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Altered mitochondrial biogenesis and its fusion gene expression is involved in the high-altitude adaptation of rat lung

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ABSTRACT

Intermittent hypobaric hypoxia-induced preconditioning (IHH-PC) of rat favored the adaption of lungs to severe HH conditions, possibly through stabilization of mitochondrial function. This is based on the data generated on regulatory coordination of nuclear DNA-encoded mitochondrial biogenesis; dynamics, and mitochondrial DNA (mtDNA)-encoded oxidative phosphorylation (mtOXPHOS) genes expression. At 16th day after start of IHH-PC (equivalent to 5000 m, 6 h/d, 2 w of treatment), rats were exposed to severe HH stimulation at 9142 m for 6 h. The IHH-PC significantly counteracted the HH-induced effect of increased lung: water content; tissue damage; and oxidant injury. Further, IHH-PC significantly increased the mitochondrial number, mtDNA content and mtOXPHOS complex activity in the lung tissues. This observation is due to an increased expression of genes involved in mitochondrial biogenesis (PGC-1α, ERRα, NRF1, NRF2 and TFAM), fusion (Mfn1 and Mfn2) and mtOXPHOS. Thus, the regulatory pathway formed by PGC-1α/ERRα/Mfn2 axes is required for the mitochondrial adaptation provoked by IHH-PC regimen to counteract subsequent HH stress.

1. Introduction

The reduced partial pressure of oxygen (O_2) at high altitude has several consequences for the O_2 economy of the body. In particular, the major consequences of hypoxia are related to the limitation in cellular energy supply that it imposes through the arrest of mitochondrial respiratory chain electrons transport and the resulting ATP depletion. So, the essence of acclimatization is the enlargement of this limiting link to restore ATP production per unit of tissue mass. This is essential in restoring the behavioral and physical activity of the organism. In lieu of acclimatization, several medications are available that effectively decrease susceptibility to altitude illness (Hackett and Roach, 2001). However, none of these pharmaceutical interventions (e.g., acetazolamide, nifedipine and sildenafil) except dexamethasone directly improves physical work performance of the individual thus, unable to prevent the exacerbating altitude induced work impairment. Hence, altitude acclimatization remains the best approach to negating the detrimental effects of altitude on health and human performance since it promotes the development of natural mechanisms of adaptation. But, acclimatization to altitude is a relatively slow process, usually attained by staying several days or weeks at progressively higher altitudes. Hence, intermittent exposure to hypobaric hypoxia (IHH) in hypobaric chambers has been used as an alternative procedure to induce acclimation to altitude (Yoshino et al., 1990; Richalet et al., 1992; Lukyanova et al., 1995), and also to improve performance (Vallier et al., 1996; Ricart et al., 2000) in the event of real exposure to HH conditions.

Transient repetitive exposure of any organism to moderate levels of stress establishes a sustained, protective response against its lethality is termed as preconditioning (PC). Two forms of PC have been described. Immediate PC occurs within minutes of stimulus initiation which are attributed to cellular changes related to the activity or functioning of tissue enzymes, response of second messengers, and alterations in ion channels. While delayed PC principally is due to the de novo protein synthesis which takes several hours to develop, and such altered physiology will persist for several days (Zhang et al., 2000; Busija et al., 2008). Several studies have demonstrated that whole body PC with IHH or intermittent hypoxia leads to increased tolerance to severe hypoxic/HH/ischemic effect on various organs like, heart, brain and lung (Lukyanova et al., 1995; Lin et al., 2011; Wang et al., 2012). However, most of

Abbreviations: ATP6/8, ATP synthetase6/8; Cyt b, cytochrome b; CO I/II/III, cytochrome c oxidase I/II/III; CS, citrate synthase; Drp-1, dynamin-related protein-1; ERRα, estrogen-related receptor-α; HH, hypobaric hypoxia; IHH, intermittent hypobaric hypoxia; Mfn1/2, mitofusin1/2; mtDNA, mitochondrial DNA; ND1 to 6* 4L, NADH-dehydrogenase-ubiquinone reductase1 to 6* 4L; nDNA, nuclear DNA; NRF1/2, nuclear respiratory factor 1/2; OXPHOS, oxidative phosphorylation; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PC, preconditioning; Fis1, fission 1; ROS, reactive oxygen species; RNS, reactive nitrogen species; TFAM, mitochondrial transcription factor A.

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such studies with PC of lungs have focused on the restoration of systemic adaptive physiology like, enhanced hypoxic ventilatory response, improved gas-exchange function, increase in blood O_2 transport activity through erythropoiesis, contractile response of tracheal smooth muscle, arterial O_2 saturation and decreased pulmonary vascular permeability (Rodriguez et al., 1999; Zhang et al., 2004; Chakrabarty and Fahim, 2005; Guner et al., 2007). But such adaptations in lungs are not only the result of increased strength of external respiratory systems which transport O_2 from the environment to the tissue mitochondria. Other important factors in such adaptation are changes that follow at the cellular level, which is the increased ability of tissue to utilize the low O_2 content of blood and increased efficiency of mitochondrial system to maintain cellular energy homeostasis for workload of hypoxic ventilation. In turn, mitochondrial function is influenced by the coordinated expression of genes involved in mitochondrial biogenesis as well as its dynamics and mitochondrial oxidative phosphorylation (mtOXPHOS).

The role of nDNA-encoded "transcription co-regulator" called peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) along with nuclear receptor, like, estrogen-related receptor-α (ERRα) is important in regulating cellular energy homeostasis and mitochondrial biogenesis (Wu et al., 1999; Mootha et al., 2004; Schreiber et al., 2004). Nuclear respiratory factors (NRF1/2) are shown to act downstream of "PGC-1α-ERRα" axes in driving the transcription and replication of the mitochondrial genome through activating mitochondrial transcription factor A (TFAM) (Wu et al., 1999; Ekstrand et al., 2004). Hence, through these regulatory factors nuclear DNA (nDNA) is involved in controlling the expression of mitochondrial DNA (mtDNA)-encoded genes, like, seven subunits of NADH-dehydrogenase-ubiquinone reductase (ND1–ND6 and ND4L), cytochrome b (Cyt b) of ubiquinol-cytochrome c reductase, three subunits of cytochrome c oxidase (CO I, CO II and CO III) and two subunits of ATP synthetase (ATP6 and 8) involved in mtOXPHOS (Fernandez-Silva et al., 2003). In addition to mitochondrial biogenesis, the coordination of mitochondrial function is dependent on dynamics nature of mitochondria, which is controlled by two opposing processes, mitochondrial fission and fusion. In mammals, mitofusin 1 and 2 (Mfn1 and Mfn2) control the mitochondrial fusion process, whereas, mitochondrial fission 1 (Fis1) and the dynamin-related protein 1 (Drp1) are involved in fission of mitochondria (Hales and Fuller, 1997; Smirnova et al., 1998; James et al., 2003).

In our previous study, we have shown that exposure of rats to severe HH conditions decreased the expression of ERRα, which apparently resulted in the decreased level of mitochondrial number, mtDNA content and mtOXPHOS in the lung tissue (Chitra and Boopathy, 2013). Along with these results, the emerging paradigms suggest that, during high altitude acclimatization, multiple adaptive pathways through the regulation of nDNA-encoded regulators like ERRα are required to sustain tissue mitochondrial respiratory function. However, at present there is paucity of information with respect to functional significance and molecular mechanisms underlying such changes in lung under IHH exposure-induced PC (IHH-PC) conditions. It is a well known fact that these adaptive changes are accompanied by the activation of nucleic acid and protein synthesis in various tissues. Hence, we hypothesized that IHH-PC co-ordinately up-regulates nDNA-encoded mitochondrial regulatory genes and even the mitochondrial number in lung, as part of an adaptive response to sustain normal physiology. Therefore, the aim of the present study was to examine the effects of HH (exposure to 9142 m for 6 h) on alveolar edema, cellular toxicity and oxidative stress in rat lung subjected with or without IHH-PC regimen (exposure to 5000 m for 6 h/day for 14 days). Also, to determine whether the beneficial effect of IHH-PC is accompanied by alteration in transcript levels of nDNA-encoded regulators of mitochondrial biogenesis, dynamics and mtDNA-encoded mtOXPHOS genes. Such an understanding would link the efficiency of mtOXPHOS and respiratory function to sustained lung function in response to an increased HH stress.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (160–180 g) were used in the study. The animals were maintained in the Animal house of the institute at 28 °C ± 2 °C and subjected to a 12–12 h light–dark cycle with free access to food and water. The experiments were carried out in accordance with the guidelines of the ethics committee of the institute Bharathiar University, Coimbatore, India.

2.2. Hypobaric hypoxic exposure

The rats were randomly assigned to four experimental groups ($n = 6$): normobaric normoxia (NN); HH; IHH-PC regimen; and IHH-PC regimen followed by HH exposure. IHH-PC regimen was imposed by exposure of rats to simulated altitude of 5000 m for 6 h/day lasting for 14 days. After the next day, beginning on the 16th day of IHH-PC regimen, rats were continued in NN or exposed to severe simulated altitude of 9142 m in animal decompression chamber at 28 °C for 6 h. The airflow in the chamber was 2 L/min with relative humidity maintained at 50–55%. Rats were provided with food and water ad libitum during the experimental protocol. All the animals survived the high-altitude exposure. After exposure to HH, the animals were sacrificed by cervical dislocation and lungs were dissected out. One portion of fresh lung tissue from the various experimental groups was used for extraction of total RNA and isolation of mitochondria. The second portion was snap frozen and stored at −80 °C for biochemical and enzyme assay.

2.3. Analysis of edema formation in lung

Lung water content was used as an index of edema formation. The water content of the lung tissue was calculated as the difference between wet weight and dry weight and expressed as mg of water per mg of dry tissue (Shukla et al., 2011).

2.4. Preparation of lung tissue

All the lung tissue obtained for biochemical measurements was homogenized as described previously (Chitra and Boopathy, 2013). The protein concentrations of the tissue homogenates were determined using BSA as standard (Lowry et al., 1951).

2.5. Assay of lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity in the lung homogenate was quantified using reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (Abe and Matsuki, 2000).

2.6. Measurement of reactive nitrogen species (RNS)

The total of nitric oxide (NO) present in the supernatant of lung homogenate was assayed using Griess reagent (Sigma) in accordance with the manufacturer's instructions (Boyaci et al., 2006).