Behavioural Brain Research 305 (2016) 65–75

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Protective effect of resveratrol against chronic intermittent hypoxia-induced spatial memory deficits, hippocampal oxidative DNA damage and increased p47Phox NADPH oxidase expression in young rats

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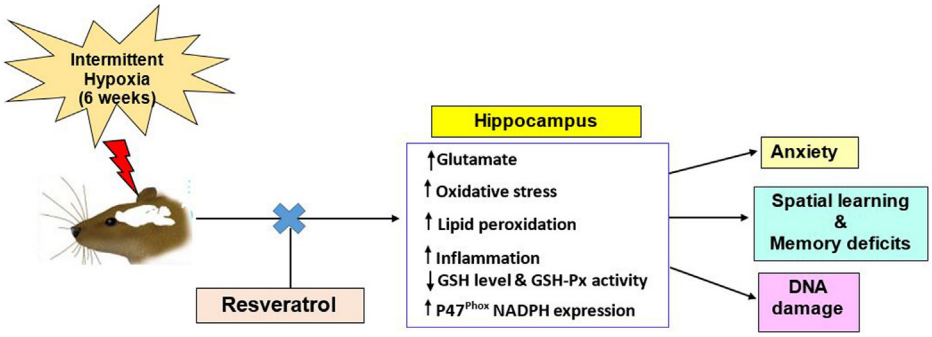
HIGHLIGHTS

- Long-term intermittent hypoxia (IH) induces memory deficits and hippocampal oxidative stress.
- Resveratrol ameliorates IH-induced spatial memory deficits in young rats.
- Resveratrol reduces IH-induced hippocampal oxidative stress challenge.
- Resveratrol protects against IH-induced hippocampal Oxidative DNA damage.
- Resveratrol attenuates the IH-induced expression of $P47^{Phox}$ subunit of NADPH oxidase.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 23 February 2016
Accepted 26 February 2016
Available online 3 March 2016

Keywords:
Resveratrol
Sleep apnea
Intermittent hypoxia
DNA damage
NADPH oxidase

ABSTRACT

Long-term intermittent hypoxia (IH) is a characteristic hallmark of obstructive sleep apnea (OSA) and causes most of the neurological aspects of OSA, such as spatial memory and learning deficits. These deficits are accompanied by an increase in oxidative stress and inflammation in brain areas involved in cognition, such as the hippocampus, particularly in children. Resveratrol is a natural polyphenolic compound with potent antioxidant, anti-inflammatory and neuroprotective properties.

Aim: The aim of this work is to study the possible protective effect of resveratrol against IH-induced neurobehavioral deficits and to investigate the possible mechanism of this protective effect in the young rat model of OSA.

Methods: The effect of resveratrol (5 and 10 mg/kg, orally) on anxiety, spatial memory and learning deficits in young rats exposed to IH for 6 weeks and the corresponding biochemical changes were studied.

Results: Resveratrol attenuated IH-induced anxiety and spatial memory deficits, as indicated by the elevated plus maze and Morris water maze tests, respectively, in a dose-dependent manner. In addition,

Abbreviations: IH, intermittent hypoxia; OSA, obstructive sleep apnea; NADPH, nicotinamide adenine dinucleotide phosphate; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2-deoxy guanosine.

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http://dx.doi.org/10.1016/j.bbr.2016.02.030

resveratrol antagonized IH-induced increases in hippocampal glutamate, TBARS and 8-OHdG levels and p47Phox expression and decreases in GSH levels and GSH-Px activity in the hippocampus of IH-exposed young rats.
Conclusion: Resveratrol ameliorates IH-induced anxiety and spatial learning deficits through multiple beneficial effects on hippocampal oxidative pathways that involve decreased expression of the p47Phox subunit of NADPH oxidase. Hence, the potential therapeutic role of resveratrol in OSA may be utilized in the near future and deserves further exploration.

1. Introduction

Obstructive sleep apnea (OSA) is a respiratory disorder that is characterized by repeated episodes of upper airway obstruction and respiratory cessation during sleep, resulting in severe hypoxia. These episodes are interspersed with reoxygenation, resulting in intermittent hypoxia (IH) [1]. This clinical respiratory disorder is currently recognized as a significant and highly prevalent public health problem that can lead to more serious cardiovascular and neurocognitive morbidities in all age groups [2]. Neurocognitive impairments, particularly memory and learning impairments, are increasingly observed in patients who suffer from OSA and are exposed to long-term IH, particularly young children. Many previous studies of experimental animals showed that these neurocognitive deficits are strictly associated with regional brain damage, particularly in the hippocampus, with apoptosis [3] likely mediated by the overproduction of reactive oxygen species (ROS) under IH conditions [4]. Cellular oxidative stress resulting from mitochondrial dysfunction is a central factor in IH-induced neurobehavioral disorders and hippocampal biochemical changes [5]. In addition, increased systemic levels of oxidative stress and inflammation markers have been recognized in patients who suffer from OSA [6]. Consistent with this concept, ROS scavengers and the pharmacological blockade of oxidative stress and inflammation could alleviate IH-induced apoptosis and spatial memory deficits in experimental animals [7–10]. Previous studies showed that the Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme contributes to the cognitive deficits induced by exposure to chronic IH [5]; thus, this enzyme has attracted attention as an indicator of cognitive deterioration in animal models of OSA.

Studies performed using animal models of hypoxia/reoxygenation patterns that resemble OSA in patients explored many of the neurocognitive and biochemical hallmarks of the disease in humans, such as increased oxidative stress and inflammatory signaling. Such studies helped to improve our understanding of the pathogenesis of the disease and promoted research for its treatment [11]. Studies of these models have revealed the effectiveness of some natural plant products with antioxidant and anti-inflammatory effects, such as *Ginkgo Biloba* [12], Grape seed proanthocyanidin [13] and *Lycium Barbarum* polysaccharides [14], in attenuating the pathological neurocognitive and biochemical hallmarks of exposure to IH, including cognitive dysfunction and oxidative stress.

Resveratrol (3,5,4-trihydroxy-*trans*-stilbine) is a natural polyphenolic compound that is present in the skin and seeds of different plants, including grapes, grains, berries, and peanuts, and is the main component in red wine [15]. Resveratrol exists in two geometric isomers, which have a *trans*-configuration and a *cis*-configuration. *trans*-Resveratrol is a non-toxic isomer and possesses the main pharmacological and beneficial effects of resveratrol [16].

Previous studies showed that resveratrol has neuroprotective activity against many neurodegenerative diseases, such as Parkinson's disease, cerebral ischemia, Huntington's disease and Alzheimer's disease (AD) [17,18]. The exact mechanism that underlies the neuroprotective effect of resveratrol is not clearly understood. The available studies relate the neuroprotective effects of resveratrol to its potent antioxidant and anti-inflammatory effects [19].

The antioxidant potency of resveratrol is related to its capacity to enhance the expression of antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase [20] and hemeoxygenase-1 [21]. Recent studies revealed that resveratrol modulates genes related to redox pathways. Resveratrol stimulated the expression of the transcription factor erythroid 2-related factor 2 (Nrf2) in a rat model of ischemia [22]. Moreover, the ability of resveratrol to provide neuroprotection against oxidative stress is at least partially mediated by the activation of the SIRT1 pathway. SIRT1 is an enzyme that deacetylates proteins that contribute to cellular regulation. SIRT1 can transfer from the cytoplasm to the nucleus, where it begins to deacetylate nucleosomal histones and contributes to telomere maintenance [23]. In addition, SIRT1 deacetylates other nonhistone cellular components, including components of the DNA repair machinery, such as poly (ADP-ribose) polymerase (PARP) [24] and Ku70 [25], as well as many transcription factors that regulate cellular energetic metabolism, such as hypoxia-inducible factor 1α (HIF-1α) [26], PGC1-α [27], and peroxisome proliferator-activated receptor-α (PPAR-α) [28]. In addition, SIRT1 deacetylates other nuclear transcription factors, such as Forkhead box (FOXO) family transcription factors (Brunet et al., 2011), p53 [29] and nuclear factor kappa B (NF-κB) [30], which play important roles in oxidative stress and inflammation. Moreover, resveratrol has partial agonistic activity for estrogen receptor alpha [31], through which resveratrol can also affect the transcription of inflammatory, redox and nuclear factors.

The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a membrane-bound enzyme complex that can be found in the plasma membrane and in the membranes of phagosomes in neutrophils. The enzyme is composed of six subunits: a Rho guanosine triphosphatase subunit and five "phox" (phagocytic oxidase) subunits (gp91-phox, p22phox, p40phox, p47phox and p67phox). NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling those electrons to molecular oxygen to produce the superoxide anion, which is a reactive free radical. Although recent studies demonstrated the involvement of NADPH oxidase in the neuroprotective effect of resveratrol against AD [32], the role of NADPH oxidase in the neuroprotective effect of resveratrol against IH-induced neurocognitive disorders has not been studied previously.

Hence, the aim of this work was to study the possible protective effect of resveratrol against the anxiety, cognitive deficits and hippocampal biochemical changes that are induced by exposure to chronic IH. Using this approach, we aimed to explore the possible mechanism of resveratrol in a young rat model of OSA. In addition, this study aimed to investigate the possible involvement of NADPH oxidase in this phenomenon by studying the hippocampal mRNA expression of the $P47^{Phox}$ subunit of NADPH oxidase.