitochondrial function shown with HT supplementation. Taken together, these findings suggest that HT is an efficient maternal nutrient protecting neurogenesis and cognitive function in prenatally stressed offspring.

Keywords: Prenatal stress; Cognitive dysfunction; Hydroxytyrosol; Oxidative stress; Mitochondrial function

1. Introduction

Stress can occur throughout the entire lifespan. Acute stress may be initially adaptive to a new allostasis, but excessive, repeated or chronic stress, especially during critical phases of development, may have detrimental long-term effects on body functions. Early life experiences, including those in utero, can have long-term consequences for the unborn child. Recent findings in human studies show that prenatal stress leads to maladaptive consequences for the offspring, including negative birth outcomes [1], altered physiological stress responses, behavior problems [2,3] and impaired cognitive and motor development [4]. Prenatal stress may also increase the risk for several adult onset chronic diseases, including cardiovascular disease, type 2 diabetes, obesity and hypertension [5,6]. Because the brain plays a key role in the processing of stressors and in the regulation of the ensuing behavioral and physiological stress responses [7], studies in animal experiments also suggest that prenatal stress impairs brain development, strengthens behavioral abnormalities and provokes neurological diseases such as depression and schizophrenia in offspring [8,9].

Maternal nutritional factors during pregnancy have been linked to fetal brain development and subsequent offspring behavior. Maternal supplementation with docosahexaenoic acid or wolfberry showed protective effects on learning and memory, oxidative status and mitochondrial function in prenatally stressed rats [10,11]. Recent reports have indicated that the protective role of a Mediterranean diet during pregnancy for the health of the mother and child is mainly due to the antioxidants supplied by components of this diet, such as olive oil [12,13]. Hydroxytyrosol (HT), one of the most effective antioxidants, is a natural, well-known phenolic compound from virgin olive oil. It has been reported that HT has multiple biological functions, including anticancer [14], anti-inflammation [15] and neuroprotective effects [16,17]. Our previous study also demonstrated that HT could activate phase II enzymes and mitochondrial biogenesis to promote cell survival [18–20]. Despite the limited reports of HT protection on neurons, the *in vivo* effects of HT on cognitive function in prenatally stressed rats remain unknown.

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http://dx.doi.org/10.1016/j.jnutbio.2014.10.006
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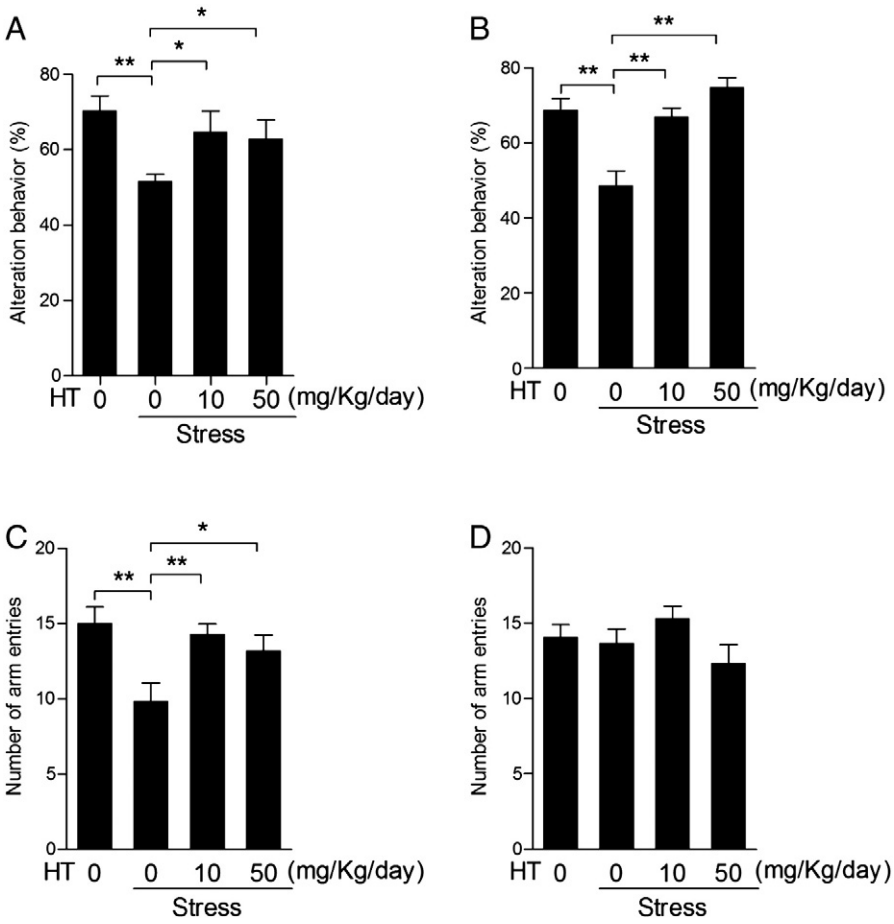

Fig. 1. Effects of prenatal stress and HT supplementation on offspring cognitive function in the T-maze test. Alternation behavior percentages in male rats (A) and female rats (B). Total number of goal arm entries in male rats (C) and female rats (D). Values are mean values±S.E.M. $n \geq 10$, $^{*}P<.05$, $^{**}P<.01$.

Prenatal restraint stress (PRS) in rats is one of the most common models of early stress known to induce long-lasting neurobiological and behavioral alterations, including impaired feedback mechanisms of the hypothalamo-pituitary-adrenal (HPA) axis, altered neuroplasticity and cognitive dysfunction [21]. Further, the hippocampus is sensitive to stress, and it is an important component of the stress response, which is involved in the regulation of learning and memory. Therefore, in the current study, we aimed to investigate the potential benefit of HT on brain function with a PRS model. We particularly focused on the protective effects of maternal HT administration on cognitive function and biochemical function in the hippocampus, including HPA, oxidative stress and mitochondrial dysfunction, which can contribute to the pathophysiology of both stress and neurodegenerative disorders. Here, we first determined the protection function of HT on learning and memory to prenatally stressed rats and explored its potential mechanism.

2. Materials and methods

2.1. Reagents and antibodies

The anti-actin antibody was obtained from Sigma (St. Louis, MO, USA). Anticomplex I, II, III, IV, and V antibodies were obtained from Invitrogen (Carlsbad, CA, USA). Anti-brain-derived neurotrophic factor (BDNF), anti-Forkhead box protein (FOX) O1, anti-FOXO3, anti-HO-1, anti-Nrf2and anti-SOD2 antibodies were from Santa Cruz (Santa Cruz, CA, USA). The Reverse Transcription System kit was purchased from Promega (Mannheim, Germany). SYBR green was purchased from Takara (Otsu, Japan). Polymerase chain reaction (PCR) primers were synthesized by Baiaoke Biotech (Beijing, China). TRIzol and other reagents were purchased from Invitrogen (Carlsbad, CA, USA). HT was purchased from APP-Chem Bio (Xi'an, China).

2.2. Animals

Specific pathogen-free (SPF) Sprague–Dawley rats were purchased from a commercial breeder (SLAC, Shanghai, China). The rats were housed in a temperature (23–26°C)- and humidity (60%)-controlled animal room and maintained on a 12-h light/12-h dark cycle (light from 08:00 a.m. to 08:00 p.m.) with food and water provided during the experiments. Female rats weighing from 230–250 g and male rats weighing 280–350 g were used. At the beginning, female rats were randomly assigned to the following experimental groups: control, stress, HT treatment groups (10 and 50 mg/kg/day). HT was administered through gavage 2 weeks before mating, and the doses were chosen based on our previous study [22]. The day on which a vaginal smear was determined to be sperm-positive was set as embryonic day 0. Each pregnant rat was then housed separately.

2.3. Stress procedure

The stress protocol was following our previous study [11]. The pregnant rats in the stress and HT treatment groups were exposed to restraint stress during embryonic days 14 to 20. The restraint was performed using a transparent plastic tube of 6.8 cm in diameter for 2 h during the time periods 08:00 a.m. to 11:00 a.m., 11:00 a.m. to 2:00 p.m., and 04:00 p.m. to 07:00 p.m.. The pregnant rats in the control group were left undisturbed. All offspring were weaned on day 21 after birth, and female and male pups were housed separately until testing at 1 month of age. All procedures were carried out in accordance with the United States Public Health Services Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at School of Life Science and Technology, Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and their suffering.

2.4. Spontaneous alternation performance in the T-maze

Spontaneous alternation is the phenomenon that rats and mice in a T maze have a natural tendency to alternate choice of goal arm (left or right arm) during exploration. The size of the maze was referring the parameters for rats in a previous publication [23]. The rats were confined to the start area for 30 s before testing; then, the sliding