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PRACA ORYGINALNA

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The effect of intermittent hypoxic training on lung and heart tissues of healthy rats

Wpływ interwałowego treningu hipoksycznego na płuca i serca zdrowych szczurów

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Abstract

Introduction: Recently, particular attention has been focused on the problem of the beneficial influence of intermittent hypoxia (IH) on the human organism. However, knowledge regarding the negative effects of intermittent hypoxic training (IHT) on cellular adaptive mechanisms remains limited.

The aim of the present study was to investigate: 1) lung and heart ultrastructural changes under IHT; and 2) the adequateness of morphological and morphometric methods to determine the constructive and destructive displays of hypoxia.

Material and methods: Adult male Wistar rats underwent IHT every day for 7–28 days. Lung and heart tissues were assessed by morphological and cellular morphometric methods.

Results: We observed evident ultra structural changes of the lung air-blood barrier (LABB) by the 7–10th day of training. Structural damage of LABB was most considerable after 2 weeks of IHT exposure, its ultrastructure partially normalized by the end of the IHT 4-weeks course: there was diminishing of LABB hydration and disappearance of areas of its destruction. The structural changes in the heart blood-tissue barrier (HBTB) were considerably less marked compared with those in LABB during the 1st and 2nd weeks of training. Heart tissue structural changes increased by the end of the fourth week of IHT. Both tissue cells revealed no significant necrotic damage of mitochondria after IHT, while changes relating to the energy-directed restructuring of mitochondria were observed. We hypothesized that acute moderate hypoxia promotes a specific type of mitosis in lung and heart tissues.

Conclusions: Ultrastructural changes in the rat lung and heart tissues depend on IHT duration. The phenomenon of "micro-mitochondria within mitochondria" is an additional adaptive mechanism for IH exposure.

Key words: intermittent hypoxic training, ultra-structural lung and heart tissues changes, biological barriers thickness, mitochondria, micro-mitochondria.

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Introduction

Recently, particular attention has been focused on the problem of the positive influence of IH on the human organism [1–5]. Clinical application of IHT was suggested based on this experience [6, 7].

Favourable effects of IHT deal with the adaptive changes in organs and tissues responsible for oxygen uptake and transport as well as with the increase in vasodilatory factors (nitric oxide, particularly) with anti-hypertensive effects, adaptive changes in the immune system, and the detoxication system of the liver associated with the cytochrome P-450 system activation [6].

IHT is characterized by a progressive increase in ventilation, haemodynamics, and erythropoesis to enhance oxygen delivery to tissues and optimize oxygen utilization. IHT improves the energy production by increasing of mitochondria morphogenesis, activating electron flux through mitochon-

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drial respiratory complex I, and increasing the efficiency of oxidative phosphorylation [5, 6].

The IHT adaptation-compensatory reactions include both tissue and cellular mechanisms. Ultrastructural changes in mitochondria apparatus and energy metabolism resulting from hypoxia seem to be of great importance [7–11]. These effects are crucial for the adaptive response to hypoxia. However, knowledge regarding the negative effects of IHT on cellular adaptive mechanisms remains limited.

The aim of this study was to investigate: 1) lung and heart ultrastructural changes under IHT; and 2) the adequateness of morphological and morphometric methods to determine the constructive and destructive displays of hypoxia.

Materials and methods

Forty-two adult male Wistar rats with body mass 250–300 g were enrolled to the study. The influence of IHT upon the ultrastructure of lung and heart tissues was assessed. All experimental procedures with animals relating to this study comply with the guiding principles for experimental procedures as set forth in the Declaration of Helsinki (2000) and the „European Convention about defence of vertebrates used for experiments or in another scientific aims" (Strasburg, 1986).

Adult male Wistar rats underwent IHT every day for 7–28 days. The study of IHT effects was conducted in three groups of rats on 7–10 days, 2 and 4 weeks after the beginning of training. Each group consisted of 10 rats.

Animals were placed in an impermeable chamber, through which a hypoxic gas mixture or atmospheric air passed at a constant rate (carbon dioxide absorption was assessed continuously). The IHT session included alternating periods of hypoxia (12% O_2 in inhaled gas mixture) followed by a subsequent oxygenating period (breathing with ambient air). Each exposure time lasted for 15 min and each session was composed of 5 periods. The control group of rats (12 animals) was placed into a similar chamber, breathing with ambient air.

Tissues were prepared by generally accepted methods and examined by electron microscopy [12]. Tissue specimens from the apex of the heart and peripheral areas of the right lung were processed by 2.5% glutaraldehyde and 1% OsO_4 with the subsequent dehydration in the increasing concentrations of ethanol and then embedded in Epson-araldite. Ultrathin slices (40-60 nm) were contrasted by uranyl acetate and lead citrate and examined by electron microscope JEM 100 — CX (Japan). Reagent kits were purchased from SIGMA--ALDRICH (Germany).

The morphometric evaluation of lung and heart tissue specimens was executed by Image Tool Version 3 (USA) with recognition of Ewald Weibel approaches [13, 14]. At every point of the research project we carried out 80–100 morpho- and stereometric measuring. The results were analysed by Student's t-test, and $p < 0.05$ was considered as statistically significant.

Results

Considerable ultrastructural changes of LABB (total and local oedema, vacuolization, etc.) were marked by the 7–10th days of training. The mean arithmetic thickness of LABB increased 1.5–2 fold, mainly due to the thickened alveolar epithelium (Tab. 1). The structural lesions of LABB remained constant at the end of the second week of IHT exposure and were accompanied by local destruction of its separate layers or the whole barrier. The thicknesses of all layers of LABB increased with the observed tendency of alveolar epithelium oedema to decline. The changes in mitochondria structure were attributed to the optimal ratio between aerobic oxidation and glycolysis. Partial normali-

Table 1. Dynamics of changes in mean arithmetic thickness (τ) of lung air-blood barrier (LABB) and its layers (nm) under intermittent hypoxic training (M ± m)

Duration of experiment	τ			
	LABB	Alveolar	Interstitial layer epithelium layer	Capillary endothelium layer
Control (n = 12; a = 100)	163 ± 8	71 ± 5	49 ± 3	63 ± 7
7–10 days of IHT (n = 10; a = 87)	326 ± 26*	118 ± 21*	66 ± 9*	94 ± 7*
2 weeks of IHT (n = 10; a = 80)	339 ± 19*	90 ± 9*	96 ± 11*	91 ± 8*
4 weeks of IHT (n = 10; a = 93)	215 ± 12*^{0}	78 ± 8^0	111 ± 13*^{0}	82 ± 5*

n — number of animals; a — amount of measuring; *significant difference between IHT and control values ($p < 0.05$); 0 — significant difference between the beginning (7–10 days of IHT) and the end (4 weeks of IHT) of training

Table 2. Dynamics of changes in mean arithmetic thickness (τ) of heart blood-tissue barrier (HBTB) and its layers (nm) under intermittent hypoxic training (M ± m)

Duration of experiment	τ		
	HBTB	Pericapillary spaces	Capillary endothelium
Control (n = 12; a = 100)	221 ± 14	126 ± 15	86 ± 9
7–10 days of IHT (n = 10; a = 80)	287 ± 10*	163 ± 18*	112 ± 19
2 weeks of IHT (n = 10; a = 87)	298 ± 23*	170 ± 20*	116 ± 13*
4 weeks of IHT (n = 10; a = 95)	394 ± 36*^{0}	235 ± 27*^{0}	186 ± 21*^{0}

n — number of animals; a — amount of measuring; *significant difference between IHT and control values ($p < 0.05$); 0 — significant difference between the beginning (7–10 days of IHT) and the end (4 weeks of IHT) of training

zation of the ultrastructure of LABB was observed after 4 weeks of IHT sessions. The thickness of LABB considerably decreased (by 51–57%) as compared to the beginning of IHT.

On the 1st and 2nd weeks of training the ultrastructural changes in HBTB were considerably less expressed than those in LABB. Separate areas of precapillary oedema were detected. The amplification of endothelial pinocytosis was accompanied by a 30-35% increase in HBTB thickness deriving from the involvement both of pericapillary spaces and capillary endothelium (Tab. 2).

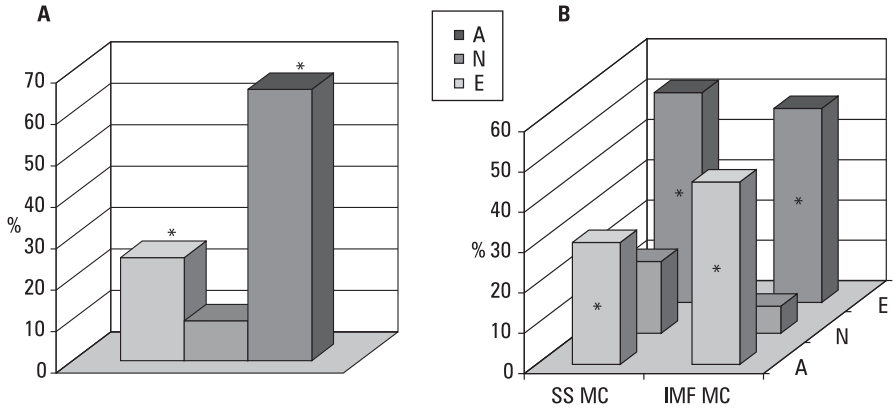
By the 4th week of IHT we observed considerable intensification of structural changes, especially in the mitochondria of cardiomyocytes. Various degrees of mitochondria damage were revealed (from moderate swelling to the complete destruction of mitochondria). The mean arithmetic thickness of HBTB increased more than 1.8 fold compared with the control values due to the increased oedema.

The features of the structural damage of mitochondria evoked by hypoxia allow specification of the type of changes [15, 16]. We classified the alterations in mitochondria ultrastructure as apoptotic, necrotic, and those related to the metabolic rate increase.

We revealed no significant necrotic damage in the lung and myocardial tissue mitochondria relating to hypoxia exposure, while numerous changes resulting from the energy-directed remodelling of their ultrastructure were detected in both tissues (Fig. 1).

Mitochondria of cardiomyocytes have demonstrated an increased proportion of apoptotic (mitoptotic) changes under hypoxia exposure. Previous studies have proven this process to be physiological and positive, deriving from different impacts on the organism [8, 15–18].

The current study evidenced a new type of mitoptosis in the lung and heart tissues under acute moderate hypoxia exposure: the formation



Zero level corresponds to control; A — apoptotic changes, N — necrotic changes, E — energy-directed changes, SS — subsarcolemmal subpopulation of mitochondria (SS MC), IMF — intramyofibrillar subpopulation of mitochondria (IMF MC), *Significant difference between IHT and control values ($p < 0.05$)

Figure 1. Types of structural changes of mitochondria under intermittent hypoxia in lung (a) and heart (b) tissues after 4 weeks of IHT

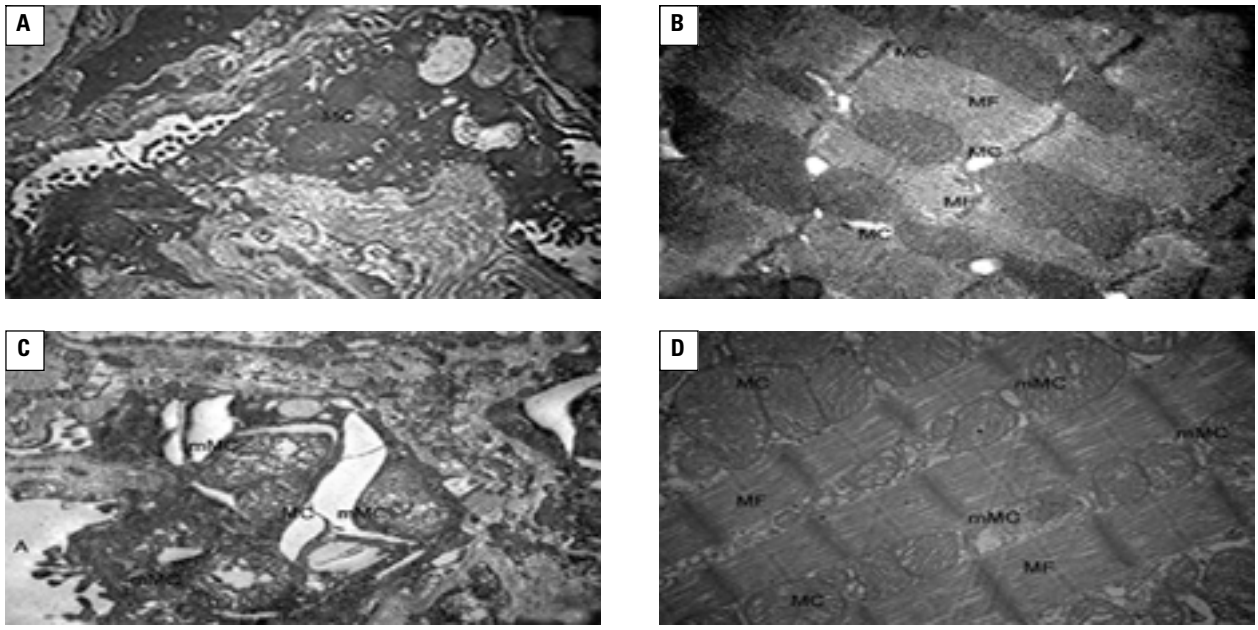


Figure 2. Ultrastructure of mitochondria in lung and heart tissue; **A** — lung tissue in control animal, **B** — heart tissue in control animal, **C** — lung tissue in animal after 4 weeks of IHT, **D** — heart tissue in animal after 4 weeks of IHT; MC — mitochondria, MF — myofibrils, A — alveoli, mMC — "mitochondria in mitochondria"; × 9600

of micro-mitochondria within the mitochondria (Fig. 2 C, D; 3 A, B).

Discussion

It was shown that ultrastructural changes in the rat lung and heart tissues depend on IHT duration.

In the initial stages of IHT (7–10th days of training) the intermittent hypoxia impact on the organism is not different from that of the acute hypoxia exposure [8], resulting in damage of the cells and separate organelles structure.

The results of our study demonstrated that structural changes of the lung under IHT developed much earlier than those of heart tissue, whereas the substantial normalization of lung ultrastructure occurred during a training period. The most evident damages were detected in the heart tissue by the end of intermittent hypoxia session. The marked changes of ultrastructure of lung and heart tissues developing under the influence of IHT, from one side, are connected with the violations that are characteristic to hypoxic hypoxia (oedema and partial destruction of biological barriers, structural changes of mitochondria with violation of regularity and integrity of crests, partial or complete vacuolisation, etc.) [8]. On the other hand, organospecificity in time and expression of the ultrastructure changes took place: more early and expressive signs of violations are marked in lung tissue, while at the same time, practically complete normalization of its ultrastructure was registered at the end of the 4th week of IHT with maintenance of insignificantly increasing LABB thickness. The ultrastructure of the heart tissue was not normalized until the end of the training period.

The current study revealed the redistribution of mitochondria ultrastructure that could be a result of the intensification of energy production due to IHT. The above-specified could serve as an example of a positive training effect during intermittent hypoxia and an explanation of the beneficial impact of mild hypoxia exposure on tissues aimed at supporting energy exchange under unfavourable conditions [6].

The phenomenon of "intramitochondrial mitochondria" formation under IH exposure verifies the recently described effects of prolonged anoxia (6–72 h) on myocardial tissues in vitro [17––20]. This condition accounted for the micromitochondria structure and cytochrome C function maintenance.

The formation of small mitochondria within the damaged organelles is observed only under hypoxic hypoxia but not under any of the other conditions resulting in hypoxia (for example, blood loss or stress) [8]. Possibly, mitoptosis starts only at the decrease of oxygen concentration in inspired air and oxygen tension in the arterial blood. This is not attributed to the secondary tissue hypoxia due to the circulatory or chemical dama-

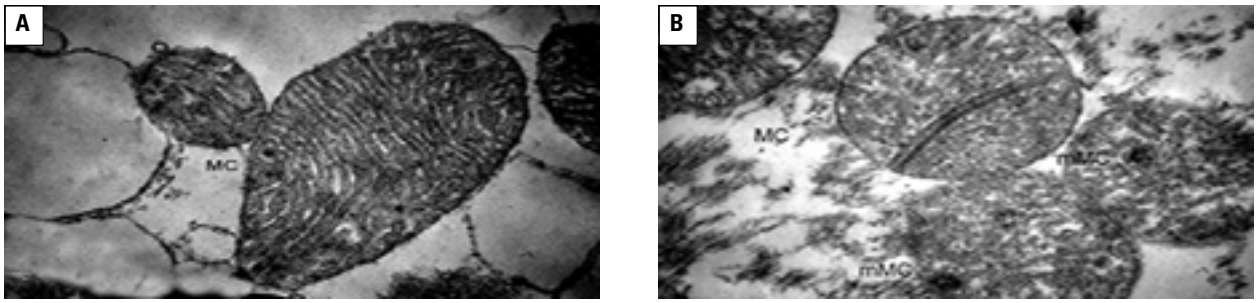


Figure 3. Example of micromitochondria formation in the separately taken mitochondria, **A** — control mitochondria, **B** — mitochondria, containing micromitochondria; MC — mitochondria, mMC — "mitochondria in mitochondria"; × 20000

ge resulting in the reduced oxygen delivery to tissues. Our findings define mitoptosis as both a self-destructive and self-reproductive process, simultaneously. Such structural alterations of mitochondria are more common for heart tissues; however, they are also detected in the lung.

We assume the intramitochondrial mitochondria formation to be one of the mitoptosis mechanisms to support energy production in mitochondrial apparatus under hypoxia exposure, and the additional adaptive mechanism in the hypoxic training.

Conclusions

1. Intermittent hypoxic training provokes morphological and functional changes in the lung and heart tissues with different degrees of expression.
2. The mitochondria morphofunctional restructuring of the lung and heart tissues may be one of the important mechanisms of hypoxic training, resulting in the optimization of energy production.
3. Intramitochondrial mitochondria formation is one of the mitoptosis pathways to support energy production in mitochondrial apparatus under hypoxia exposure, and the additional adaptive mechanism in the hypoxic training.

Conflict of interest

Authors do not declare current or perceived conflict of interst.

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