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Research Report

Mitochondrial function in rat cerebral cortex and hippocampus after short- and long-term hypobaric hypoxia

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ABSTRACT

Taking into account the importance of aerobic metabolism in brain, the aim of the present work was to evaluate mitochondrial function in cerebral cortex and hippocampus in a model of sustained hypobaric hypoxia (5000 m simulated altitude) during a short (1 mo) and a long (7 mo) term period, in order to precise the mechanisms involved in hypoxia acclimatization.

Hippocampal mitochondria from rats exposed to short-term hypobaric hypoxia showed lower respiratory rates than controls in both states 4 (45%) and 3 (41%), and increased NO production (1.3 fold) as well as eNOS and nNOS expression associated to mitochondrial membranes, whereas mitochondrial membrane potential decreased (7%). No significant changes were observed in cortical mitochondria after 1 mo hypobaric hypoxia in any of the mitochondrial functionality parameters evaluated.

After 7 mo hypobaric hypoxia, oxygen consumption was unchanged as compared with control animals both in hippocampal and cortical mitochondria, but mitochondrial membrane potential decreased by 16% and 8% in hippocampus and cortex respectively. Also, long-term hypobaric hypoxia induced an increase in hippocampal NO production (0.7 fold) and in eNOS expression. A clear tendency to decrease in H_2O_2 production was observed in both tissues.

Results suggest that after exposure to hypobaric hypoxia, hippocampal mitochondria display different responses than cortical mitochondria. Also, the mechanisms responsible

Abbreviations: $DiOC_6$, 3, 3''-dihexyloxacarbocyanine iodide; EDTA, ethylenediaminetetraacetic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HRP, horseradish peroxidase; H_2O_2, hydrogen peroxide; $^{\bullet}NO$, nitric oxide; ROS, reactive oxygen species; SOD, superoxide dismutase

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for acclimatization to hypoxia would be time-dependent, according to the physiological functions of the brain studied areas. Nitric oxide metabolism and membrane potential changes would be involved as self-protective mechanisms in high altitude environment.

1. Introduction

The mechanisms of adaptation to low O_2 pressures have been a puzzle for many years. The well-known systemic responses are not energetically economic mechanisms, can only buffer but not prevent the fall in mixed venous blood PO_2 below normal limits, and are mostly absent or attenuated in organisms genotypically adapted to high altitude, suggesting that complete adaptation would take place at cellular level, mainly through changes in O_2 utilization by mitochondria. However, although *in vivo* oxidative functions are O_2 dependent at relatively high arterial concentrations, affinity of isolated mitochondria for O_2 was reported to be too high to be involved in the control of O_2 consumption, and measurements of mitochondrial respiration and oxidative phosphorylation, as well as the activity of respiratory complexes, were mostly unchanged in animals acclimatized to hypoxia (Costa, 2007). The finding that nitric oxide ($^{\bullet}$NO) has an essential role as a physiological regulator of the respiratory chain provides a hypothesis to fill the gap between the observations previously mentioned. The $^{\bullet}$NO-inhibited respiration lowers the steepness of intracellular O_2 gradients and allows O_2 to diffuse further along its gradient, extending the space of adequate tissue oxygenation away from the blood vessel. Endogenous$^{\bullet}$NO production has been shown to inhibits tissue O_2 consumption in hippocampal slices at physiological O_2 concentration, strongly supporting the current paradigm for O_2 and $^{\bullet}$NO interplay in the regulation of cellular respiration (Ledo et al., 2010).

Previous studies of our research group showed that nitric oxide synthase (NOS) was modulated in heart mitochondria during acclimatization to hypobaric hypoxia in association with cardioprotection (La Padula et al., 2008). Brain is particularly sensitive to oxygen deficiency. Morphological changes in the hippocampus have been observed by Hale et al. in hypobaric hypoxia (Shukitt-Hale et al., 1996). Hypoxia adaptative responses such as metabolic reprogramming and reactive oxygen species (ROS) neutralization depends on the different brain regions and cell types (Van Elzen et al., 2010). Cerebral cortex exposed to acute hypobaric hypoxia (3, 6, 12 and 24 h) is less vulnerable to hypoxia than hippocampus due to its high content of antioxidant enzymes (Sharma et al., 2013). Maiti and colleagues (2006) reported that hippocampus and striatum are more susceptible to hypoxia than cerebral cortex after animal exposure for three or seven days to a simulated altitude of 6100 m, showing increased free radicals production, $^{\bullet}$NO levels, lipid peroxidation and decreased amount of antioxidant defenses (Maiti et al., 2006). Furthermore, the level of oxidative stress is time-dependent. Exposure to a simulated altitude of 6100 m during 14 days was found to reduce the oxidative stress in rat hippocampus when compared to seven days of exposure (Hota et al., 2007).

Mitochondrial oxygen consumption is required for ATP generation, and cell survival is threatened when cells are deprived of oxygen. Due to the fact that mitochondria represent the final step of the interaction with molecular oxygen, they are able to sense the concentration gradient of oxygen arriving from the environment (Guzy and Schumacker, 2006; Lukyanova, 2013). Then, the mitochondrial respiratory chain acts as a signal-transforming metabolic system which activates the functional response to hypoxia.

Previous data from hypobaric hypoxia models have shown enhanced $^{\bullet}$NO production with the implication of nNOS in the CNS. Acute hypobaric hypoxia has been demonstrated to increase nNOS mRNA expression in Purkinje cells (Prabhakar et al., 1996) and $^{\bullet}$NO levels production in extracts from whole brain (Malyshev et al., 1999), in the supraoptic and paraventricular nuclei (Luo et al., 2000) and in cerebellum (Serrano et al., 2003).

Studies in chronic hypoxic conditions compatible with acclimatization are scarce, even though clinically relevant, because adaptation to hypoxia renders protection to nerve cells of brain (Goryacheva et al., 2010; Manukhina et al., 2008; Mashina et al., 2006). Taking into account the importance of mitochondria in oxygen homeostasis, the aim of the present work was to evaluate mitochondrial function in cerebral cortex and hippocampus in a model of sustained hypobaric hypoxia (1 and 7 months) that simulates high-altitude (5000 m), in order to precise the mechanisms involved in hypoxia acclimatization.

2. Results

2.1. *Oxygen consumption*

Malate-glutamate dependent oxygen consumption was measured in state 4 (resting or controlled respiration) and in state 3 (active respiration, the maximal physiological rate of O_2 uptake and ATP synthesis) (Boveris et al., 1999). The respiratory control ratio (the most sensitive indicator of mitochondrial oxidative phosphorylation coupling) was calculated as the relationship between state 3/state 4 respiration rates.

Table 1 shows oxygen consumption rates of cortical and hippocampal intact mitochondria isolated from different experimental groups. Hippocampal mitochondria from animals exposed to short-term hypobaric hypoxia showed lower respiratory rates than controls, 45% in state 4 ($p<0.05$) and 41% in state 3 ($p<0.05$). No significant changes were observed in state 4 and state 3 respiratory rates in cortical mitochondria. Respiratory control of cortical and hippocampal mitochondria from 1 mo hypoxic rats were similar to control