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Original article

PERK Signaling of Unfolded Protein Response Activated in Acute Hypobaric Hypoxia and Effect of Ginsenoside Rb_1

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ABSTRACT

Objective To observe pancreatic ER kinase (PERK) signaling of unfolded protein response (UPR). **Methods** The rats were divided into control, model, and ginsenoside Rb_1 (Rb_1) groups, and put into hypoxia chamber to establish the acute plateau stress model. The water solutions of Rb_1 were given to rats in Rb_1 group for 7 d. After that, The behavior of rats was observed by Y-maze and passive avoidance test and the rats were sacrificed in a batch for detection by Western blotting. **Results** Hypobaric hypoxia mediated UPR pathway accompanied the activation of protective pathways such as PERK-eIF2a-ATF4 and Grp78/Bip pathways. On the other hand, Rb_1, the extract from herbal medicine ginseng, increased on the expression of PERK, eIF2a, ATF4, and Grp78/Bip in rats. **Conclusion** The results indicate that PERK-eIF2a-ATF4-GRP78 pathway is a potential target for therapeutic applications in high altitude diseases and Rb_1 can attenuate the injury to memory function caused by hypobaric hypoxia neurotoxicity.

Key words

ginsengoside Rb_1; hypobaric hypoxia; pancreatic ER kinase pathway

1. Introduction

High altitude areas are the most inhospitable on earth. According to WHO in 1966, there were approximately 140 million people living at altitudes over 2500 m and there were several areas of permanent habitation at over 4000 m. It is generally accepted that most nervous changes occur in the first days following arrival at high altitude, and this is the period when acute mountain sickness may occur (Norboo et al, 2011). Recent studies have highlighted the changes of the cognition following high altitude exposure (Virues et al, 2008), but cellular mechanism of these changes is not completely understood. Endoplasmic reticulum (ER) stress triggers a cellular stress response called unfolded protein response (UPR) and intended to protect the cell from injury (Roussel et al, 2013). pancreatic ER kinase (PERK) is one of the main stress kinases that can inhibit protein synthesis (Harding et al, 2000). Activated PERK phosphorylates eukaryotic translation initiation factor 2 subunit a (eIF2a). After stress-induced phosphorylation of eIF2a, global protein translation of normal cellular mRNAs is repressed (Boyce et al, 2005). In parallel, the translational initiation of transcription factor ATF4 is selectively stimulated. ATF4 induces the expression of downstream target genes such as GRP78 and others (Ye and

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Koumenis, 2009). Under physiological conditions, the ER chaperone GRP78 is bound to PERK in ER membrane. When unfolded proteins accumulate in the ER lumen, GRP78 is attracted to bind to these unfolded proteins to support correct protein folding and is thereby released from PERK, which is consequently activated (Rao et al, 2002; Pfaffenbach and Lee, 2010). UPR activation results in an overall decrease in translation, increased protein degradation, and increased levels of ER chaperones, including GRP78 (Maattanen et al, 2010). Once sufficient GRP78 protein is available for binding and inactivation of PERK, the UPR will be shut down and ER functioning restored. So, the activation of UPR could protect the cells against the toxic misfolded proteins and make ER homeostasis return to normal.

Ginseng has long been used as a tonic and agent for prolonging life span in traditional Chinese medicine (TCM). Using modern technology, ginsenoside Rb_1 (Rb_1) was proved to be the main active compontent in ginseng. This compound showed the effect in improving learning and memory, increasing M-cholinergic receptors in central nerve system and accelerating cerebral protein and acetylcholine biosynthesis (Wang et al, 2001; Su et al, 2007; Liu et al, 2015). However, Rb_1 had immunoregulatory action in aged rats as well as enhanced the expression of IL-2 receptor alpha chain and inhibited the release of soluble IL-2 receptor (Liu and Zhang, 1996). On the other hand, Rb_1 had anti-oxidant activity (Lin et al, 2008) and anti-stress effect in antagonizing acute, chronic, and repeated stress induced reduction of sexual behavior and decrease of plasma andogen or estrogen (Lu et al, 2009).

However, the role of UPR pathway, e.g. PERK signaling pathway, has not been elucidated in ER stress mediated hypobaric hypoxia. Here, we demonstrate that the selective activation of PERK pathway is an early event of ER stress induced by exposure to hypobaric hypoxia. PERK-eIF2a pathway promotes the induction of ER chaperones and confers resistant to aggregated protein toxicity in neuronal cells. In the present study, the major objectives were to reveal the intrinsic mechanism of cellular changes in exposure to a hypoxic environment and observe the effect of ginsenoside Rb (Rb) on it.

2. Materials and methods

2.1 Animals

A total number of 60 male Sprague-Dawley (SD) rats (220–250 g), provided by Experimental Animal Center of Lanzhou General Hospital were used in this study. Animals were housed in stainless steel cages under a 12 h light/dark cycle at 22 ^{o}C, with free access to food and water. Animals were allowed to acclimate for at least 10 d prior to the experiment. All the experiments were conducted in accordance with the regulations of Lanzhou General Hospital for the use of experimental animals in research, and in conformity with the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Experimental design

Sixty male SD rats were randomly divided into three groups such as control, model, and Rb_1 groups. The rats in model and Rb_1 groups (n = 40) were directly put into hypoxia chamber and ascended to a height of 5000 m to establish the acute plateau stress model. The rats in the control and model groups (n = 20) received ig administration of distilled water. The water solutions of Rb_1 (20 mg/kg) were ig given to the rats in Rb_1 group each day. These rats were treated for 7 d. After 7 d, the learning and memory were measured by the spatial working memory test and passive avoidance test. Then, the rats were sacrificed for detection by Western blotting.

2.3 Behavioral tests

Spatial working memory was assessed by recording spontaneous alternation behavior in a Y-maze 7 d after the Aβ42 peptide injection and 12 h after the last administration of TSG or vehicles. Each rat was placed at the end of one random arm and allowed to move freely in the maze during an 8-min session. The series of arm entries, including possible returns into the same arm, were automatically recorded by a computer. An alternation was defined as entries into all three arms consecutively. The number of maximum alternations was therefore the total number of arm entries minus two. The percentage of alternation was calculated as actual alternations/ maximum alternations.

The step-down passive avoidance behavior was also examined to assess the long-term memory (Norboo et al, 2011; Ye and Koumenis, 2009). Training was carried out one day after the Y-maze test and consisted of two similar sessions at one day interval. Each rat was placed on a wooden platform set at the center of a cage with a grid floor. When the rat stepped down, electric shocks (0.5 mA) were delivered using an isolated pulse stimulator (Model 2100, AM Systems, WA). The retention test was performed one day later. Each rat was placed again on the platform and the step down latencies were recorded with the timeout of 300 s.

The animals were trained to criterion in the passive avoidance apparatus. On the basis of additional experience gained with other animals in this paradigm, training parameters were as follows: a 0.5 mA foot shock for 1 s was used in this experiment and animals were kept in the dark chamber for 15 s before being returned to their home cages between sessions. Training continued until an avoidance criterion for 300 s was achieved. Animals were tested for retention only once.

2.4 Western blotting

Proteins of the subcellular fractions were extracted as described previously and transferred to nitrocellulose membrane after SDS-PAGE. Membranes were blocked with 5% nonfat dry milk in buffer containing 1% BSA, 0.1 mol/L NaCl, 0.01 mol/L Tris-HCl (pH 7.5), and 0.1% Tween 20. Membranes were then probed with antibodies directed against