Time course of differential mitochondrial energy metabolism adaptation to chronic hypoxia in right and left ventricles

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Abstract

Objective: The present study was designed to characterize mitochondrial adaptation to chronic hypoxia (CH) in the rat heart. Mitochondrial energy metabolism was differentially examined in both left and right ventricles since CH selectively triggers pulmonary hypertension and right ventricular hypertrophy.

Methods: Rats were exposed to a hypobaric environment for 2 or 3 weeks and compared with rats maintained in a normoxic environment. Oxidative capacity (oxygen consumption and ATP synthesis) was measured in saponin-skinned fibers with glutamate or palmitoyl carnitine as substrates. Enzymatic activities of mitochondrial respiratory chain complexes were measured on tissue homogenates. Morphometric analysis of mitochondria was performed on electron micrographs. Mitochondrial DNA was quantified using Southern blot analysis.

Results: Whereas oxidative capacity of both ventricles was decreased following 21 days of CH, oxygen consumption and ATP synthesis was maintained with the glutamate substrate in the right ventricle following 14 days of CH. As for the oxidative capacity, enzyme activities were decreased only in the left ventricle following 14 days of CH and in both ventricles following 21 days of CH. These functional alterations were associated with an increase in numerical density and a decrease in size of mitochondria without a change in volume density in both ventricles. Finally, 21 days of CH also decreased the ratio of mitochondrial DNA to nuclear DNA in both ventricles.

Conclusions: CH alters morphometry and function of mitochondria in the heart, but this effect is delayed in the right compared to the left ventricle, suggesting some adaptive processes at the onset of right ventricular hypertrophy.

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Keywords: Chronic hypoxia; Energy metabolism; Mitochondria; Oxidative phosphorylation

1. Introduction

Chronic hypoxia (CH) occurs under either physiologic (high altitude) or pathologic conditions (e.g., chronic pulmonary diseases). Previous studies in animals and humans have shown complex mechanisms of metabolic adaptation during exposure to CH [1]. One of these initial adaptive mechanisms is a marked suppression of adenosine 5’-triphosphate (ATP) demand and supply pathways [2]. This regulation allows ATP level to remain constant, even while ATP turnover rate greatly declines. Thus, many ATP-dependent processes, like ion pumping or protein synthesis, are down-regulated during exposure to CH [3]. Whereas CH is characterized by a reduction in oxygen supply, it selectively induces a chronic functional overload of the
right ventricle (RV) due to pulmonary hypertension that requires additional energy to overcome this increase in pulmonary vascular resistance.

In the heart, mitochondria provide, through oxidative phosphorylation, more than 95% of the energy supply in the form of ATP. In the course of oxidative phosphorylation, electrons are transferred through the respiratory enzymatic complexes of the mitochondrial inner membrane, thus releasing energy used to generate a proton gradient across the membrane. This protonmotive force allows the synthesis of ATP from adenosine 5'-diphosphate (ADP) and phosphate by the ATP-synthase. Mitochondrial metabolism is involved in CH adaptation via energy regulation, generation of reactive oxygen species, and apoptosis [4–7]. Most of the studies have examined the global cardiac response to CH despite the selective effect of CH on the RV. In this connection, few recent data from our, as well as other, laboratories have indicated that the adaptive mechanism to CH was different in left ventricle (LV) and RV. LV appeared very sensitive to CH and exhibited a rapid decrease in the oxidative capacity, whereas RV appeared not affected [8,9].

To examine whether ventricular hypertrophy was responsible for the maintained oxidative capacity of RV, we have compared mitochondrial energy metabolism adaptation to CH in RV and LV in the rat heart over an extended period of time of 21 days. Indeed, regarding the time course of CH-induced pulmonary hypertension, we have previously observed that RV hypertrophy increases linearly with the duration of exposure to CH from 1 to 3 weeks and then remains stable up to 4 weeks [10]. Our results indicate that CH decreases ATP synthesis as a consequence of an alteration in mitochondrial respiratory chain complexes in both ventricles but that this effect is delayed in the RV suggesting some specific, but transient, adaptive processes at the onset of right ventricle hypertrophy.

2. Materials and method

2.1. Chronic hypoxia

Adult male Wistar rats (aged 8–10 wk, weighing 220–240 g) were separated into two groups. One group (hypoxic rats) was exposed to a simulated altitude of 5000 m (barometric pressure 380 mmHg) in a well-ventilated, temperature-controlled hypobaric chamber for 14 or 21 days. The chamber was opened twice a week for a few minutes to clean the cages. The other control group (normoxic rats) was kept in the same room but not in the hypobaric chamber, with the same 12–12 h light–dark cycle. Free access to a standard rat diet and water was allowed throughout the experimental period.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996) and European Directives (86/609/CEE).

2.2. Heart preparation

Animals were killed by cervical dislocation, and the heart was quickly removed in a normoxic (i.e., equilibrated with air) cooled (4 °C) relaxing solution (solution 1: 10 mM EGTA, 3 mM Mg²⁺, 20 mM taurine, 0.5 mM dithiothreitol, 5 mM ATP, 15 mM phosphocreatine, 20 mM imidazole, and 0.1 M K⁺ 2-[N-morpholino]ethane sulfonic acid, pH 7.2); chemicals were from Sigma Chemical Company (St Louis, MO). The heart was then dissected and weighed. Pulmonary hypertension was assessed by measuring the ratio of RV free wall weight to the sum of septum plus LV free wall (LVS) weight [10–12].

2.3. Oxidative phosphorylation

Bundles of fibers (non-isolated cardiomyocytes) between 5 and 10 mg were excised from the endocardial surface of both LV and RV then permeabilized in solution 1 added with saponin 50 μg/ml. The bundle was then washed twice for 10 min each time in solution 2 (10 mM EGTA, 3 mM Mg²⁺, 20 mM taurine, 0.5 mM dithiothreitol, 3 mM phosphate, 1 mg/ml fatty acid-free bovine serum albumin, 20 mM imidazole, and 0.1 M K⁺ 2-[N-morpholino]ethane sulfonic acid, pH 7.2) to remove saponin. All procedures were carried out at 4 °C with extensive stirring. The success of the permeabilization procedure was estimated by determining the activity of the cytosolic lactate dehydrogenase and the mitochondrial citrate synthase in the medium. After 15–20 min of permeabilization, more than 60% of the cytosolic lactate dehydrogenase was found in the external medium, and the mitochondrial citrate synthase activity in the medium remained below 5% [13]. The oxygen consumption rate was measured polarographically at 30 °C using a Clark-type electrode (Hansatec, UK) connected to a PC computer that displayed on-line the respiration rate value (original software developed in the laboratory). Solubility of oxygen in the medium was considered to be equal to 450 nmol O/ml. Respiratory rates were determined in a 1-ml oxygraph cuvette containing one bundle of fibers in solution 2 with 10 mM malate plus 10 mM glutamate or 20 μM palmitoyl carnitine as substrates; 50 μM diadenosine 5′-pentaphosphate, 20 μM EDTA and 1 mM iodoacetate were also added to the cuvette to inhibit extramitochondrial ATP synthesis (via the glycolysis or the adenylate kinase) and ATP hydrolysis [14]. ADP-stimulated respiration, associated with ATP synthesis, was determined in the presence of 1 mM ADP. Basal respiration without ATP synthesis was measured after addition of 70 μM atractyloside and 1 μM oligomycin. After measurements, fibers were removed, dried on a precision wipe and weighted. Results were expressed in nanomoles of atom oxygen consumed per minute and per milligram wet weight of fiber. Under identical conditions, the mitochondrial ATP synthesis rate in skinned fibers was determined by bioluminescence measurement (luciferine-luciferase system) of the ATP produced after addition of 1
mM ADP [15]. The ATP Bioluminescence Assay Kit HS II from Roche Diagnostics GmbH (Mannheim, Germany) was used. At various time intervals after addition of ADP, 10-μl aliquots were withdrawn from the oxygraph chamber, quenched in 100 μl DMSO, and diluted in 5 ml ice-cold distilled water. Standardization was performed with known quantities of ATP measured under the same conditions. ATP synthesis rate was expressed in nanomoles ATP produced per minute and per milligram wet weight of fiber. The efficiency of oxidative phosphorylation was taken as the ratio of ATP synthesis rate to oxygen consumption rate (ATP/O) [14].

2.4. Enzyme activity

About 100 mg of LV or RV were minced and homogenized with a glass Potter homogenizer in a ice-cold medium (10% w/v) containing 225 mM mannitol, 75 mM sucrose, 10 mM Tris-HCl, 0.10 mM EDTA, pH 7.2. The homogenate was then centrifuged for 20 min at 650 g. The supernatant was collected and the protein concentration was determined [16]. Enzymatic activity was assessed using previously described spectrophotometric procedures (model UVIKON 940, KONTRON) and expressed in nanomoles of substrate transformed per minute and per milligram of protein. The enzyme activity of citrate synthase was measured as described by Srere in the presence of 4% Triton (vol/vol) by monitoring at 412 nm wavelength at 30 °C the formation of thionitrobenzoate dianion from the reaction of coenzyme A and 5,5′-dithiobis(2-nitrobenzoic acid) [17]. The enzyme activity of complex I, reduced nicotinamide adenine dinucleotide (NADH) ubiquinone reductase, was measured as described by Birch-Machin et al. [18]. The oxidation of NADH by complex I was recorded using the ubiquinone analog decylubiquinone as the electron acceptor. The decrease in absorption resulting from NADH oxidation was measured at 340 nm at 30 °C. Complex I activity was calculated from the difference in the rate before and after the addition of rotenone (2 μM), a specific inhibitor of complex I. The Complex II (succinate dehydrogenase) specific activity was measured by monitoring the reduction of 2,6-dichlorophenol indophenol at 600 nm at 30 °C, in the presence of phenazine methosulfate [19]. The oxidation of ubiquinol (UQH₂) by complex III (ubiquinol cytochrome c reductase) was determined using cytochrome c(III) as the electron acceptor [18]. The reduction of cytochrome c(III) was recorded at 550 nm at 30 °C. Complex IV (cytochrome c oxidase) was measured by the method described by Wharton and Tzagoloff using cytochrome c(II) as substrate [20]. The oxidation of cytochrome c was monitored at 550 nm at 30 °C.

2.5. Morphometric analysis

Three blocks were taken from each heart, and 10–20 electron micrographs of randomly chosen fields were obtained from each block. Myocardial fibers for electron microscopic examination were fixed using 2.5% glutaraldehyde and post-fixed in osmium tetroxide; they were then embedded in epoxy resin. Ultrathin sections were stained with saturated uranyl acetate and lead citrate. Mitochondrial volume density (Vv), numerical density (Nv) and mean volume (V) were determined in final prints of 161 cm² at magnification×17,250 according to the method of Weibel et al. [21] as follows.

- Vv is the relative volume fraction of the mitochondria determined as the relative surface fraction of the unit area comprised by all mitochondrion slices.
- Nv depends on mitochondria sections (NA) counted per standard measuring surface, on the size distribution (K) and, finally on the shape of the mitochondria, writing Nv=K.NA^3/2/β.Vv/k. The form coefficient β for ellipsoidal structures is a function of the longitudinal and transverse diameter. On the basis of previous studies it was assumed to be β=2 in the heart [22]. K is determined by the size distribution of the objects. According to previous studies in chronic hypoxic heart, it was assumed to be K=1.1 in the present study [22].
- V is the mean volume per mitochondria obtained by simple division of Vv by Nv.

2.6. Southern blot analysis

Total genomic DNA was isolated from homogenates of muscle tissue according to standard procedures. Approximately 5 μg of DNA was digested with XhoI (New England Biolabs, Inc.). Samples were resolved on 0.7% agarose gels by electrophoresis, transferred to nylon membranes and hybridized simultaneously with 32P-labeled probes for mtDNA and the nuclear 18S rRNA gene [23]. Signals were quantified with a phosphorimager using ImageQuant™ software (Molecular Dynamics, Inc.). To correct for quantitative variations among the samples, mtDNA signals were normalized relative to nuclear DNA signals.

Table 1

<table>
<thead>
<tr>
<th>Effects of chronic hypoxia on physical characteristics</th>
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<tr>
<td>Normoxia</td>
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<td>BW (g)</td>
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<td>RWV (mg)</td>
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<td>RVW/RWV (mg/g)</td>
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<td>LSVW/RWV (mg/g)</td>
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Data are mean±SE (n=20 in normoxic group, n=10 in each hypoxic group). Data were obtained from animals whose hearts were used for measurement of substrate oxidation. BW=body weight; RVW=right ventricular free wall weight; LSVW=left ventricular free wall plus septum weight, *P<0.05 versus normoxic animals.
Effects of chronic hypoxia on mitochondrial oxidative phosphorylation

<table>
<thead>
<tr>
<th>Glutamate/Malate</th>
<th>ATP synthesis</th>
<th>ATP/O</th>
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<td></td>
<td>−ADP</td>
<td>+ADP</td>
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<tr>
<td>Glutamate Malate</td>
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<td>Normoxia</td>
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<tr>
<td>RV</td>
<td>3.2±0.2</td>
<td>14.1±1.2</td>
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<tr>
<td>LV</td>
<td>3.5±0.2</td>
<td>15.0±1.1</td>
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<tr>
<td>14 days Hypoxia</td>
<td>2.7±0.3</td>
<td>11.9±1.1</td>
</tr>
<tr>
<td>RV</td>
<td>2.9±0.1*</td>
<td>11.5±0.8*</td>
</tr>
<tr>
<td>LV</td>
<td>19.7±1.9*</td>
<td>1.8±0.1</td>
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<tr>
<td>21 days Hypoxia</td>
<td>3.2±0.2</td>
<td>10.4±0.8*</td>
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<tr>
<td>RV</td>
<td>9.3±0.6*</td>
<td>17.6±2.4*</td>
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<tr>
<td>LV</td>
<td>1.9±0.3</td>
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</table>

Data are mean±SE (n=20 in normoxic group, n=10 in each hypoxic group). RV=right ventricular free wall; LV=left ventricular free wall. Experimental conditions are described in “Material and Methods”. Basal, without adenosine diphosphate (ADP), and ADP-stimulated oxygen consumption rates supported by glutamate or palmitoyl carnitine, in the presence of malate, are expressed in nmol atom oxygen.min−1.mg wet weight−1. ATP synthesis rate is expressed in nmol ATP.min−1.mg wet weight−1. ATP-to-oxygen ratio (ATP/O) is calculated as the ratio of the rate of ATP synthesis to the rate of the concomitant respiration in the presence of ADP. *P<0.05 versus normoxic animals, 1P<0.05 versus right ventricle under the same conditions.

2.7. Data analysis

Results were expressed as mean±SE. Data were plotted and analyzed using SigmaPlot 8.0 and Systat 10.0 (SPSS, Chicago, IL). Differences between groups were tested using analysis of variance with post hoc Dunnett’s test. Paired Student’s t-test was used to compare data of LV and RV in the same animals. All P values were two-tailed, and a P value of less than 0.05 was required to reject the null hypothesis.

3. Results

3.1. Physical characteristics

There was little change in body weight and the heart-to-body weight ratio remained constant in normoxic animals throughout the experiments. In contrast, in rats exposed to CH for 14 days, cardiac mass increased significantly (1.65±0.06 g and 1.05±0.04 g in hypoxic and normoxic animals, respectively, P<0.05) with also a significant increase in the heart-to-body weight ratio (0.54±0.09/100 g and 0.29±0.03/100 g, respectively, P<0.05). The hypertrophy of hypoxic heart was associated with an increase in the RV mass (348±16 mg and 146±4 mg in 14 days hypoxic and normoxic animals, respectively, P<0.05), and in the RV-to-LVS ratio (Table 1). In rats exposed to hypobaric hypoxia for 21 days, the RV-to-LVS ratio was almost threefold the control value (0.62±0.02 and 0.22±0.01 in 21 days hypoxic and normoxic animals, respectively, P<0.05).

3.2. Oxidative capacity

In normoxic rats, no difference was found in any respiratory parameter between RV and LV with either glutamate or palmitoyl carnitine as substrates. On glutamate, mitochondrial oxygen consumption and ATP synthesis significantly decreased at 14 days of CH in the LV without change in ATP/O ratio (Table 2). A similar decrease was still observed in the LV following 21 days of CH. Likewise, oxidative capacity of the RV was decreased following 21 days of CH. However, unlike in LV, oxidative phosphorylation on glutamate remained consistent in RV at 14 days of CH (Table 2). The results look slightly different with
Palmitoyl carnitine as substrate (Table 2). Mitochondrial respiration and ATP synthesis were significantly decreased in both ventricles following 14 days of CH. At 21 days of CH, oxidative capacities tended to return to normoxic values. The enzyme activity of the respiratory chain showed comparable time-dependent changes according to the ventricle. Citrate synthase and the four enzyme complexes of the respiratory chain decreased following 14 days of CH in the LV, but not in the RV (Table 3). The amplitude of these changes represented an averaged reduction of 30% of the enzymatic activities. Nearly all these activities (except complex I) were reduced in both ventricles following 21 days of CH (Table 3). However, it is noteworthy that the ratio of the activity of each respiratory chain complex to citrate synthase activity were not significantly different, suggesting a global decrease in mitochondrial enzymes (Fig. 1).

3.3. Morphometric analysis

Following 14 days of CH, mitochondrial Vv, i.e., the relative mitochondrial fraction of the sarcoplasm, decreased

![Fig. 1. Ratio of the enzyme activity of the respiratory chain complexes to the citrate synthase activity. Experimental conditions are described in “Material and Methods.” Enzyme activities are reported in Table 3. Values are means±SE (n=20 in normoxic group, n=10 in each hypoxic group).](https://academic.oup.com/cardiovascres/article-abstract/66/1/132/463015)

![Fig. 2. Electron micrographs of mitochondria in rat heart (×17,250): mitochondria in normoxic left ventricle (A), in right ventricle from rat exposed to 14 days of chronic hypoxia (B) and in left ventricle from rat exposed to 21 days of chronic hypoxia (C). There was no change at 14 days of chronic hypoxia. On the contrary, there was a significant increase in numerical density and a decrease in size of mitochondria after 21 days of chronic hypoxia.](https://academic.oup.com/cardiovascres/article-abstract/66/1/132/463015)
3.4. Mitochondrial DNA

In normoxic animals, the ratio of mtDNA to nuclear DNA (18S) was similar in RV and LV. Following 14 days of CH, this ratio was markedly reduced only in LV (Fig. 3) as was the oxidative capacity in the same ventricle for the same duration of CH. In contrast, following 21 days of CH, mtDNA to nuclear DNA ratio was reduced in both ventricles, without change in the signal of 18S spot.

4. Discussion

The purpose of the present investigation was to examine the time course of the differential mitochondrial energy metabolism adaptation to chronic hypoxia in right and left ventricles in the rat heart taking advantage that, whereas CH is characterized by a global reduction in oxygen supply, it selectively induces a functional overload of the RV due to pulmonary hypertension. Our results indicate that CH decreases ATP synthesis as a consequence of an alteration in mitochondria function in both ventricles but that this effect is delayed in the RV. However, when RV hypertrophy was fully developed, mitochondrial energy metabolism was decreased as in the LV, suggesting some specific adaptive processes at the onset of RV hypertrophy.

CH leads to pulmonary hypertension which induces a marked RV hypertrophy. In the present study rats were exposed to CH for 2 and 3 weeks on the basis of previous experiments which have shown that such duration leads to an alteration of LV and the full development of RV hypertrophy, respectively [8,10,24]. When RV hypertrophy reaches its maximal amplitude, following 3 weeks of CH, mean pulmonary arterial pressure rises from, on average, 10 (control condition) to 32 mmHg and the ratio RVW/LVSW increases, on average, up to 0.6 [24], a value close to that found in the present study (Table 1).

Oxidation of glutamate and most enzyme activities of the respiratory chain decreased in both ventricles following 21 days of CH. At that stage, the change in oxidative capacity was characterized by a similar decline in ATP synthesis and in oxygen consumption. The normal ATP/O ratio indicated that the yield of oxidative phosphorylation was not modified. Similar findings have been reported in heart and liver mitochondria of rats acclimatized to a 4400-m simulated altitude. No changes in the oxygen or ADP dependence of mitochondrial respiration was shown as a mechanism of adaptation to chronic hypoxia and the ATP/O ratio was similar in heart mitochondria from hypoxic or normoxic animals [25–27]. Rumsey et al. have shown that oxidation of pyruvate and glutamate was compromised in LV as early as 7 days of hypoxic exposure in a 10% O₂ atmosphere [9]. In terms of adaptation of enzymes involved in cardiac energetics, total creatine kinase activity and mitochondrial creatine kinase were also reduced in LV of rats submitted 28 days to a simulated altitude of 5500 m [28]. Collectively, these results suggest that decrease in oxidative capacity is an early adaptation mechanism to CH, at least in the LV.

The mechanism of the decrease in oxidative phosphorylation can be related to the reduction in the enzyme activities of the different respiratory chain complexes of the mitochondrial inner membrane [29,30]. In the present study, we have observed a global decrease in all enzyme complexes and in the citrate synthase activity. Normalization of enzyme activities of the respiratory chain complexes by citrate synthase (a mitochondrial enzyme) yields no changes during hypoxia. These data suggest that CH induces a reduction in the functional mitochondrial mass. The decrease in oxidation observed in skinned fibers could be explained by the reduction in mitochondrial mass rather than a decrease in the specific activity of the complexes This hypothesis is compatible with our morphometric data demonstrating a reduction in the mean volume of mitochondria following 21 days of CH and is also in agreement with previous reports regarding the effect on mitochondria of both hypobaric hypoxia (4400 m) for 9–11 months [25] and normobaric hypoxia (FIO₂ at 8%) [22]. The increase in mitochondria number should be considered as a mechanism of adaptation to hypoxia [25,31]. It favors the mitochondrial transport of oxygen by increasing the surface-to-volume ratio of mitochondria.

The reduction of the mitochondrial mass can be ascribed to a decrease in mitochondrial protein synthesis.
explained, at least in part, by mtDNA down regulation as observed in the present study. However, the respiratory chain complexes depend on both mitochondrial and nuclear genes. If 13 sub-units of respiratory complexes are encoded by mtDNA, nuclear genes contribute to the machinery required for maintenance, replication and expression of mtDNA. Among the nuclear transcriptional regulators implicated in mitochondrial biogenesis, the nuclear respiratory factors (NRF-1 and NRF-2) play an integrative role [32]. In mice, the loss of function of NRF-1 is associated with a lethal phenotype and a dramatic decrease in the amount of mtDNA [33]. NRF-1 is involved in the maintenance of the respiratory apparatus. Since NRF gene expression is down-regulated by hypoxia, it is likely involved in the alteration of the electron transfer chain and in the decrease in mtDNA observed during CH [3]. Moreover, protein synthesis is an ATP-consuming process that is also down-regulated during CH [2]. Therefore, a global decline in cellular protein synthesis could also at least in part, contribute to the decrease in mitochondrial proteins.

The results concerning the time course of CH-induced alteration in energy metabolism in the RV deserve further discussion. As above discussed, we report a decrease in ATP synthesis in RV at 21 days of CH with all substrates in agreement with a previous study showing a decrease in mitochondrial respiration with pyruvate as substrate after 3 weeks of exposure to hypoxic conditions (FiO2 10%) [34]. In contrast, Rumsey et al. have shown an alteration of the oxidative metabolism in RV only with palmitoyl carnitine as substrate but not with pyruvate nor glutamate [9]. This difference could be due to difference in the hypoxic model and/or the techniques used. In the present study, saponin-skinned fibers from each ventricle were used to directly assess the mitochondrial metabolism. The decrease in food utilization following long-term exposure to chronic hypoxia can also in part explain the changes in cardiac energy metabolism [35]. However, these alterations affect both ventricles and cannot totally account for the differences between RV and LV [36].

Oxidation of palmitoyl carnitine was decreased in both ventricles following 14 days of CH. The mechanism of the reduction of fatty acid catabolism is not still elucidated. Recent studies on mechanically overload hearts revealed that the development of cardiac hypertrophy is frequently associated with an impaired fatty oxidation [37]. Therefore, during the development of cardiac hypertrophy, a shift from fatty acid to glucose utilization may occur [38]. This alteration could be due to a decrease in long-chain fatty acid oxidation with alteration in transporters or decline in fatty acid beta oxidation pathway. Moreover, acclimation to hypoxia might affect the relative contribution of lipids to energy metabolism with also a shift toward higher utilisation of carbohydrate [39]. Hypoxia per se induced a rise in hexokinase activity and a fall of hydroxy-acyl CoA-dehydrogenase activity in both myocardial ventricles [40]. Probably, the effects of both overload heart and chronic hypoxia contribute to the observed decrease in palmitoyl carnitine metabolism in RV at 14 days of CH. The fact that fatty acid oxidation tended to return to baseline value by 21 days of hypoxia in the same time as the activities of all respiratory complexes decrease remain difficult to explain. Several hypotheses can be proposed: a specific alteration in fatty acids transport, a slight uncoupling of oxidative phosphorylation or a differential effect of CH of sub-populations of heart mitochondria (subsarcolemmal and interfibrilar mitochondria) as shown for aging [41]. Further studies are necessary to understand fatty acid catabolism during prolonged hypoxia [9,42].

Interestingly, whereas at 21 days of CH the mitochondrial metabolism was decreased in both ventricles, the oxidative capacity of the RV was maintained on glutamate following 14 days of CH, i.e., CH-induced alteration in RV energy metabolism was delayed. We suggest that the compensatory increase in RV mass could, in part, explain that oxidative capacity is maintained at 14 days of CH. In this connection, it has been shown that increasing energy demand enhances mitochondrial cytochrome content in skeletal muscle and the heart [43]. Alternatively, a recent study in rats submitted to hypobaric hypoxia (450 mmHg corresponding to an FiO2 of ≈12%) has indicated that acclimatizing to a simulated altitude may have a tissue-specific effect on protein expression [44]. Whatever, the mechanism, it is noteworthy when RV hypertrophy was fully developed, following 21 days of CH [10,24], mitochondrial energy metabolism was decreased as in the LV. The present results suggest that cardiac hypertrophy only transiently compensates for the hypoxia-induced alteration in mitochondrial energy metabolism. In this connection, we have previously observed, at the site of the pulmonary vasculature, a similar transient adaptive phenomenon at the onset of RV hypertrophy, the occurrence of rhythmic contractions [10]. Very recent studies indicate that CH-induced pulmonary hypertension and RV hypertrophy can be prevented, at least in the rat, by administering in vivo, in the course of exposure to hypoxia, a variety of drugs including serotonin transporter inhibitors [45], sildenafil [46,47] or DHEA [48]. Whether, under such conditions, antagonism of RV hypertrophy would accelerate CH-induced alteration in RV energy metabolism remains to be examined to better understand the differential mitochondrial energy metabolism adaptation to chronic hypoxia in right and left ventricles.

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References


