Free Radical Biology and Medicine 75 (2014) 156–166

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

AMPK activation prevents prenatal stress-induced cognitive impairment: Modulation of mitochondrial content and oxidative stress

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ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form
10 July 2014
Accepted 23 July 2014
Available online 1 August 2014

Keywords:
AMPK
Resveratrol
Mitochondrial biogenesis
Oxidative stress
Prenatal stress
Cognitive function

ABSTRACT

Prenatal stress induces cognitive functional impairment in offspring, an eventuality in which mitochondrial dysfunction and oxidative stress are believed to be closely involved. In this study, the involvement of the AMP-activated protein kinase (AMPK) pathway was investigated. A well-known activator, resveratrol (Res), was used to induce AMPK activation in SH-SY-5Y cells. Significant mitochondrial biogenesis and phase II enzyme activation, accompanied by decreased protein oxidation and GSSG content, were observed after Res treatment, and inhibition of AMPK with Compound c abolished the induction effects of Res. Further study utilizing a prenatal restraint stress (PRS) animal model indicated that maternal supplementation of Res may activate AMPK in the hippocampi of both male and female offspring, and that PRS-induced mitochondrial loss in the offspring hippocampus was inhibited by Res maternal supplementation. In addition, Res activated Nrf2-mediated phase II enzymes and reduced PRS-induced oxidative damage in both male and female offspring. Moreover, PRS markedly decreased mRNA levels of various neuron markers, as well as resultant offspring cognitive function, based on spontaneous alternation performance and Morris water maze tests, the results of which were significantly improved by maternal Res supplementation. Our results provide evidence indicating that AMPK may modulate mitochondrial content and phase II enzymes in neuronal cells, a process which may play an essential role in preventing PRS-induced cognitive impairment. Through the coupling of mitochondrial biogenesis and the Nrf2 pathway, AMPK may modulate oxidative stress and be a promising target against neurological disorders.

Introduction

Prenatal stress during gestation has many deleterious effects on both the development and the behavior of offspring [1]. Clinical studies demonstrate that exposure of pregnant mothers to stressful conditions increases the susceptibility of their offspring to mental disorders, such as depression, schizophrenia, and cognitive deficits [2]. Animal studies show that prenatal stress causes alterations of both the hypothalamic–pituitary adrenocortical axis and the brain neurotransmitter systems and also impairs hippocampal-dependent spatial learning and memory abilities in offspring [3,4]. Pregnant rodents that have suffered restraint stress represent a valid model of stress with neurobiological and behavioral consequences [5,6]. Although the specific mechanisms of prenatal restraint stress (PRS) remain unclear, evidence suggests that both oxidative stress and mitochondrial dysfunction may be involved in PRS-induced neurological damage and cognitive impairment [7,8]. However, the detailed mechanisms regulating each of these processes have not been elucidated in the rodent model.

The involvement of cellular energy metabolism in different conditions has become an area of intense interest [9]. As a sensor of cellular energy status, AMP-activated protein kinase (AMPK) is an attractive target for a range of diseases, such as cancer [10], diabetes [11], and cardiovascular disease [12]. Studies also implicate a neuroprotective

Abbreviations: AMPK, AMP-activated protein kinase; Arc, activity-regulated cytoskeleton-associated protein; BDNF, brain-derived neurontrophic factor; EMX2, empty spiracles homeobox 2; GAP43, growth-associated protein-43; GCL, glutamate-cysteine ligase; HO-1, heme oxygenase 1; NMDAR, *N*-methyl-D-aspartic acid receptor; Nrf2, NF-E2-related factor; NQO-1, NAD(P)H dehydrogenase (quinone 1); PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PRS, prenatal restraint stress; Res, resveratrol; SCG10, stathmin-like 2.

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http://dx.doi.org/10.1016/j.freeradbiomed.2014.07.029
0891-5849/

effect of AMPK both *in vitro* [13] and *in vivo* [14]. However, whether AMPK plays a role in PRS-induced neurological impairment is still unknown. It has been demonstrated that AMPK activity correlates strongly with mitochondrial function by promoting a mitochondrial biogenesis pathway. Activation of AMPK is dependent on the upregulation of peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) and nuclear respiratory factor 1 (NRF1) expression in rat visual cortical neurons [15]. In addition to mitochondrial dysfunction, oxidative stress was also involved in PRS-induced neurological damage and cognitive impairment [7,8]. Induction of phase II detoxifying enzymes is one of the most important pathways for cells to fight against oxidative stress. Nuclear factor erythroid-2-related factor-2 (Nrf2) is an antioxidant transcription factor mediating the expression of antioxidant enzymes, such as NADPH quinineoxido reductase-1 (NQO1) and heme oxygenase-1 (HO-1). It has a wide range of activities in regulating redox state and energy metabolism in cells [16]. The response of AMPK to oxidative stress has been recently reported, but the downstream signals of this response are largely unknown. The potential for cross talk between the AMPK and the Nrf2 cascades has been reported in *Caenorhabditis elegans* [17], in human endothelial cells [18], and in mammalian inflammatory systems [19]. However, no information about the potential for convergence between the AMPK and the Nrf2 pathways or the subsequent exertion of a neuroprotective effect exists.

Resveratrol (3,4′,5 trihydroxystilbene, Res), a naturally occurring phytoalexin compound present in almost 70 plant species (such as grape, peanut, and soya beans), is considered one of the most effective known antioxidants. Similar to other chemical activators of AMPK, such as 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and metformin, Res has been widely accepted as a natural AMPK activator [20,21]. The impact of Res on the prevention of prenatal stress-induced cognitive impairment has been recently reported, but the exact mechanisms involved in its neuroprotective effects are still poorly characterized [22–24].

In the present study, to investigate the potential regulatory effect of AMPK on mitochondrial biogenesis and phase II enzyme induction, and their subsequent involvement in prenatal stress-induced cognitive dysfunction, an AMPK activator was employed both in SH-SY-5Y cells and in a PRS animal model. We propose that the upregulation of mitochondrial biogenesis and Nrf2 pathways by AMPK activation may play an important role in promoting neuron survival and related improvement in cognitive function.

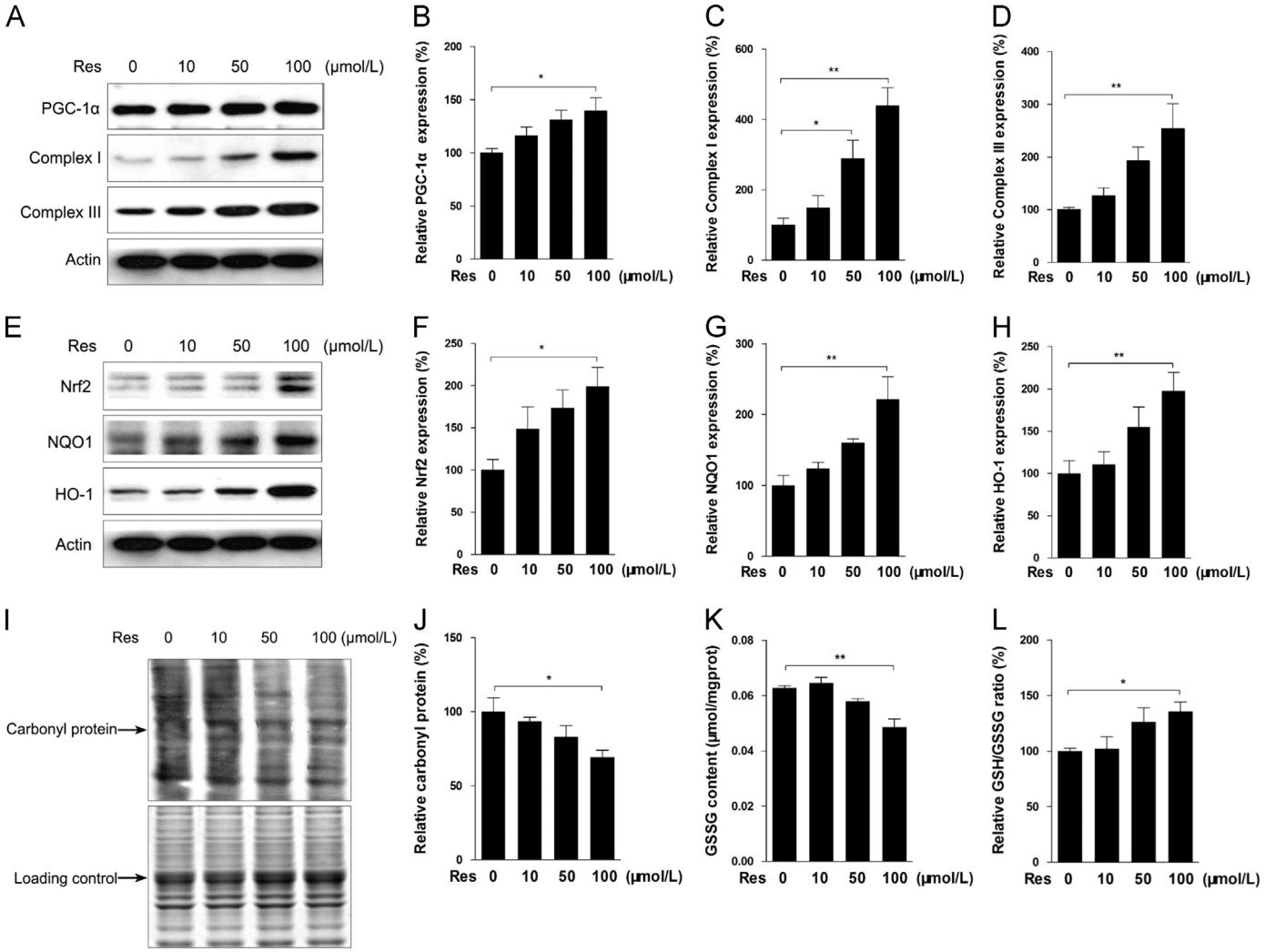

Fig. 1. Effects of Res on mitochondria and phase II enzymes in SH-SY-5Y cells. Cells were treated with Res at doses of 10, 50, and 100 μM for 24 h. Mitochondrial biogenesis-related proteins were analyzed by Western blot (A, Western blot image; B, statistical analysis of PGC-1α; C, statistical analysis of Complex I; D, statistical analysis of Complex III). Phase II enzyme-related proteins were determined by Western blot (E, Western blot image; F, statistical analysis of Nrf2; G, statistical analysis of NQO1; H, statistical analysis of HO-1). Protein oxidation was determined by measuring carbonyl protein content (I, Western blot image; J, statistical analysis). GSSG was evaluated (K). GSH/GSSG ratio was calculated (L). Values are means ± SEM from at least three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.